IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Marija Vrljic	Art Unit	:	1791
Patent No.	:	10,039,306	Examiner	:	Elizabeth A. Gwartney
Issue Date	:	August 7, 2018	Conf. No.	:	8501
Serial No.	:	14/796,970			
Filed	:	July 10, 2015			
Title	:	METHODS AND COMPOSITIONS FOR CONSUMABLES			

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PETITION TO ACCEPT UNINTENTIONALLY DELAYED
BENEFIT CLAIMS UNDER 37 C.F.R. §§ 1.78(c) AND 1.78(e)

Applicant hereby submits a petition to the Office under 37 C.F.R. §§ l.78(c) and 1.78(e) to accept the unintentionally delayed claims for benefit of prior-filed applications as noted in the Supplemental Application Data Sheet and Request for Certificate of Correction filed concurrently herewith. Pursuant to 37 C.F.R. §§ l.78(c)(3) and l.78(e)(3), the entire delay between the date the priority claims were due and the date the priority claims were filed was unintentional.

U.S. Application No. 14/796,970 (the "present application") was filed on July 10, 2015, and was issued on August 7, 2018, as U.S. Patent No. 10,039,306 (the "present patent"). This Petition Under 37 C.F.R. §§ l.78(c) and 1.78(e) (the "Petition") is being filed on May 30, 2023, more than two years after the date the domestic benefit claim was due. Thus, under 37 CFR §§ 1.78(c)(3) and 1.78(e)(3), the Director may require additional information where there is a question whether the delay was unintentional.

Applicant first became aware of unintentional omissions in the benefit claim of the present patent after detailed analysis of the petitions for *Inter Partes* Review of U.S. Patent Nos. 9,943,096 (IPR2023-00206), 11,224,241 (IPR2023-00207), 11,013,250 (IPR2023-00208), and 10,039,306 (IPR2023-00209), all filed on December 7, 2022.

The CROSS-REFERENCE TO RELATED APPLICATIONS on page 1 of the specification of the present application states:

> This application claims priority to U.S. Application Serial No. 13/941,211, filed July 12, 2013, U.S. Application Serial No. 61/908,634, filed November 25, 2013, and to U.S. Application Serial No. 61/751,816, filed January 11, 2013; and is

> related to the following co-pending patent applications: Application Serial No. PCT/US12/46560; Application Serial No PCT/US12/46552; Application Serial No. 61,876,676, filed September 11, 2013; Application Serial No. 61/751,818, filed January 11, 2013, and Application Serial No. 61/611,999, filed March 16, 2012, all of which are incorporated herein by reference.

On September 15, 2015, a Preliminary Amendment and Request for Corrected Official Filing Receipt was filed to amend the CROSS-REFERENCE TO RELATED APPLICATIONS as shown below:

> This application is a Continuation of, and claims the benefit of priority under 35 U.S.C. § 120 to, PCT/US2014/011361, which is a Continuation of, and claims the benefit of priority under 35 U.S.C. § 120 to, U.S. Application Serial No. 13/941,211, filed July 12, 2013, which claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Application Serial No. 61/908,634, filed November 25, 2013, and to U.S. Application Serial No. 61/751,816, filed January 11, 2013; and this application is related to the following co-pending patent applications: Application Serial No. PCT/US2012/46560; Application Serial No PCT/US2012/46552; Application Serial No. 61[[,]] / 876,676, filed September 11, 2013; Application Serial No. 61/751,818, filed January 11, 2013, and Application Serial No. 61/611,999, filed March 16, 2012, all of which are incorporated herein by reference.

It appears that this benefit claim was based on CROSS-REFERENCE TO RELATED

APPLICATIONS on page 1 of the specification of PCT/US2014/011361, which states:

> This application claims priority to U.S. Application Serial No. 13/941,211, filed July 12, 2013, U.S. Application Serial No. 61/908,634, filed November 25, 2013, and to U.S. Application Serial No. 61/751,816, filed January 11, 2013; and is related to the following co-pending patent applications: Application Serial No. PCT/US12/46560; Application Serial No PCT/US12/46552; Application Serial No. 61,876,676, filed September 11, 2013; Application Serial No. 61/751,818, filed January 11, 2013, and Application Serial No. 61/611,999, filed March 16, 2012, all of which are incorporated herein by reference.

The CROSS REFERENCE on page 1 of the specification of U.S. Application No. 13/941,211

states:

> This application is a continuation-in-part of International Application No. PCT/US2012/046560, filed July 12, 2012, which claims the benefit of priority of U.S. Provisional Application 61/572,205, filed July 12, 2011 and is a non-

provisional of and claims the benefit of U.S. Provisional Application 61/671,069, filed July 12, 2012, all of which are incorporated herein by reference.

The omission of the priority claim in the present application to U.S. Application No. 61/671,069, PCT/US2012/046560, and U.S. Application No. 61/572,205 was made unintentionally, based on the priority claim in PCT/US2014/011361. This error was inadvertently carried through later applications that claim the benefit of the present application.

As noted above, applicant first became aware of omissions in the benefit claim of the present patent after detailed analysis of the petitions for *Inter Partes* Review of U.S. Patent Nos. 9,943,096 (IPR2023-00206), 11,224,241 (IPR2023-00207), 11,013,250 (IPR2023-00208), and 10,039,306 (IPR2023-00209), all filed on December 7, 2022. The time period from receipt of the IPR petitions to filing of the Petitions for Correction in related family members reflects a reasonable period of time for applicant to conduct a diligent investigation into the challenges in each of IPR2023-0206, -0207, -0208 and -0209 petitions, including invalidity and priority issues, investigation of the extensive patent family and prosecution histories of each family, as well as consulting with IPR counsel to take into consideration the procedural complexities presented by co-pending litigation.

On March 20, 2023, applicant filed Patent Owner's Preliminary Responses in U.S. Patent Nos. 9,943,096 (IPR2023-00206), 11,224,241 (IPR2023-00207), 11,013,250 (IPR2023-00208), and 10,039,306 (IPR2023-00209), along with exhibits including petitions under 37 C.F.R. § 1.78 filed on March 20, 2023, in related U.S. Application Nos. 15/786,776 and 14/797,006. Between March 21, 2023, and May 22, 2023, applicant requested and was granted leave to seek certificates of correction in U.S. Patent Nos. 9,943,096 (IPR2023-00206), 11,224,241 (IPR2023-00207), 11,013,250 (IPR2023-00208), and 10,039,306 (IPR2023-00209).

Between March 24, 2023, and March 28, 2023, petitions under 37 C.F.R. § 1.78 and requests for certificates of correction were filed in related U.S. Application Nos. 15/624,505, 15/624,513, 15/839,994, 15/913,018, 15/912,763, 16/238,769, 17/081,938, 17/531,442, 17/081,908, and 17/081,922.

Decisions on some but not all of the petitions under 37 C.F.R. § 1.78 have been received. For instance, on April 19, 2023, a Decision on Petition under 37 CFR 1.78(c) and 1.78(e) was received for U.S. Application No. 14/797,006, noting reasons for dismissal, including an inconsistency between the benefit claim of U.S. Patent Application No. 13/941,211 and PCT/US2012/046560. In particular, the Decision noted that U.S. Application No. 13/941,211 does not directly claim the benefit of U.S. Application No. 61/671,069.

On April 28, 2023, a petition to accept unintentionally delayed benefit claims under 37 C.F.R. § 1.78(c) was filed to correct what appeared to be a clerical error on the Application Data Sheet filed with U.S. Application No. 13/941,211, and thereby align the benefit claim with the CROSS-REFERENCE on page 1 of the specification for U.S. Application No. 13/941,211, which states:

> This application is a continuation-in-part of International Application No. PCT/US2012/046560, filed July 12, 2012, which claims the benefit of priority of U.S. Provisional Application 61/572,205, filed July 12, 2011 and is a non-provisional of and claims the benefit of U.S. Provisional Application 61/671,069, filed July 12, 2012, all of which are incorporated herein by reference.

The Order Granting Patent Owner's Motion for Leave to Seek a Certificate of Correction in IPR2023-00206 (Patent 9,943,096), IPR2023-00207 (Patent 11,224,241), IPR2023-00208 (Patent 11,013,250), and IPR2023-00209 (Patent 10,039,306) (the "Order") was issued on May 22, 2023. The Order granted Patent Owner's motions for leave to request a certificate of correction for the challenged patent in each of the above-identified proceedings, and ordered Patent Owner to file with the Director a Petition for Certificate of Correction within five (5) business days of the entry of the order.

On May 25, 2023, a Decision on Petition under 37 CFR 1.78(c) and 1.78(e) was issued for U.S. Application No. 15/786,776, noting reasons for dismissal.

This Petition is being filed on May 30, 2023, as required by the Order.

As explained above, the entire delay between the date the claim for benefit of prior-filed applications was due and the date the claim was filed was unintentional.

Applicant and Applicant's representatives submit the following:

- The present patent was issued from U.S. Application No. 14/796,970, filed on July 10, 2015, which is a continuation of PCT/US2014/011361, filed on January 13, 2014, which is a continuation of and claims priority to U.S. Application No. 13/941,211, filed on July 12, 2013, and claims priority to U.S. Application No. 61/908,634, filed on November 25, 2013, and to U.S. Application No. 61/751,816, filed on January 11, 2013.
- In the CROSS-REFERENCE TO RELATED APPLICATIONS of the present patent, PCT/US2012/046560, PCT/US2012/046552, U.S. Application No. 61/876,676, U.S. Application No. 61/751,818, and U.S. Application No. 61/611,999, were identified as "related to" the present application.
- An Application Data Sheet ("ADS") including a claim to priority under 35 U.S.C. § 120 was filed concurrently with the present application on July 10, 2015, and later revised on September 15, 2015. However the corresponding Application Data Sheets, as well as the corresponding CROSS-REFERENCE TO RELATED APPLICATIONS paragraphs, omitted the priority claims to prior-filed U.S. Application No. 61/671,069, PCT/US2012/046560, and U.S. Application No. 61/572,205.
- The omission of the priority claims to U.S. Application No. 61/671,069, PCT/US2012/046560, and U.S. Application No. 61/572,205 initially occurred in the filing of this application (U.S. Application No. 14/796,970), in which priority claims to U.S. Application Nos. 61/671,069 and 61/572,205 under 35 U.S.C. § 119(e) and to PCT/US2012/046560 under 35 U.S.C. § 120 were unintentionally omitted. This error was inadvertently carried through later-filed applications that claim the benefit of U.S. Application No. 14/796,970.
- Applicant first became aware of unintentional omissions in the benefit claim of the present patent (U.S. Patent No. 10,039,306) after detailed analysis of the petitions for *Inter Partes* Review of U.S. Patent Nos. 9,943,096 (IPR2023-00206), 11,224,241

(IPR2023-00207), 11,013,250 (IPR2023-00208), and 10,039,306 (IPR2023-00209), all filed on December 7, 2022.

- On April 28, 2023, a Petition under 37 C.F.R. § l.78(c) was filed to correct the priority claim of U.S. Application No. 13/941,211 to clarify that U.S. Application No. 13/941,211 claims the benefit of U.S. Application No. 61/671,069, filed on July 12, 2012, and is a continuation-in-part of PCT/US2012/046560, filed on July 12, 2012, which claims the benefit of U.S. Application No. 61/572,205, filed on July 12, 2011.
- U.S. Application No. 14/796,970, from which the present patent was issued, was examined under the pre-AIA first to invent provisions. Thus, the correction sought with this petition would not change the statutory framework under which U.S. Application No. 14/796,970 was examined, and further examination would not be required.

A Supplemental Application Data Sheet and Request for Certificate of Correction are being filed with this Petition to include the references required by 37 C.F.R. §§ l.78(c)(1) and 1.78(e)(1). A Petition for Expedited Review Under 37 C.F.R. § 1.182 is also being filed with this Petition.

As required by the ORDER Granting Patent Owner's Motion for Leave to Seek a Certificate of Correction entered May 22, 2023, in IPR2023-00206 (U.S. Patent No. 9,943,096), IPR2023-00207 (U.S. Patent No. 11,224,241), IPR2023-00208 (U.S. Patent No. 11,013,250) and IPR2023-00209 (U.S. Patent No. 10,039,306), the following documents are also being filed with this Petition:

- EXHIBIT 1: PETITIONER'S OPPOSITION TO PATENT OWNER'S MOTION PURSUANT TO 37 C.F.R. § 1.323 for *Inter Partes* Review No. 2023-00209 (Paper No.11), dated April 21, 2022 [*sic*];

- EXHIBITS 1A-1H, corresponding to Motif EX1160-EX1167 filed with PETITIONER'S OPPOSITION TO PATENT OWNER'S MOTION PURSUANT TO 37 C.F.R. § 1.323 for *Inter Partes* Review No. 2023-00209; and

- EXHIBIT 2: ORDER Granting Patent Owner's Motion for Leave to Seek a Certificate of Correction entered May 22, 2023, in IPR2023-00206, IPR2023-00207, IPR2023-00208, and IPR2023-00209 (Paper No. 15)

The required petition fee as set forth in 37 C.F.R. § 1.17(m) is submitted herewith to satisfy 37 C.F.R. §§ l.78(c)(2) and 1.78(e)(2).

And, as required by 37 C.F.R. §§ l.78(c)(3) and 1.78(e)(3), the entire delay between the date the benefit claim was due under paragraph (a)(4) or (d)(3) of this section and the date the benefit claim is being filed was unintentional.

Petitions to correct priority claims and corresponding Requests for Certificate of Correction are also being filed in U.S. Patent No. 11,013,250, as well as other patents that claim the benefit of U.S. Application No. 13/941,211.

Accordingly, Applicant respectfully requests that the Office grant this Petition, and grant the Request for Certificate of Correction filed concurrently herewith.

The fee of $2,100 required by 37 C.F.R. § 1.17(m) is being paid with this request. Please apply any other necessary charges or credits to Deposit Account No. 06-1050, referencing the above-indicated Attorney Docket Number.

Respectfully submitted,

Date: May 30, 2023

/Heather L. Flanagan/
Heather L. Flanagan
Reg. No. 54,101

Customer Number 26191
Fish & Richardson P.C.
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Marija Vrljic	Art Unit	:	1791
Patent No.	:	10,039,306	Examiner	:	Elizabeth A. Gwartney
Issue Date	:	August 7, 2018	Conf. No.	:	8501
Serial No.	:	14/796,970			
Filed	:	July 10, 2015			
Title	:	METHODS AND COMPOSITIONS FOR CONSUMABLES			

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PETITION FOR EXPEDITED REVIEW UNDER 37 C.F.R. § 1.182

Pursuant to 37 C.F.R. § 1.182, Applicant petitions for expedited review of the accompanying Petition to Accept Unintentionally Delayed Benefit Claims Under 37 C.F.R. §§ 1.78(c) and 1.78(e) (the "37 C.F.R. § 1.78 Petition").

As noted in the 37 C.F.R. § 1.78 Petition, Applicant first became aware of unintentional omissions in the benefit claim of the present patent (U.S. Patent No. 10,039,306) after detailed analysis of the petitions for *Inter Partes* Review of U.S. Patent Nos. 9,943,096 (IPR2023-00206), 11,224,241 (IPR2023-00207), 11,013,250 (IPR2023-00208), and 10,039,306 (IPR2023-00209), all filed on December 7, 2022. Concurrently with this Petition Under 37 C.F.R. § 1.182, Applicant has submitted the 37 C.F.R. § 1.78 Petition along with a Supplemental Application Data Sheet and a Request for Certificate of Correction for this patent (U.S. Patent No. 10,039,306) as well as other U.S. patents claiming the benefit of U.S. Application No. 13/941,211.

Expedited review of the 37 C.F.R. § 1.78 Petition is necessary to prevent delay of the IPR proceedings for U.S. Patent Nos. 11,013,250 (IPR2023-00208) and 10,039,306 (IPR2023-00209). Thus, Applicant requests an expedited review of the 37 C.F.R. § 1.78 Petition filed with this Petition Under 37 C.F.R. § 1.182.

The fee of $420 required by C.F.R. § 1.17(f) is being paid with this request. Please apply any other necessary charges or credits to Deposit Account No. 06-1050, referencing the above-indicated Attorney Docket Number.

Respectfully submitted,

Date: May 30, 2023

/Heather L. Flanagan/
Heather L. Flanagan
Reg. No. 54,101

Customer Number 26191
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Telephone: (612) 335-5070
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	38767-0010001
	Application Number	14/796,970

Title of Invention	METHODS AND COMPOSITIONS FOR CONSUMABLES

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Application Information:

Title of the Invention	METHODS AND COMPOSITIONS FOR CONSUMABLES		
Attorney Docket Number	38767-0010001	**Small Entity Status Claimed** ☐	
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	9	**Suggested Figure for Publication (if any)**	

Filing By Reference:

Only compete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., “Domestic Benefit/National Stage Information” and “Foreign Priority Information”).

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing Date (YYYY-MM-DD)	Intellectual Property Authority or Country

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Enter either Customer Number or complete the Representative Name section below. If both sections are completed the Customer Number will be used for the Representative Information during processing.

Please Select One:	☒ Customer Number	☐ U.S. Patent Practitioner	☐ Limited Recognition (37 CFR 11.9)
Customer Number	26191		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the “Application Number” field blank.

Prior Application Status	~~Pending~~ Expired		
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (**YYYY-MM-DD**)
14796970	Continuation of	PCTUS2014011361	2014-01-13

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	38767-0010001
	Application Number	14/796,970
Title of Invention	METHODS AND COMPOSITIONS FOR CONSUMABLES	

Prior Application Status	~~Pending~~ Abandoned		
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (**YYYY-MM-DD**)
PCTUS2014011361	Continuation of	13941211	2013-07-12

Prior Application Status	Expired		
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (**YYYY-MM-DD**)
PCTUS2014011361	Claims benefit of	61908634	2013-11-25

Prior Application Status	Expired		
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (**YYYY-MM-DD**)
PCTUS2014011361	Claims benefit of	61751816	2013-01-11

Prior Application Status	Expired		
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (**YYYY-MM-DD**)
13941211	Continuation-in-part of	PCTUS2012046560	2012-07-12

Prior Application Status	Expired		
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (**YYYY-MM-DD**)
PCTUS2012046560	Claims benefit of	61572205	2011-07-12

Prior Application Status	Expired		
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (**YYYY-MM-DD**)
13941211	Claims benefit of	61671069	2012-07-12

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b).

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Heather L. Flanagan/			Date (**MM/DD/YYYY**)	May 30, 2023
First Name	Heather	Last Name	Flanagan	Registration Number	54,101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Marija Vrljic	Art Unit	:	1791
Patent No.	:	10,039,306	Examiner	:	Elizabeth A. Gwartney
Issue Date	:	August 7, 2018			
Serial No.	:	14/796,970	Conf. No.	:	8501
Filed	:	July 10, 2015			

Title : METHODS AND COMPOSITIONS FOR CONSUMABLES

Attention Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION

A Certificate of Correction is requested in the above-identified patent (the "Subject Patent"). The exact locations of the errors appearing in the Subject Patent are as follows:

On the Title Page

Item (63), delete:

"Continuation of application No. PCT/US2014/011361, filed on Jan. 13, 2014, which is a continuation of application No. 13/941,211, filed on Jul. 12, 2013."

And insert:

-- Continuation of application No. PCT/US2014/011361, filed on Jan. 13, 2014, which is a continuation of application No. 13/941,211, filed on Jul. 12, 2013, now abandoned, which claims the benefit of application No. 61/671,069, filed on July 12, 2012, and is a continuation-in-part of application No. PCT/US2012/046560, filed on July 12, 2012, which claims the benefit of application No. 61/572,205, filed on July 12, 2011. –

In the Specification

In Column 1, CROSS-REFERENCE TO RELATED APPLICATIONS, delete:

"This application is a Continuation of, and claims the benefit of priority under 35 U.S.C. 120 to, PCT/US2014/011361, which is a Continuation of, and claims the benefit of priority under 35 U.S.C. § 120 to, U.S. application Ser. No. 13/941,211, filed Jul. 12, 2013, which claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Application Ser. No. 61/908,634, filed Nov. 25, 2013, and to U.S. Application Ser. No. 61/751,816, filed Jan. 11, 2013; and this application is related to the following co-pending patent applications: Application Serial No. PCT/US2012/46560; Application Ser. No. PCT/US2012/46552; Application Ser. No. 61/876,676, filed Sep. 11, 2013; Application Ser. No. 61/751,818, filed Jan. 11, 2013, and Application Ser. No. 61/611,999, filed Mar. 16, 2012, all of which are incorporated herein by reference."

And insert:

-- This application (1) is a continuation of and claims priority to PCT/US2014/011361, filed on Jan. 13, 2014, which is incorporated by reference and is a continuation of and claims priority to U.S. Application No. 13/941,211, filed on Jul. 12, 2013, now abandoned, which is incorporated by reference and claims the benefit of U.S. Application No. 61/671,069, filed on Jul. 12, 2012, and is a continuation-in-part of PCT/US2012/046560, filed on Jul. 12, 2012, which is incorporated by reference and claims the benefit of U.S. Application No. 61/572,205, filed on Jul. 12, 2011; (2) claims priority to U.S. Application No. 61/908,634, filed on Nov. 25, 2013, which is incorporated by reference; (3) claims priority to U.S. Application No. 61/751,816, filed on Jan. 11, 2013, which is incorporated by reference; and (4) is related to the following patent applications, which are incorporated by reference: PCT/US2012/046552, filed on Jul. 12, 2012; U.S. Application No. 61/876,676, filed on Sep. 11, 2013; U.S. Application No. 61/751,818, filed on Jan. 11, 2013; and U.S. Application No. 61/611,999, filed on Mar. 16, 2012. --

Because the Subject Patent claims and previously claimed priority to U.S. Application No. 13/941,211, which itself claims the benefit of U.S. Application No. 61/671,069 and is a continuation-in-part of PCT/US/2012/046560, filed on July 12, 2012, which claims the benefit of U.S. Application Serial No. 61/572,205, filed on July 12, 2011, it is clear from the record that the revised priority claims are appropriate.

Applicant submits that correction of these errors does not require the reexamination of the Subject Patent. It is therefore requested that a Certificate of Correction reflecting the corrections noted on the attached form PTO/SB/44 be issued.

This request is accompanied by a Supplemental Application Data Sheet, a Petition to Accept Unintentionally Delayed Benefit Claims under 37 C.F.R. §§ 1.78(c) and 1.78(e), and a Petition for Expedited Review Under 37 C.F.R. § 1.182.

The fee of $160 required by 37 C.F.R. § 1.20(a) is being paid with this request. Please apply any other necessary charges or credits to Deposit Account No. 06-1050, referencing the above-indicated Attorney Docket Number.

Respectfully submitted,

Date: May 30, 2023

/ Heather L. Flanagan /
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PTO/SB/44 (09-07)
Approved for use through 08/31/2013. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 10,039,306

APPLICATION NO.: 14/796,970

ISSUE DATE : August 7, 2018

INVENTOR(S) : Marija Vrljic, Sergey Solomatin, Rachel Fraser, Patrick O'Reilly Brown, Jessica Karr, Celeste Holz-Schietinger, Michael Eisen and Ranjani Varadan

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page

Item (63), delete:

"Continuation of application No. PCT/US2014/011361, filed on Jan. 13, 2014, which is a continuation of application No. 13/941,211, filed on Jul. 12, 2013."

And insert:

-- Continuation of application No. PCT/US2014/011361, filed on Jan. 13, 2014, which is a continuation of application No. 13/941,211, filed on Jul. 12, 2013, now abandoned, which claims the benefit of application No. 61/671,069, filed on July 12, 2012, and is a continuation-in-part of application No. PCT/US2012/046560, filed on July 12, 2012, which claims the benefit of application No. 61/572,205, filed on July 12, 2011. –

In the Specification

In Column 1, CROSS-REFERENCE TO RELATED APPLICATIONS, delete:

"This application is a Continuation of, and claims the benefit of priority under 35 U.S.C. 120 to, PCT/US2014/011361, which is a Continuation of, and claims the benefit of priority under 35 U.S.C. § 120 to, U.S. application Ser. No. 13/941,211, filed Jul. 12, 2013, which claims the benefit of priority under 35 U.S.C. § 119(e) to

MAILING ADDRESS OF SENDER (Please do not use customer number below):
Fish & Richardson P.C.
P.O. Box 1022
Minneapolis, MN 55440-1022

This collection of information is required by 37 CFR 1.322, 1.323, and 1.324. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1.0 hour to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Attention Certificate of Corrections Branch, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 10,039,306

APPLICATION NO.: 14/796,970

ISSUE DATE : August 7, 2018

INVENTOR(S) : Marija Vrljic, Sergey Solomatin, Rachel Fraser, Patrick O'Reilly Brown, Jessica Karr, Celeste Holz-Schietinger, Michael Eisen and Ranjani Varadan

U.S. Application Ser. No. 61/908,634, filed Nov. 25, 2013, and to U.S. Application Ser. No. 61/751,816, filed Jan. 11, 2013; and this application is related to the following co-pending patent applications: Application Serial No. PCT/US2012/46560; Application Ser. No. PCT/US2012/46552; Application Ser. No. 61/876,676, filed Sep. 11, 2013; Application Ser. No. 61/751,818, filed Jan. 11, 2013, and Application Ser. No. 61/611,999, filed Mar. 16, 2012, all of which are incorporated herein by reference."

And insert:

-- This application (1) is a continuation of and claims priority to PCT/US2014/011361, filed on Jan. 13, 2014, which is incorporated by reference and is a continuation of and claims priority to U.S. Application. No. 13/941,211, filed on Jul. 12, 2013, now abandoned, which is incorporated by reference and claims the benefit of U.S. Application No. 61/671,069, filed on Jul. 12, 2012, and is a continuation-in-part of PCT/US2012/46560, filed on Jul. 12, 2012, which is incorporated by reference and claims the benefit of U.S. Application No. 61/572,205, filed on Jul. 12, 2011; (2) claims priority to U.S. Application No. 61/908,634, filed on Nov. 25, 2013, which is incorporated by reference; (3) claims priority to U.S. Application No. 61/751,816, filed on Jan. 11, 2013, which is incorporated by reference; and (4) is related to the following patent applications, which are incorporated by reference: PCT/US2012/046552, filed on Jul. 12, 2012; U.S. Application No. 61/876,676, filed on Sep. 11, 2013; U.S. Application No. 61/751,818, filed on Jan. 11, 2013; and U.S. Application No. 61/611,999, filed on Mar. 16, 2012. --

MAILING ADDRESS OF SENDER (Please do not use customer number below):
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This collection of information is required by 37 CFR 1.322, 1.323, and 1.324. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1.0 hour to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Attention Certificate of Corrections Branch, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and selection option2.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

MOTIF FOODWORKS, INC.,

Petitioner,

v.

IMPOSSIBLE FOODS INC.,
Patent Owner.

U.S. Patent No. 10,039,306

"METHODS AND COMPOSITIONS FOR CONSUMABLES"

Inter Partes Review No. 2023-00209

PETITIONER'S OPPOSITION TO PATENT OWNER'S MOTION PURSUANT TO 37 C.F.R. § 1.323

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Petitioner respectfully opposes PO's Motion because it fails to show there is a "sufficient basis" that the alleged mistake in priority claim is correctable. To the contrary, PO's proposed "correction" is legally defective because it repeats (and exploits) an existing error in the priority claim where one application is linked to another as a "continuation" despite the two applications having entirely different specifications and figures.

Moreover, during prosecution, PO made a deliberate choice—the opposite of a mistake—to identify the prior-filed applications as being merely "related to" the Challenged Patents instead of claiming "priority to" them under 35 U.S.C. §§119-120, as it did for other patent applications. The timing of PO's attempted "correction"—in an attempt to defeat Petitioner's grounds based on PO's own prior art ("Brown") disclosure—reveals PO's proposed changes are strategic rather than fixing an innocent mistake. PO's 7-year delay in seeking a priority change further reveals that the entire delay in seeking correction was not unintentional. Even if PO supposedly only learned of its "mistake" after Petitioner filed IPRs, PO offers no explanation for why it waited 3.5 months to provide notice or seek correction.

PO's request has serious, unforeseeable consequences for Petitioner—if PO is granted leave and the Board gives retroactive effect to any Certificate of Correction ("CoC") issued by the Director, PO will argue that multiple grounds in the IPRs are obviated. However, Petitioner cannot now pursue different, additional

grounds in this proceeding. And absent Board authorization, Petitioner will not have an opportunity to show that the applications Petitioner now seeks priority to fail to provide written description support for the Challenged Claims.

PO's motion to cede jurisdiction should be denied.

I. THE BOARD SHOULD MAINTAIN EXCLUSIVE JURISDICTION

PO incorrectly contends that the rules only require a motion to cede jurisdiction after trial has been instituted. Mot. 3-5. Notwithstanding PO's misstatement of the law, PO's contention is moot. The Board directed the parties to submit briefing concerning PO's request and PO has now filed its Motion pursuant to § 1.323. Petitioner opposes PO's motion and requests that the Board maintain its exclusive jurisdiction under 37 C.F.R. § 42.3(a) and deny PO's request for leave.

II. NO "SUFFICIENT BASIS" FOR CORRECTION

To prevail on its motion, PO must show that "there is sufficient basis … that the mistake may be correctable." *Honeywell Int'l v. Arkema, Inc.*, 939 F.3d 1345, 1351 (Fed. Cir. 2019). PO cannot do so for multiple reasons.

First, to correct priority via a CoC, it "must be clear from the record of the patent and the parent application(s) that priority is appropriate." MPEP § 1481.03.II.A; *see also* 35 U.S.C. §§ 119(e), 120. As explained below, the proposed change to the priority claim fails to satisfy this standard.

The existing priority claim for the Challenged Patents has a defect that

forecloses the priority claim changes that PO now proposes. Specifically, the challenged '096 and '241 patents ("Fraser Patents") currently claim priority through multiple continuation applications to PCT/US2014/011347 ("PCT '347"). PCT '347 is allegedly a ***continuation*** of U.S. application no. 13/941,211 ("'211 App."). However, PCT '347 is not actually a continuation of the '211 App. because they have ***completely different*** specifications and figures. *Compare* EX1160 (PCT '347) *with* EX1161 ('211). Indeed, a redline comparison illustrates that there is virtually no overlap between these two applications. EX1162 (redline comparison) (non-overlapping text shown in blue/red). The same is true for the challenged '250 and '306 patents ("Vrljic Patents"), which currently claim priority to PCT/US2014/011361 ("PCT '361") (instead of PCT '347) as allegedly being a continuation of the '211 App. Here again, PCT '361 and the '211 App. have substantially different specifications and completely different figures. *Compare* EX1163 (PCT '361) *with* EX1161 ('211); *see also* EX1164 (redline comparison). This contravenes the well-known rule that "[b]y definition, ***a continuation adds no new matter***." *Applied Materials, Inc. v. Advanced Semiconductor Materials America, Inc.*, 98 F.3d 1563, 1579 (Fed. Cir. 1996)[1]; MPEP § 201.07; *see also Medtronic CoreValve, LLC v. Edwards Lifesciences Corp.*, 741 F.3d 1359, 1363-67

[1] All emphasis added unless otherwise stated herein.

(Fed. Cir. 2014) (rejecting priority claim where intervening applications failed to accurately identify every patent in the chain per 35 U.S.C. § 120).[2]

This underlying defect in the existing priority claims is critical to the Board's determination here because PO seeks to expand on and exploit the defect by further extending the priority claim back from the '211 App. to earlier-filed applications, including to PCT/US2012/46560 ("Brown"), which Petitioner asserts as prior art to the Fraser Patents.[3] PO's proposed changes to the priority claim are improper as a matter of law, *Applied Materials*, 98 F.3d at 157, and demonstrate that the alleged

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[2] The overlap between PCT '347 and the '211 application is so deficient that PCT '347 could not even be properly characterized as a CIP of the '211 application, which would require PCT '347 or PCT '361 to "***repeat[] some [] substantial portion*** or all" of the '211 application. *Litton Sys., Inc. v. Whirlpool Corp.*, 728 F.2d 1423, 1436-37 (Fed. Cir. 1984) (citing MPEP § 211.08). To be clear, PO has *not* asserted a CIP relationship between PCT '347/PCT '361 and the '211 application and instead has repeatedly made clear that it intends to file the same CoC it recently filed in other related patents that rely on the "continuation" designation discussed above.

[3] PO's proposed priority claim changes also include prov. apps. 61/572,205 and 61/671,069, both of which purport to claim priority through the '211 application. Brown and the '069 and '205 applications are the "Prior-filed Applications."

mistake is not correctable.

Second, 35 U.S.C. § 255 allows a patent owner to correct a "mistake" only if it was made in "good faith." In the context of reissue proceedings, the Federal Circuit has distinguished a correctable "mistake" from an uncorrectable "***deliberate***, but subsequently found to be disadvantageous, choice." *In re Serenkin*, 479 F.3d 1359, 1364 (Fed. Cir. 2007); *id.*, 1362-63 ("the deliberate action of an inventor or attorney … generally fails to qualify as a correctable error under § 251").

Here, it is readily apparent that PO deliberately chose not to claim priority to Brown from the outset. In the first paragraph of the Challenged Patents, PO distinguished Brown from other applications it chose to include in the priority claim:

> This application is a continuation of…14/797,006 filed on Jul. 10, 2015 which is a continuation of and ***claims priority*** to PCT/US2014/011347 which ***claims priority*** to…13/941,211, filed Jul. 12, 2013, … 61/908,634, filed Nov. 25, 2013, and to … 61/751,816, filed Jan. 11, 2013, and is ***related to*** the following patent applications: Application Serial No. ***PCT/US12/46560 [Brown]***; Application Serial No PCT/US12/46552 …"

See, e.g., '096 patent, 1:7-15. PO mischaracterizes the above recitation as an "omission," but in fact PO did not omit Brown—PO deliberately characterized Brown as merely "***related to***" the Challenged Patents. Tellingly, PO carefully reviewed and previously corrected a date within the '096 patent's priority claim after issuance, but left the "related to" language for Brown undisturbed, reinforcing that PO intentionally excluded Brown from its priority claim. EX1165. PO now finds its

choice unhelpful, but this choice was not a mistake and cannot now be corrected.[4]

Third, the "entire delay" in seeking correction to a priority claim must be "unintentional." 37 C.F.R. § 1.78(c), (e). The Office treats a delay of more than two years as an "extended period of delay" that "raises a question as to whether the entire delay was unintentional." EX1166 (85 Federal Register 12222). In such cases, PO is "required to provide an additional explanation of the circumstances surrounding the delay that establishes that the entire delay was unintentional" beyond the simple (but required) "statement that the entire delay was unintentional." *Id.*; *see also* 37 C.F.R. § 1.58(e)(3) ("The Director may require additional information where there is a question whether the delay was unintentional.").

PO fails to provide the required explanation. *See* EX2001. Rather, PO merely asserts that the alleged omission "initially occurred" seven years ago in the first-filed application and "[t]his error was inadvertently carried through later-filed applications." *Id.*, 6-7; Mot. 6. This fails to explain why PO identified Brown as

[4] PO's motivations for making this choice are unknown but could have been two-fold: (1) PO recognized that it could not claim priority to the Prior-filed Applications (see point 1, *supra*), and/or (2) PO chose not to claim priority to the Prior-filed Applications in an effort to extend the life of the patent. Either way, PO's prior deliberate choice forecloses its proposed changes. *In re Serenkin*, 479 F.3d at 1364.

merely "related to " the Challenged Patents instead of claiming priority to Brown. Moreover, even accepting as true PO's assertion that it first learned of the alleged mistake from the IPR Petitions filed on December 7, 2022, PO fails to explain why it intentionally waited 3.5 months to provide notice or request leave to file CoCs.[5]

Fourth, to correct a priority claim, and "[t]o be entitled to the benefit of the filing date of an earlier-filed application, … the disclosure of the prior-filed application must provide adequate support … for the claimed subject matter of the later-filed application." MPEP § 211.05. Here, the Petitions against the Fraser Patents establish that the '816 Provisional, to which the Challenged Patents claim priority, fails to adequately support the claimed sugar and sulfur compounds. *See, e.g.*, IPR2023-00206, Paper 7 (Pet.), 5-10. PO did not dispute this. *See generally id.*, Paper 8 (POPR). The Prior-filed Applications suffer from the same defect. *See, e.g.*, EX2012 (no disclosure of claimed sugar compounds except glucose); EX1012 (same); EX1167 (no claimed sugar disclosed). Hence, PO's concession that the '816 Provisional fails to provide written description support reasonably extends to the

[5] At a minimum, the Board should permit discovery on PO's delays "to determine whether there is sufficient basis supporting Patent Owner's position that the mistake may be correctable." *Honeywell*, 939 F.3d at 1351.

Prior-filed Applications and, thus, there is no sufficient basis for PO's corrections.[6]

III. EFFECT ON PENDING IPR PROCEEDINGS

Even if the Board grants leave to PO, any issued CoCs cannot be applied retroactively and, hence, they would have no effect on these proceedings. Specifically, 35 U.S.C. § 255 states that a CoC "shall have the same effect and operation in law on the trial of actions for causes ***thereafter arising*** as if the same had been originally issued in such corrected form." The Federal Circuit has affirmed that this language only allows "the PTO to make ***prospectively*** effective corrections, and the PTO was given no authority to correct the claims retroactively." *Novo Indus., L.P. v. Micro Molds Corp.*, 350 F.3d 1348, 1356 (Fed. Cir. 2003). The Board has also reached the same conclusion. *Emerson Electric Co. v. SIPCO, LLC*, IPR2016-00984, Paper 52 at 17-20 ("[T]he logical and natural reading of § 255 is that … a [CoC] for an applicant's mistake [] does not receive retroactive application."); *id.* at 20-21 (distinguishing § 255 from § 256 as the latter would apply retroactively).

Notwithstanding, even if 35 U.S.C. § 255 allowed the Board the option to give retroactive effect to any issued CoC (it does not), the Board should decline to do so as it "could impede the Board's ability to meet its statutory deadlines." *See Election*

[6] At bottom, the combination of all of the above issues shows that PO's mistake is anything but "minor [in] character" and requires a reissue application. MPEP § 1481.

Sys. & Software, LLC v. Hart Intercivic, Inc., PGR2020-00031, Paper 18 at 7-9 (denying retroactive effect to CoC).

IV. PREJUDICE TO PETITIONER AND REQUEST FOR BRIEFING

Petitioner would be irreparably prejudiced if the Board grants PO leave and gives retroactive effect to any issued CoC for IPR2023-00206 or IPR2023-00207. Half of the grounds against the '096 patent's independent claims and a third of the grounds against the '241 patent's independent claims rely on Brown as prior art.[7] Because retroactively applying PO's proposed changes to the priority chain would at least *on its face* remove Brown as prior art,[8] Petitioner will be prejudiced if dozens of pages in those two proceedings' Petitions are rendered meaningless. And Petitioner could not have known PO would attempt to "correct" priority for the Challenged Patents—otherwise, Petitioner could have addressed the lack of written description support for such a claim and/or pursued different, additional grounds. Without the ability to foresee PO's alleged "mistake" and IPR-driven strategy, Petitioner should not have to "bear any consequences incident to the issuing of a certificate of correction … which would be the possible result if that correction were

[7] The Petitions against the Vrljic Patents do not rely on Brown.

[8] Petitioner maintains that because the Prior-filed Applications fail to provide written description support for the Challenged Claims, Brown is still prior art.

to receive retroactive application." *Emerson*, Paper 52 at 23-24.

For this reason, if the Board grants leave to PO to file CoC petitions and considers giving retroactive effect to any CoCs that may issue for the Fraser Patents, Petitioner requests a further 15-page brief to show that the Fraser Patents are not entitled to the benefit of the filing dates of the Prior-filed Applications (and, thus, can still be invalidated by Brown) because the Prior-filed Applications fail to provide written description support to the Fraser Patents' claims. *See Lockwood v. American Airlines, Inc.*, 107 F.3d 1565, 1571-72 (Fed. Cir. 1997) (finding great-grandparent patent anticipated child patent where priority chain was broken due to lack of continuity of disclosure). Given that any authorized CoC here may issue (if approved) just days or weeks before the institution deadline in these proceedings, Petitioner requests that such briefing be granted now to allow the Board to consider Petitioner's arguments ahead of the institution decision deadline.

Finally, if the Board *does* grant leave to PO to file CoC petitions, Petitioner requests that PO be allowed to file only one CoC petition for each Challenged Patent and also be ordered "in the interests of transparency" to append to its CoC petitions the parties' briefing and any Board order that may issue concerning PO's Motion for leave. *See Arkema Inc. v. Honeywell Int'l Inc.*, PGR2016-00011, Paper 57 at 13; *IP Holding B.V. v. Kokusai Elec. Corp.,* IPR2019-00378, Paper 17 at 5–6); *see also Election*, Paper 12 at 4-5 (allowing only one request).

DATED: April 21, 2022

Respectfully submitted,

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CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. § 42.6(e), the undersigned hereby certify that Petitioner's Opposition to Patent Owner's Motion Pursuant to § 1.323, and all exhibits and other documents filed together with the Petitioner's Opposition, were served on Patent Owner on April 21, 2023 by e-mailing copies to:

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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(54) **Title**: METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES

(57) **Abstract**: This document relates to food products containing highly conjugated heterocyclic rings complexed to an iron ion and one or more flavor precursors, and using such food products to modulate the flavor and/or aroma profile of other foods. The food products described herein can be prepared in various ways and can be formulated to be free of animal products.

METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 13/941,211, filed July 12, 2013, U.S. Application Serial No. 61/908,634, filed November 25, 2013, and to U.S. Application Serial No. 61/751,816, filed January 11, 2013, and is related to the following co-pending patent applications: Application Serial No. PCT/US12/46560; Application Serial No PCT/US12/46552; Application Serial No. 61,876,676, filed September 11, 2013; and Application Serial No. 61/751,818, filed January 11, 2013, all of which are incorporated herein by reference.

TECHNICAL FIELD

This invention relates to food products and more particularly, to food products that include a highly conjugated heterocyclic ring complexed to iron such as a heme-cofactor and one or more flavor precursor molecules.

BACKGROUND

Food is any substance that is either eaten or drunk by any animal, including humans, for nutrition or pleasure. It is usually of plant or animal origin, and can contain essential nutrients, such as carbohydrates, fats, proteins, vitamins, or minerals. The substance is ingested by an organism and assimilated by the organism's cells in an effort to produce energy, maintain life, or stimulate growth.

Food typically has its origin in a photosynthetic organism, such as a plant. Some food is obtained directly from plants, but even animals that are used as food sources are raised by feeding them food which is typically derived from plants.

In most cases, the plant or animal food source is fractionated into a variety of different portions, depending upon the purpose of the food. Often, certain portions of the plant, such as the seeds or fruits, are more highly prized by humans than others and these

are selected for human consumption, while other less desirable portions, such as the stalks of grasses, are typically used for feeding animals.

Current plant-based meat substitutes have largely failed to cause a shift to a vegetarian diet. Meat substitute compositions are typically extruded soy/grain mixtures which largely fail to replicate the experience of cooking and eating meat. Common limitations of plant-based meat substitute products are a texture and mouth-feel that are more homogenous than that of equivalent meat products. Furthermore, as these products must largely be sold pre-cooked, with artificial flavors and aromas pre-incorporated, they fail to replicate the aromas, flavors, and other key features, such as texture and mouth-feel, associated with cooking or cooked meat. As a result, these products appeal largely to a limited consumer base that is already committed to vegetarianism/veganism, but have failed to appeal to the larger consumer segment accustomed to eating meat. It would be useful to have improved plant-based meat substitutes which better replicate the aromas and flavors of meat, particularly during and/or after cooking.

SUMMARY

Provided herein are methods and compositions for modulating the flavor and/or aroma profile of consumable food products, including animal- or non-animal (e.g., plant) based food products, or mixtures of animal- and non-animal-based food products. In some embodiments, the methods and compositions are useful for modulating the flavor and/or aroma profile of a consumable food product during and/or after the cooking process. In some embodiments, the methods and compositions are used to generate one or more chemical compounds that modulate the flavor and/or aroma profile of the consumable food product during and/or after the cooking process.

As provided herein, and without being bound by theory, certain characteristic meaty flavors and/or aromas (e.g., beefy, bacony, umami, savory, bloody, brothy, gravy, metallic, bouillon-like; see Tables 2, 7, and 11), including one or more specific chemical compounds associated with the same (see Tables 3, 8, 9, 12, 14, 16, or 17), are believed to be produced during the cooking process of a consumable food product by chemical reaction of one or more flavor precursor molecules or compositions catalyzed by the

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presence of a highly conjugated heterocyclic ring complexed to an iron ion (e.g., a heme moiety; or a porphyrin; a porphyrinogen; a corrin; a corrinoid; a chlorin; a bacteriochorophyll; a corphin; a chlorophyllin; a bacteriochlorin; or an isobacteriochlorin moiety complexed to an iron ion). Such highly conjugated heterocycylic moieties include heterocyclic aromatic rings composed of one or more (2, 3, or 4 more) pyrrole, pyrrole-like, and/or pyrroline subunits. The highly conjugated heterocyclic ring complexed to an iron ion is referred to herein as an iron complex. In some embodiments, the heme moiety can be a heme cofactor such as a heme moiety bound to a protein; a heme moiety bound to a non-proteinaceous polymer; a heme moiety bound to a solid support; or a heme moiety encapsulated in a liposome. In some embodiments, the flavors and/or aromas are not generated in the absence of the iron complex (e.g., in the absence of a ferrous chlorin) or are not generated in the absence of a heme-cofactor (e.g., in the absence of a heme-containing protein). Accordingly, as described herein, the iron complexes such as isolated chlorin-iron complexes or heme-cofactors (e.g., heme-containing proteins) can be used to generate meaty flavors and/or aromas in a variety of food products, such as during the cooking process.

Combining one or more iron complexes such as a heme-cofactor (e.g., a heme-containing protein, including, for example a plant-derived heme protein such as a plant leghemoglobin (legH)), with one or more flavor precursor molecules or compositions (see, e.g., Table 1 or Table 13) can generate or provide a range of savory and meaty aromas and tastes (see, e.g., Tables 2, 7, and/or 11) in a cooked consumable food product. Flavor precursor molecules or compositions can be added to the uncooked food product in purified form and/or can be derived from ingredients in the uncooked consumable food product that contain and/or are enriched with one or more of the particular flavor precursors or compositions, including, for example, yeast extract, vegetable oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, canola oil, sunflower oil, coconut oil, mango oil, or an algal oil. The resultant flavor and/or aroma profile can be modulated by the type and concentration of the flavor precursors, the pH of the reaction, the length of cooking, the type and amount of iron complex (e.g., a heme cofactor such as a heme-containing protein), the

temperature of the reaction, and the amount of water activity in the product, among other factors.

One or more flavor precursor molecules or compositions can be added along with a iron complex (e.g., ferrous chlorophyllin or a heme cofactor such as a heme-containing protein), to an uncooked food product, before and/or during the cooking process, to give the cooked consumable food product a particular meaty taste and smell, for example, the taste and smell of beef, bacon, pork, lamb, or chicken. Consumable food products can be animal or non-animal based (e.g., plant) food products, or combinations of an animal and non-animal based food product. For example, a plant based veggie burger or an animal-based burger, such as a chicken burger, can be modified with the compositions and methods of the present disclosure to result in a burger having a cooked flavor and/or aroma profile that is more meat like, e.g., beef-like, lamb-like, pork-like, turkey-like, duck-like, deer-like, yak-like, bison-like or other desirable meat flavor.

Food products for use in the present disclosure include those that have an iron-complex (e.g., a heme cofactor such as a heme-containing protein), and one or more flavor precursor molecules included therein. The iron-complex such as a heme cofactor (e.g., a heme-containing protein) and the one or more flavor precursor molecules can be homogenously or heterogeneously included in the food products. A heme protein can be isolated and purified prior to inclusion in the food product. Non-limiting examples of consumable food products which can include an iron complex such as a heme-cofactor (e.g., a heme-containing protein) and one or more flavor precursor molecules include animal-based or non-animal (e.g., plant-based), or combinations of animal-based and non-animal-based, food products in the form of hot dogs, burgers, ground meat, sausages, steaks, filets, roasts, breasts, thighs, wings, meatballs, meatloaf, bacon, strips, fingers, nuggets, cutlets, or cubes.

Consumable food products for use in the present disclosure can be flavor additive compositions, e.g., for addition to another consumable food product before, during, or after its cooking process. A flavor additive composition can include an iron complex such as a heme-cofactor (e.g., a heme-containing protein), and one or more flavor precursors.

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A flavor additive composition can include a heme protein, e.g., an isolated and purified heme protein; such a flavor additive composition can be used to modulate the flavor and/or aroma profile of a consumable food product that comprises one or more flavor precursor molecules or compositions. A flavor additive composition can include one or more flavor precursor molecules or compositions; such a flavor additive composition can be used to modulate the flavor and/or aroma profile of a consumable food product that comprises the heme protein, e.g., an isolated and purified heme protein.

A flavor additive composition can be in the form, of but not limited to, soup or stew bases, bouillon, e.g., powder or cubes, flavor packets, or seasoning packets or shakers. Such flavor additive compositions can be used to modulate the flavor and/or aroma profile for a variety of consumable food products, and can be added to a consumable food product before, during, or after cooking of the consumable food product.

In some embodiments, a flavor additive composition such as one including an iron complex (e.g., ferrous chlorin or a heme protein) and one or more flavor precursors can be reacted (e.g., *in vitro*) with heating to generate a particular flavor and/or aroma profile of interest and the resultant product mixture can be added to the consumable food product of interest, which can then be eaten as-is or can be additionally modified, e.g., by additional cooking. In some embodiments, the iron complex can be removed from the resultant product mixture before adding the product mixture to the consumable food product of interest. For example, the iron complex can be removed from the product mixture using chromatographic techniques such as column chromatography, e.g., a column containing heme or iron-chlorin.

In some embodiments, the iron complex such as a heme-cofactor, e.g., a heme-protein, and the one or more flavor precursor flavor additive compositions can be soy-free, wheat-free, yeast-free, MSG-free, and free of protein hydrolysis products, and can taste meaty, highly savory, and without off odors or flavors.

In one aspect, this document features a food product that includes an iron complex such as a heme moiety, or a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochorophyll, a corphin, a chlorophyllin, a bacteriochlorin, or an isobacteriochlorin

moiety complexed to an iron ion and one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, sunflower oil, canola oil, olive oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone. The heme moiety can be a heme-containing protein, a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support. The heme-containing protein can be a plant, mammalian, a yeast or filamentous fungi, or bacterial heme-containing protein. The food product can include two to one hundred, two to fifty flavor precursors, two to forty flavor precursors, two to thirty-five flavor precursors, two to ten flavor precursors, or two to six flavor precursors. In some embodiments, the one or more flavor precursor molecules are selected from the group consisting of glucose, ribose, cysteine, a cysteine derivative, thiamine, alanine, methionine, lysine, a lysine derivative, glutamic acid, a glutamic acid derivative, IMP, GMP, lactic acid, maltodextrin, creatine, alanine, arginine, asparagine, aspartate, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, linoleic acid, and mixtures thereof. The heme-containing protein can be a non-symbiotic hemoglobin or a leghemoglobin (e.g., a plant leghemoglobin such as one from soybean, alfalfa, lupin, pea, cow pea, or lupin). The heme-containing protein can include an amino acid sequence having at least 80% sequence identity to a polypeptide set forth in SEQ ID NOs:1-26. The heme-containing protein can be isolated and purified. The food product further can include a food-grade oil, a seasoning agent, a flavoring agent, a protein, a protein concentrate, an emulsifier, a gelling agent, or a fiber. The food product can be a meat

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substitute, a soup base, stew base, snack food, bouillon powder, bouillon cube, a flavor packet, or a frozen food product. Any of the food products can be free of animal products. The food product can be sealed within a packet or shaker.

This document also features a method for producing a flavor compound. The method can include combining an iron complex (e.g., a heme moiety, a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochorophyll, a corphin, a chlorophyllin, a bacteriochlorin, or an isobacteriochlorin complexed to an iron) and one or more flavor precursor molecules to form a mixture, the one or more flavor precursor molecules selected from the group consisting of glucose, fructose, arabinose, ribose glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, canola oil, olive oil, sunflower oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture to form one or more flavor compounds selected from the group consisting of phenylacetaldehyde, 1-octen-3-one, 2-n-heptylfuran, 2-thiophenecarboxaldehyde, 3-thiophenecarboxaldehyde, butyrolactone, 2-undecenal, pyrazine, methyl-, furfural, 2-decanone, pyrrole, 1-octen-3-ol, 2-acetylthiazole, (E)-2-octenal, decanal, benzaldehyde, (E)-2-nonenal, pyrazine, 1-hexanol, 1-heptanol, dimethyl trisulfide, 2-nonanone, 2-pentanone, 2-heptanone, 2,3-butanedione, heptanal, nonanal, 2-octanone, 1-octanol, 3-ethylcyclopentanone, 3-octen-2-one, (E,E)-2,4-heptadienal, (Z)-2-heptenal, 2-heptanone, 6-methyl-, (Z)-4-heptenal, (E,Z)-2,6-nonadienal, 3-methyl-2-butenal, 2-pentyl-furan, thiazole, (E, E)-2,4-decadienal,, hexanoic acid, 1-ethyl-5-methylcyclopentene, (E,E)-2,4-nonadienal, (Z)-2-decenal, dihydro-5-pentyl-2(3H)-furanone, trans-3-nonen-2-one, (E,E)-3,5-octadien-2-

one, (Z)-2-octen-1-ol, 5-ethyldihydro-2(3H)-furanone, 2-butenal, 1-penten-3-ol, (E)-2-hexenal, formic acid, heptyl ester, 2-pentyl-thiophene, (Z)-2-nonenal, 2-hexyl-thiophene, (E)-2-decenal, 2-ethyl-5-methyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine, 2-ethyl-1-hexanol, thiophene, 2-methyl-furan, pyridine, butanal, 2-ethyl-furan, 3-methyl-butanal, trichloromethane, 2-methyl-butanal, methacrolein, 2-methyl-propanal, propanal, acetaldehyde, 2-propyl-furan, dihydro-5-propyl-2(3H)- furanone, 1,3-hexadiene, 4-decyne, pentanal, 1-propanol, heptanoic acid, trimethyl-ethanethiol, , 1-butanol, 1-penten-3-one, dimethyl sulfide, 2-ethyl furan, 2-pentyl-thiophene, 2-propenal, 2-tridecen-1-ol, 4-octene, 2-methyl thiazole,, methyl-pyrazine, 2-butanone, 2-pentyl-furan, 2-methyl-propanal, butyrolactone,, 3-methyl-butanal, methyl-thiirane, 2-hexyl-furan, butanal,, 2-methyl-butanal, 2-methyl-furan, furan, octanal, 2-heptenal, 1-octene, formic acid heptyl ester, 3-pentyl-furan, and 4-penten-2-one. The heme moiety can be a heme-containing protein, a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support. The method can include combining cysteine, ribose, lactic acid, lysine, and/or thiamine with the heme-containing protein.

In another aspect, this document features a method for producing a flavor compound. The method includes combining an iron complex, such as a heme-containing protein, and one or more flavor precursor molecules to form a mixture, the one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, methionine, cysteine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture to form one or more

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flavor compounds set forth in Tables 3, 8, or 9. For example, the flavor precursors can include cysteine, a sugar, and one or more other precursors.

This document also features a method for imparting a meat like flavor (e.g., beef-like, chicken like, pork-like, lamb-like, turkey-like, duck-like, deer-like, or bison-like) to a food product. The method includes contacting the food product with a flavoring composition, the flavoring composition comprising i) an iron complex, such as a heme moiety (e.g., a heme-containing protein); and ii) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; wherein after heating the food product and the flavoring composition together, a meat like flavor (e.g., beef-like, chicken like, pork-like, lamb-like, turkey-like, duck-like, deer-like, or bison-like) is imparted to the food product. In some embodiments, the iron complex is removed from the food product. The flavoring composition further can include a seasoning agent, a flavoring agent, a protein, a protein concentrate, or an emulsifier. The flavoring composition can be sealed within a packet or shaker.

In another aspect, this document features a method of making a food product. The method includes combining an isolated heme-containing protein and one or more flavor precursor molecules to form a mixture, the one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin,

inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, sunflower oil, coconut oil, canola oil, flaxseed oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims. The word "comprising" in the claims may be replaced by "consisting essentially of" or with "consisting of," according to standard practice in patent law.

DESCRIPTION OF THE DRAWINGS

FIG. 1 contains amino acid sequences of exemplary heme-containing proteins.

FIG. 2 is a bar graph of the beefiness rating of the meat replica with or without the Magic Mix, both samples in triplicate with 1% w/v LegH protein. Tasters rated beefiness on a scale from 1-7, with 1 being not beefy at all and 7 being exactly like ground beef.

DETAILED DESCRIPTION

This document is based on methods and materials for modulating the taste and/or aroma profile of food products. As described herein, compositions containing one or

more flavor precursors and one or more highly conjugated heterocyclic rings complexed to an iron (referred to herein as an iron complex) can be used to modulate the taste and/or aroma profile of food products. Such iron complexes include heme moieties or other highly conjugated heterocylic rings complexed to an iron ion (referred to as an iron complex). "Heme" refers to a prosthetic group bound to iron (Fe^{2+} or Fe^{3+}) in the center of a porphyrin ring. Thus, an iron complex can be a heme moiety, or a porphyrin, porphyrinogen, corrin, corrinoid, chlorin, bacteriochorophyll, corphin, chlorophyllin, bacteriochlorin, or isobacteriochlorin moiety complexed to iron ion. The heme moiety that can be used to modulate the taste and/or aroma profile of food products can be a heme cofactor such as a heme-containing protein; a heme moiety bound to a non-peptidic polymer or other macromolecule such as a liposome, a polyethylene glycol, a carbohydrate, a polysaccharide, a cyclodextrin, a polyethylenimine, a polyacrylate, or derivatives thereof; a siderophore (i.e., an iron chelating compound); or a heme moiety bound to a solid support (e.g., beads) composed of a chromatography resin, cellulose, graphite, charcoal, or diatomaceous earth.

In some embodiments, the iron complexes catalyze some reactions and produce flavor precursors without heating or cooking. In some embodiments, the iron complex destabilizes upon heating or cooking and releases the iron, e.g., the protein is denatured, so flavor precursors can be generated.

Suitable flavor precursors include sugars, sugar alcohols, sugar derivatives, oils (e.g., vegetable oils), free fatty acids, alpha-hydroxy acids, dicarboxylic acids, amino acids and derivatives thereof, nucleosides, nucleotides, vitamins, peptides, protein hydrolysates, extracts, phospholipids, lecithin, and organic molecules. Non-limiting examples of such flavor precursors are provided in Table 1.

TABLE 1

Flavor Precursor Molecules

Sugars, sugar alcohols, sugar acids, and sugar derivatives: glucose, fructose, ribose, sucrose, arabinose, glucose-6-phosphate,fructose-6-phosphate, fructose 1,6-diphosphate, inositol, maltose, molasses, maltodextrin, glycogen, galactose,

lactose, ribitol, gluconic acid and glucuronic acid, amylose, amylopectin, or xylose
Oils: coconut oil, mango oil, sunflower oil, cottonseed oil, safflower oil, rice bran oil, cocoa butter, palm fruit oil, palm oil, soybean oil, canola oil, corn oil, sesame oil, walnut oil, flaxseed, jojoba oil, castor, grapeseed oil, peanut oil, olive oil, algal oil, oil from bacteria or fungi
Free fatty acids: caprylic acid, capric acid, lauric acid, myristic acid, palmititic acid, palmitoleic acid, stearic, oleic acid, linoleic acid, alpha linolenic acid, gamma linolenic acid, arachidic acid, arachidonic acid, behenic acid, or erucic acid
Amino acids and derivatives thereof: cysteine, cystine, a cysteine sulfoxide, allicin, selenocysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, 5-hydroxytryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, or tyrosine
Nucleosides and Nucleotides: inosine , inosine monophosphate (IMP), guanosine, guanoside monophosphate (GMP), adenosine, adenosine monophosohate (AMP)
Vitamins: thiamine, vitamin C, Vitamin D, Vitamin B6, or Vitamin E
Misc: phospholipid, lecithin, pyrazine, creatine, pyrophosphate
Acids: acetic acid, alpha hydroxy acids such as lactic acid or glycolic acid, tricarboxylic acids such as citric acid, dicarboxylic acids such as succinic acid or tartaric acid
Peptides and protein hydrolysates: glutathione, vegetable protein hydrolysates, soy protein hydrolysates, yeast protein hydrolysates, algal protein hydrolysatess, meat protein hydrolysates
Extracts: a malt extract, a yeast extract, and a peptone

In some embodiments, one flavor precursor or combinations of two to one hundred flavor precursors, two to ninety, two to eighty, two to seventy, two to sixty, or two to fifty flavor precursors are used. For example, combinations of two to forty flavor

precursors, two to thirty-five flavor precursors, two to ten flavor precursors, or two to six flavor precursors can be used with the one or more iron complexes (e.g., heme co-factors such as a heme-containing proteins). For example, the one or more flavor precursors can be glucose, ribose, cysteine, a cysteine derivative, thiamine, lysine, a lysine derivative, glutamic acid, a glutamic acid derivative, alanine, methionine, IMP, GMP, lactic acid, and mixtures thereof (e.g., glucose and cysteine; cysteine and ribose; cysteine, glucose or ribose, and thiamine; cysteine, glucose or ribose, IMP, and GMP; cysteine, glucose or ribose, and lactic acid). For example, the one or more flavor precursors can be alanine, arginine, asparagine, aspartate, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, glucose, ribose, maltodextrin, thiamine, IMP, GMP, lactic acid, and creatine.

As used herein, the term "heme containing protein" can be used interchangeably with "heme containing polypeptide" or "heme protein" or "heme polypeptide" and includes any polypeptide that can covalently or noncovalently bind a heme moiety. In some embodiments, the heme-containing polypeptide is a globin and can include a globin fold, which comprises a series of seven to nine alpha helices. Globin type proteins can be of any class (e.g., class I, class II, or class III), and in some embodiments, can transport or store oxygen. For example, a heme-containing protein can be a non-symbiotic type of hemoglobin or a leghemoglobin. A heme-containing polypeptide can be a monomer, i.e., a single polypeptide chain, or can be a dimer, a trimer, tetramer, and/or higher order oligomers. The life-time of the oxygenated $Fe2^{+}$ state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption. The life-time of the unoxygenated Fe^{2+} state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption

Non-limiting examples of heme-containing polypeptides can include an androglobin, a cytoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a

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myoglobin, an erythrocruorin, a beta hemoglobin, an alpha hemoglobin, a protoglobin, a cyanoglobin, a cytoglobin, a histoglobin, a neuroglobins, a chlorocruorin, a truncated hemoglobin (e.g., HbN or HbO), a truncated 2/2 globin, a hemoglobin 3 (e.g., Glb3), a cytochrome, or a peroxidase.

Heme-containing proteins that can be used in the compositions and food products described herein can be from mammals (e.g., farms animals such as cows, goats, sheep, pigs, ox, or rabbits), birds, plants, algae, fungi (e.g., yeast or filamentous fungi), ciliates, or bacteria. For example, a heme-containing protein can be from a mammal such as a farm animal (e.g., a cow, goat, sheep, pig, ox, or rabbit) or a bird such as a turkey or chicken. Heme-containing proteins can be from a plant such as *Nicotiana tabacum* or *Nicotiana sylvestris* (tobacco); *Zea mays* (corn), *Arabidopsis thaliana*, a legume such as *Glycine max* (soybean), *Cicer arietinum* (garbanzo or chick pea), *Pisum sativum* (pea) varieties such as garden peas or sugar snap peas, *Phaseolus vulgaris* varieties of common beans such as green beans, black beans, navy beans, northern beans, or pinto beans, *Vigna unguiculata* varieties (cow peas), *Vigna radiata* (Mung beans), *Lupinus albus* (lupin), or *Medicago sativa* (alfalfa); *Brassica napus* (canola); *Triticum sps.* (wheat, including wheat berries, and spelt); *Gossypium hirsutum* (cotton); *Oryza sativa* (rice); *Zizania sps.* (wild rice); *Helianthus annuus* (sunflower); *Beta vulgaris* (sugarbeet); *Pennisetum glaucum* (pearl millet); *Chenopodium sp.* (quinoa); *Sesamum sp.* (sesame); *Linum usitatissimum* (flax); or *Hordeum vulgare* (barley). Heme-containing proteins can be isolated from fungi such as *Saccharomyces cerevisiae*, *Pichia pastoris*, *Magnaporthe oryzae*, *Fusarium graminearum*, *Aspergillus oryzae*, *Trichoderma reesei*, *Myceliopthera thermophile*, *Kluyvera lactis*, or *Fusarium oxysporum*. Heme-containing proteins can be isolated from bacteria such as *Escherichia coli*, *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Synechocistis sp., Aquifex aeolicus, Methylacidiphilum infernorum*, or thermophilic bacteria such as *Thermophilus*. The sequences and structure of numerous heme-containing proteins are known. See for example, Reedy, *et al.*, *Nucleic Acids Research*, 2008, Vol. 36, Database issue D307–D313 and the Heme Protein Database available on the world wide web at http://hemeprotein.info/heme.php.

For example, a non-symbiotic hemoglobin can be from a plant selected from the group consisting of soybean, sprouted soybean, alfalfa, golden flax, black bean, black eyed pea, northern, garbanzo, moong bean, cowpeas, pinto beans, pod peas, quinoa, sesame, sunflower, wheat berries, spelt, barley, wild rice, or rice.

Any of the heme-containing proteins described herein that can be used for producing food products can have at least 70% (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%) sequence identity to the amino acid sequence of the corresponding wild-type heme-containing protein or fragments thereof that contain a heme-binding motif. For example, a heme-containing protein can have at least 70% sequence identity to an amino acid sequence set forth in FIG. 1, including a non-symbiotic hemoglobin such as that from *Vigna radiata* (SEQ ID NO:1), *Hordeum vulgare* (SEQ ID NO:5), *Zea mays* (SEQ ID NO:13), *Oryza sativa subsp. japonica (rice)* (SEQ ID NO:14), or *Arabidopsis thaliana* (SEQ ID NO:15), a Hell's gate globin I such as that from *Methylacidiphilum infernorum* (SEQ ID NO:2), a flavohemoprotein such as that from *Aquifex aeolicus* (SEQ ID NO:3), a leghemoglobin such as that from *Glycine max* (SEQ ID NO:4), *Pisum sativum* (SEQ ID NO:16), or *Vigna unguiculata* (SEQ ID NO:17), a heme-dependent peroxidase such as from *Magnaporthe oryzae*, (SEQ ID NO:6) or *Fusarium oxysporum* (SEQ ID NO:7), a cytochrome c peroxidase from *Fusarium graminearum* (SEQ ID NO:8), a truncated hemoglobin from *Chlamydomonas moewusii* (SEQ ID NO:9), *Tetrahymena pyriformis* (SEQ ID NO:10, group I truncated), *Paramecium caudatum* (SEQ ID NO:11, group I truncated), a hemoglobin from *Aspergillus niger* (SEQ ID NO:12), or a mammalian myoglobin protein such as the *Bos taurus* (SEQ ID NO:18) myoglobin, *Sus scrofa* (SEQ ID NO:19) myoglobin, *Equus caballus* (SEQ ID NO:20) myoglobin, a heme-protein from *Nicotiana benthamiana* (SEQ ID NO:21), *Bacillus subtilis* (SEQ ID NO:22), *Corynebacterium glutamicum* (SEQ ID NO:23), Synechocystis PCC6803 (SEQ ID NO:24), Synechococcus sp. PCC 7335 (SEQ ID NO:25), or *Nostoc commune* (SEQ ID NO:26).

The percent identity between two amino acid sequences can be determined as follows. First, the amino acid sequences are aligned using the BLAST 2 Sequences (Bl2seq) program from the stand-alone version of BLASTZ containing BLASTP version

2.0.14. This stand-alone version of BLASTZ can be obtained from Fish & Richardson's web site (e.g., www.fr.com/blast/) or the U.S. government's National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov). Instructions explaining how to use the Bl2seq program can be found in the readme file accompanying BLASTZ. Bl2seq performs a comparison between two amino acid sequences using the BLASTP algorithm. To compare two amino acid sequences, the options of Bl2seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two amino acid sequences: C:\Bl2seq –i c:\seq1.txt –j c:\seq2.txt –p blastp –o c:\output.txt. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology, then the designated output file will not present aligned sequences. Similar procedures can be following for nucleic acid sequences except that blastn is used.

Once aligned, the number of matches is determined by counting the number of positions where an identical amino acid residue is presented in both sequences. The percent identity is determined by dividing the number of matches by the length of the full-length polypeptide amino acid sequence followed by multiplying the resulting value by 100. It is noted that the percent identity value is rounded to the nearest tenth. For example, 78.11, 78.12, 78.13, and 78.14 is rounded down to 78.1, while 78.15, 78.16, 78.17, 78.18, and 78.19 is rounded up to 78.2. It also is noted that the length value will always be an integer.

It will be appreciated that a number of nucleic acids can encode a polypeptide having a particular amino acid sequence. The degeneracy of the genetic code is well known to the art; i.e., for many amino acids, there is more than one nucleotide triplet that serves as the codon for the amino acid. For example, codons in the coding sequence for a given enzyme can be modified such that optimal expression in a particular species (e.g., bacteria or fungus) is obtained, using appropriate codon bias tables for that species.

Heme-containing proteins can be extracted from the source material (e.g., extracted from animal tissue, or plant, fungal, algal, or bacterial biomass, or from the culture supernatant for secreted proteins) or from a combination of source materials (e.g., multiple plant species). Leghemoglobin is readily available as an unused by-product of commodity legume crops (e.g., soybean, alfalfa, or pea). The amount of leghemoglobin in the roots of these crops in the United States exceeds the myoglobin content of all the red meat consumed in the United States.

In some embodiments, extracts of heme-containing proteins include one or more non-heme-containing proteins from the source material (e.g., other animal, plant, fungal, algal, or bacterial proteins) or from a combination of source materials (e.g., different animal, plant, fungi, algae, or bacteria).

In some embodiments, heme-containing proteins are isolated and purified from other components of the source material (e.g., other animal, plant, fungal, algal, or bacterial proteins). As used herein, the term "isolated and purified" indicates that the preparation of heme-containing protein is at least 60% pure, e.g., greater than 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% pure. Without being bound by theory, isolating and purifying proteins can allow the food products to be made with greater consistency and greater control over the properties of the food product as unwanted material is eliminated. Proteins can be separated on the basis of their molecular weight, for example, by size exclusion chromatography, ultrafiltration through membranes, or density centrifugation. In some embodiments, the proteins can be separated based on their surface charge, for example, by isoelectric precipitation, anion exchange chromatography, or cation exchange chromatography. Proteins also can be separated on the basis of their solubility, for example, by ammonium sulfate precipitation, isoelectric precipitation, surfactants, detergents or solvent extraction. Proteins also can be separated by their affinity to another molecule, using, for example, hydrophobic interaction chromatography, reactive dyes, or hydroxyapatite. Affinity chromatography also can include using antibodies having specific binding affinity for the heme-containing protein, nickel NTA for His-tagged recombinant proteins, lectins to bind to sugar moieties on a glycoprotein, or other molecules which specifically binds the protein.

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Heme-containing proteins also can be recombinantly produced using polypeptide expression techniques (e.g., heterologous expression techniques using bacterial cells, insect cells, fungal cells such as yeast, plant cells such as tobacco, soybean, or *Arabidopsis*, or mammalian cells). In some cases, standard polypeptide synthesis techniques (e.g., liquid-phase polypeptide synthesis techniques or solid-phase polypeptide synthesis techniques) can be used to produce heme-containing proteins synthetically. In some cases, *in vitro* transcription-translation techniques can be used to produce heme-containing proteins.

The protein used in the consumable may be soluble in a solution. In some embodiments, the isolated and purified proteins are soluble in solution at greater than 5, 10, 15, 20, 25, 50, 100, 150, 200, or 250g/L.

In some embodiments, the isolated and purified protein is substantially in its native fold and water soluble. In some embodiments, the isolated and purified protein is more than 50, 60, 70, 80, or 90% in its native fold. In some embodiments, the isolated and purified protein is more than 50, 60, 70, 80, or 90% water soluble.

Modulating Flavor and/or Aroma Profiles

As described herein, different combinations of flavor precursors can be used with one or more iron complexes (e.g., a ferrous chlorin, a chlorin-iron complex, or a heme-cofactor such as a heme-containing protein or heme bound to a non-peptidic polymer such as polyethylene glycol or to a solid support) to produce different flavor and aroma profiles when the flavor precursors and iron complexes are heated together (e.g., during cooking). The resultant flavor and/or aroma profile can be modulated by the type and concentration of the flavor precursors, the pH of the reaction, the length of cooking, the type and amount of iron complex (e.g., a heme-cofactor such as heme-containing protein, heme bound to non-peptidic polymer or macromolecule, or heme bound to a solid support), the temperature of the reaction, and the amount of water activity in the product, among other factors. In embodiments in which a heme moiety is bound to a solid support such as cellulose or a chromatography resin, graphite, charcoal, or diatomaceous earth, the solid support (e.g., beads) can be incubated with sugars and/or one or more other

flavor precursors to generate flavors, and then the solid support with attached heme moiety can be re-used, i.e., incubated again with sugars and/or one or more other flavor precursors to generate flavors.

Table 2 provides non-limiting examples of flavor types that can be generated by combining one or more flavor precursors and one or more heme co-factors (e.g., heme-containing proteins). See also Tables 7 and/or 11.

TABLE 2

Flavor Types	
beef	beef broth
beef dripping	cheesy
cold-cut deli meat	squash
bacon	sharp
meaty	fruity
brothy	floral
ramen	musty
egg	fried food
malty	caramel
bready	barbeque
sulfur	chocolate
fried chicken	sweet
browned	potato
pretzel	french toast
grassy	breadcrust
bloody	mushroom
broccoli	chicken
brothy	cumin
buttery	umami
metallic	raisin

yeasty	goaty
vegetable broth	

Flavor and aroma profiles are created by different chemical compounds formed by chemical reactions between the heme co-factor (e.g., heme-containing protein) and flavor precursors. Gas chromatography–mass spectrometry (GCMS) can be used to separate and identify the different chemical compounds within a test sample. For example, volatile chemicals can be isolated from the head space after heating a heme-containing protein and one or more flavor precursors.

Table 3 provides non-limiting examples of compounds that can be produced. See also Tables 8, 9, 12, and/or 14.

TABLE 3

Compounds Produced

phenylacetaldehyde	2-butenal, 2-ethyl-	1,3-hexadiene
1-octen-3-one	acetonitrile	4-decyne
2-n-heptylfuran		pentanal
2-thiophenecarboxaldehyde	(E)-2-Hexenal	1-propanol
3-thiophenecarboxaldehyde	4-ethyl-phenol,	heptanoic acid
1-octene	3-octanone	ethanethiol
butyrolactone	styrene	2-methyl-1-heptene
2-undecenal	furan, 3-pentyl-	(E)-4-octene
propyl-cyclopropane	formic acid, heptyl ester	2-methyl-2-heptene
methyl-pyrazine	(E)-2-Heptenal	pentanoic acid
1-hydroxy-propanone	6-methyl-5-hepten-2-one	nonanoic acid
acetic acid	n-caproic acid vinyl ester	1,3-dimethyl-benzene
furfural	2-ethyl-2-hexenal	
2-decanone	1-hepten-3-ol	toluene
pyrrole	1-ethyl-1-methyl-cyclopentane	1-butanol
1-octen-3-ol	3-ethyl-2-methyl-1,3-hexadiene	2,3,3-trimethyl-pentane
2-acetylthiazole	2-pentyl-thiophene	isopropyl alcohol
(E)-2-octenal	(Z)-2-nonenal	2,2,4,6,6-pentamethyl-heptane
decanal	2-n-octylfuran	phenol

benzaldehyde	2-hexyl-thiophene	1-penten-3-one
(E)-2-Nonenal	4-cyclopentene-1,3-dione	dimethyl sulfide
pyrazine	1-nonanol	thiirane
1-pentanol	(E)-2-decenal	(E)-2-octen-1-ol
trans-2-(2-pentenyl)furan	4-ethyl-benzaldehyde	2,4-dimethyl-1-heptene
1-hexanol	1,7-octadien-3-ol	1,3-bis(1,1-dimethylethyl)-benzene
1-heptanol	octanoic acid	heptane
dimethyl trisulfide	2-ethyl-5-methyl-pyrazine	4,7-dimethyl-undecane
2-nonanone	3-ethyl-2,5-dimethyl-pyrazine	acetophenone
2-pentanone	1,3,5-cycloheptatriene	tridecane
2-heptanone	2-ethyl-1-hexanol	thiophosphoramide, s-methyl ester
2,3-butanedione	4-methyl-octanoic acid	2-methyl-thiazole
heptanal	m-aminophenylacetylene	3-(1-methylethoxy)-propanenitrile,
nonanal	benzene	2,4-bis(1,1-dimethylethyl)-phenol
2-octanone	thiophene	3-ethyl-2,2-dimethyl-pentane
2-butanone	2-methyl-furan	3-ethyl-pentane
octanal	pyridine	2,3,4-trimethyl-pentane
1-octanol	furan	2,4,6-trimethyl-octane
3-ethylcyclopentanone	butanal	2,6-dimethyl-nonane
8-methyl-1-undecene	2-ethyl-furan	2-hexyl-furan
3-octen-2-one	carbon disulfide	4-methyl-5-thiazoleethanol
2,4-Heptadienal, (E,E)-	Furan, 2-hexyl-:2	4-penten-2-one
(Z)-2-heptenal	3-methyl-butanal	4-methylthiazole
6-methyl-2-heptanone	2-methyl-butanal	2-methyl-3-pentanone
(Z)-4-heptenal	methacrolein	2,3-pentanedione
(E,Z)-2,6-nonadienal	octane	(E)-2-tridecen-1-ol
3-methyl-2-butenal	ethanol	2-thiophenemethanamine
2-pentyl-furan	2-methyl-propanal	(Z)-2-nonenal,
thiazole	acetone	methyl thiolacetate
(E,E)-2,4-decadienal	propanal	methyl ethanoate
hexanoic acid	methyl-thiirane	isothiazole
1-ethyl-5-methylcyclopentene	acetaldehyde	3,3-dimethyl-hexane

(E,E)-2,4-nonadienal	2-propenal	4-methyl-heptane
(Z)-2-decenal	2-propyl-furan	2,4-dimethyl-heptane
dihydro-5-pentyl-2(3h)-furanone	dihydro-5-propyl-2(3H)-furanone	2,3,4-trimethyl-heptane
trans-3-nonen-2-one	dihydro-3-(2H)-thiophenone	2-methyl-heptane
(E,E)-3,5-octadien-2-one	2,2,6-trimethyl-decane	2-methyl-3-furanthiol
(Z)-2-octen-1-ol	3,3'-dithiobis[2-methyl-furan	4-amino-1,2,5-oxadiazole-3-carbonitrile
5-ethyldihydro-2(3h)-furanone	1-heptene	1,2-benzisothiazol-3(2H)-one
2-butenal	1,3-octadiene	2-acetyl-propen-2-ol,
1-penten-3-ol	1-nonene	1-decen-3-one
1-(ethylthio)-2-(methylthio)-buta-1,3-diene		

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein is heated in the presence of ground chicken, to increase specific volatile flavor and odorant components typically elevated in beef. For example, propanal, butanal, 2-ethyl-furan, heptanal, octanal, trans-2-(2-pentenyl)furan, (Z)-2-heptenal, (E)-2-octenal, pyrrole, 2,4-dodecadienal, 1-octanal, (Z)-2-decenal, or 2-undecenal can be increased in the presence of the heme-containing protein, which can impart a more beefy flavor to the chicken.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein is heated in the presence of cysteine and glucose or other combinations of flavor precursors to provide a different profile of volatile odorants than when any subset of the three components are used individually. Volatile flavor components that are increased under these conditions include but are not limited to furan, acetone, thiazole, furfural, benzaldehyde, 2-pyridinecarboxaldehyde, 5-methyl-2-thiophenecarboxaldehyde, 3-methyl-2-thiophenecarboxaldehyde, 3-thiophenemethanol and decanol. See, e.g., Tables 8 and 9. Under these conditions, cysteine and glucose alone or in the presence of iron salts such as ferrous glucanate produced a sulfurous, odor, but addition of heme-containing proteins reduced the

sulfurous odor and replaced it with flavors including but not limited to chicken broth, burnt mushroom, molasses, and bread.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein is heated in the presence of cysteine and ribose to provide a different profile of volatile odorants. Heating in the presence of ribose created some additional compounds as compared to when a heme-containing protein and glucose were heated together. See Tables 8 and 9.

In some embodiments, an iron complex (e.g., a ferrous chlorophillin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of thiamine and a sugar to affect the formation of 5-Thiazoleethanol, 4-methyl-furan, 3,3'-dithiobis[2-methyl-furan, and/or 4-Methylthiazole. These compounds are known to be present in meat and have beefy, meaty taste notes.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of a nucleotide such as inosine monophosphate and/or guanosine monophosphate to control the formation of flavor compounds such as (E)-4-octene, 2-ethyl-furan, 2-pentanone, 2,3-butanedione, 2-methyl-thiazole, methyl-pyrazine, tridecane, (E)-2-octenal, 2-thiopenecarboxaldehyde, and/or 3-thiopenecarboxaldehyde. These compounds are known to be present in meat and have a beefy, meaty, buttery, and or savory flavor notes.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of lysine, a sugar such as ribose, and cysteine to control the formation of flavor compounds such as dimethyl trisulfide, nonanal, 2-pentyl thiophene, 2-nonenal furfural, 1-octanol, 2-nonenal, thiazole, 2-acetylthiazole, phenylacetaldehyde, and/or 2-acetylthiazole. These compounds are known to be present in meat and some have a beefy, meaty, and or savory flavor.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of lactic acid, a sugar such as ribose, and cysteine to control the formation of the flavor compounds nonanal, thiazole, 2-acetylthiazole, and/or 8-methyl 1-undecene. These

compounds are known to be present in meat and have beefy, savory, browned, bready, and malty notes.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of amino acids, sugars such as glucose, ribose, and maltodextrin, lactic acid, thiamine, IMP, GMP, creatine, and salts such as potassium chloride and sodium chloride, to control the formation of flavor compounds such as 1,3-bis(1,1-dimethylethyl)-benzene, 2-methyl 3-furanthiol, and/or bis(2-methyl-4,5-dihydro-3-furyl) disulfide. These compounds are known to be present in meat and have beefy notes. See also Table 14.

In some embodiments, a particular type of heme-containing protein is chosen to control the formation of flavor compounds. See, for example, the results of Table 9, which shows that the addition of different types of heme-proteins (LegH, Barley, *B. myoglobin*, or *A. aeolicus*) in flavor reaction mixtures containing one or more flavor precursor compounds results in many of the same key meat flavors, including but not limited to pentanone, 3-methyl butanal, 2-methyl butanal, 2-heptenal, 1-octene, nonanal, 2-propenal, 2-decenal, 2-nonanone, 2-octanone, 2-tridecen-1-ol, 2-octanone, 2-octenal, 4-methyl-2-heptanone, octanal, 2-undecenal, butyrolactone, 1-octen-3-one, 3-methylheptyl acetate, and 2-pentyl-thiophene. These differences in flavor compounds can change the overall taste profile.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein and one or more flavor precursors can be reacted (e.g., *in vitro*) with heating to generate a particular flavor and/or aroma profile of interest and the resultant flavor additive composition can be added to the consumable food product of interest, which can then be eaten as-is or can be additionally modified, e.g., by additional cooking.

In some embodiments, any undesirable flavors can be minimized by deodorizing with activated charcoal or by removing enzymes such as lipoxygenases (LOX), which can be present in trace amounts when using preparations of plant proteins, and which can convert unsaturated triacylglycerides (such as linoleic acid or linolenic acid) into smaller and more volatile molecules. LOX are naturally present in legumes such as peas,

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soybeans, and peanuts, as well as rice, potatoes, and olives. When legume flours are fractionated into separate protein fractions, LOX can act as undesirable "time-bombs" that can cause undesirable flavors on aging or storage. Compositions containing plant proteins (e.g., from ground plant seeds) can be subjected to purification to remove LOX using, for example, an affinity resin that binds to LOX and removes it from the protein sample. The affinity resin can be linoleic acid, linolenic acid, stearic acid, oleic acid, propyl gallate, or epigalloccatechin gallate attached to a solid support such as a bead or resin. See, e.g., WO2013138793. In addition, depending on the protein component of the food product, certain combinations of antioxidants and/or LOX inhibitors can be used as effective agents to minimize off-flavor or off-odor generation especially in the presence of fats and oils. Such compounds can include, for example, one or more of β-carotene, α-tocopherol, caffeic acid, propyl gallate, or epigallocatechin gallate.

In some embodiments, specific flavor compounds, such as those described in Tables 3, 8, 9, 12, 14, 16, or 17 can be isolated and purified from the flavor additive composition. These isolated and purified compounds can be used as an ingredient to create flavors useful to the food and fragrance industry.

A flavor additive composition can be in the form, of but not limited to, soup or stew bases, bouillon, e.g., powder or cubes, flavor packets, or seasoning packets or shakers. Such flavor additive compositions can be used to modulate the flavor and/or aroma profile for a variety of food products, and can be added to a consumable food product before, during, or after cooking of the food product.

Food Products

Food products containing one or more flavor precursors and one or more heme-containing proteins can be used as a base for formulating a variety of additional food products, including meat substitutes, soup bases, stew bases, snack foods, bouillon powders, bouillon cubes, flavor packets, or frozen food products. Meat substitutes can be formulated, for example, as hot dogs, burgers, ground meat, sausages, steaks, filets, roasts, breasts, thighs, wings, meatballs, meatloaf, bacon, strips, fingers, nuggets, cutlets, or cubes.

In addition, food products described herein can be used to modulate the taste and/or aroma profile of other food products (e.g., meat replicas, meat substitutes, tofu, mock duck or other gluten based vegetable product, textured vegetable protein such as textured soy protein, pork, fish, lamb, or poultry products such as chicken or turkey products) and can be applied to the other food product before or during cooking. Using the food products described herein can provide a particular meaty taste and smell, for example, the taste and smell of beef or bacon, to a non-meat product or to a poultry product.

Food products described herein can be packaged in various ways, including being sealed within individual packets or shakers, such that the composition can be sprinkled or spread on top of a food product before or during cooking.

Food products described herein can include additional ingredients including food-grade oils such as canola, corn, sunflower, soybean, olive or coconut oil, seasoning agents such as edible salts (e.g., sodium or potassium chloride) or herbs (e.g., rosemary, thyme, basil, sage, or mint), flavoring agents, proteins (e.g., soy protein isolate, wheat glutin, pea vicilin, and/or pea legumin), protein concentrates (e.g., soy protein concentrate), emulsifiers (e.g., lecithin), gelling agents (e.g., k-carrageenan or gelatin), fibers (e.g., bamboo filer or inulin), or minerals (e.g., iodine, zinc, and/or calcium).

Food products described herein also can include a natural coloring agent such as turmeric or beet juice, or an artificial coloring agent such as azo dyes, triphenylmethanes, xanthenes, quinines, indigoids, titanium dioxide, red #3, red #40, blue #1, or yellow #5.

Food products described herein also can include meat shelf life extenders such as carbon monoxide, nitrites, sodium metabisulfite, Bombal, vitamin E, rosemary extract, green tea extract, catechins and other anti-oxidants.

Food products described herein can be free of animal products (e.g., animal heme-containing proteins or other animal products).

In some embodiments, the food products can be soy-free, wheat-free, yeast-free, MSG-free, and/or free of protein hydrolysis products, and can taste meaty, highly savory, and without off odors or flavors.

Assessment of Food Products

Food products described herein can be assessed using trained human panelists. The evaluations can involve eyeing, feeling, chewing, and tasting of the product to judge product appearance, color, integrity, texture, flavor, and mouth feel, etc. Panelists can be served samples under red or under white light. Samples can be assigned random three-digit numbers and rotated in ballot position to prevent bias. Sensory judgments can be scaled for "acceptance" or "likeability" or use special terminology. For example, letter scales (A for excellent, B for good, C for poor) or number scales may be used (1 = dislike, 2 = fair, 3 = good; 4 = very good; 5=excellent). A scale can be used to rate the overall acceptability or quality of the food product or specific quality attributes such beefiness, texture, and flavor. Panelists can be encouraged to rinse their mouths with water between samples, and given opportunity to comment on each sample.

In some embodiments, a food product described herein can be compared to another food product (e.g., meat or meat substitute) based upon olfactometer readings. In various embodiments, the olfactometer can be used to assess odor concentration and odor thresholds, odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of appreciation, or relative intensity of odors.

In some embodiments, an olfactometer allows the training and automatic evaluation of expert panels. In some embodiments, a food product described herein causes similar or identical olfactometer readings. In some embodiments, the differences between flavors generated using the methods of the invention and meat are sufficiently small to be below the detection threshold of human perception.

In some embodiments, volatile chemicals identified using GCMS can be evaluated. For example, a human can rate the experience of smelling the chemical responsible for a certain peak. This information could be used to further refine the profile of flavor and aroma compounds produced using a heme-containing protein and one or more flavor precursors.

Characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions molecules including amino acids, fats and sugars which are found in plants as well as meat. Therefore, in some embodiments, a food

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product is tested for similarity to meat during or after cooking. In some embodiments human ratings, human evaluation, olfactometer readings, or GCMS measurements, or combinations thereof, are used to create an olfactory map of the food product. Similarly, an olfactory map of the food product, for example, a meat replica, can be created. These maps can be compared to assess how similar the cooked food product is to meat.

In some embodiments, the olfactory map of the food product during or after cooking is similar to or indistinguishable from that of cooked or cooking meat. In some embodiments the similarity is sufficient to be beyond the detection threshold of human perception. The food product can be created so its characteristics are similar to a food product after cooking, but the uncooked food product may have properties that are different from the predicate food product prior to cooking.

These results will demonstrate that the compositions of the invention are judged as acceptably equivalent to real meat products. Additionally, these results can demonstrate that compositions of the invention are preferred by panelist over other commercially available meat substitutes. So, in some embodiments the present invention provides for consumables that are significantly similar to traditional meats and are more meat like than previously known meat alternatives.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1: Addition of heme-protein increases beefy qualities of replica burgers

Replica burgers containing the ingredients in Table 4 and the flavor precursors cysteine (10 mM), glutamic acid (10 mM), glucose (10 mM), and thiamine (1 mM) were prepared. Water was added to make up the balance. See, for example, U.S. Provisional Application No. 61/751,816, filed January 11, 2013. Control burgers were prepared as in Table 4 with precursors cysteine (10 mM), glutamic acid (10 mM), glucose (10 mM), and thiamine (1 mM) except LegH was omitted.

After cooking for 5 minutes at 150 C, the replica burgers were evaluated by a trained sensory panel. Panelists were served samples under red lights and each panelist individually evaluated the samples. Samples were assigned a random three-digit number and rotated in ballot position to prevent bias. Panelists were asked to evaluate cooked replica burger samples on multiple flavor, aroma, taste, texture and appearance attributes including but not limited to: beefiness, bloody quality, savory quality, and overall acceptability using a 7-point scale from 1=dislike extremely, to 7=like extremely. Panelists were encouraged to rinse their mouths with water between samples, and to fill out a survey to record their evaluation of each sample.

When replica burgers containing the LegH were compared to the control replica burgers without LegH, the samples containing LegH were rated significantly beefier, bloodier, more savory, and overall preferred compared to those that did not include LegH. See Table 5.

TABLE 4

Replica Burger Ingredients

Replica burger	% precooked w/w
Pea vicilin	3.86
Soy protein concentrate (SPC)	2.52
Bamboo fiber	0.34

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NaCl	0.54
Pea legumin	2
Soy Protein Isolate (SPI) (Solae, St. Louis, MO)	4.68
Wheat gluten	4.68
Coconut oil	15
Soy lecithin	0.1
k-carrageenan	1
LegH	1

TABLE 5

Sensory evaluation of replica burger with Heme

Attribute	Beef 20/80	No Heme	1% Heme
Beefyness			
mean	5.33	1.30	3.20
STDEV	1.58	0.67	0.79
Bloody			
mean	4.00	1.10	2.78
STDEV	1.32	0.32	1.64
Savory			
mean	4.67	3.00	5.10
STDEV	1.22	1.63	0.57

Example 2: Replica burgers with a flavor precursor mixture taste beefy and bloody

Replica burgers containing a flavor precursor mixture of glucose, cysteine, thiamine, and glutamic acid and 1% LegH pre-cooked w/w (see Table 4) were prepared as described in Example 1, and evaluated by a trained sensory panel after the burgers were cooked for 5 minutes at 150 C. Control burgers included LegH and all other ingredients except for the flavor precursor mixture.

Panelists were asked to evaluate the samples overall improvement in taste and descriptively analyze each sample using a 5-point scale from 1=dislike extremely, to 5=like extremely. Panelists were encouraged to rinse their mouths with water between samples, and to fill out a survey to record their evaluation of each sample. The replicate burgers which included LegH and the flavor precursor mixture were described as having bouillon, gravy, meaty, bloody, savory, and beefy notes on taste, and were preferred to the same replica burger with LegH but no added flavor precursor mixture. See, Table 6

TABLE 6

Improvement of overall taste with precursors added to LegH burgers

	with precursors	without precursors
Average	3.5	1.8
STDV	0.6	0.5

Example 3: Replica burgers with flavor precursor mixture resulting in a bacon taste

Replica burgers (see Table 4) were cooked with different precursor mixes (see Table 7) and 1% LegH and evaluated by a trained sensory panel after the burgers were cooked for 5 minutes at 150 C. Control burgers contained LegH and all of the other ingredients except for the flavor precursors. Panelists were asked to evaluate each sample and descriptively analyze of each sample. 5-point scale from 1=dislike extremely, to 5=like extremely. Panelists were encouraged to rinse their mouths with water between samples, and to fill out a survey to record their evaluation of each sample. A replica burger with a precursor mixture of 10 mM glucose, 10 mM ribose, 10 mM cysteine, 1 mM thiamine, 1 mM glutamic acid, 1 mM GMP, and LegH was described as having a bacon aroma and taste, and overall meatiness, savory quality, a very umami quality, a brothy quality, and slight beefy notes. See Table 7 for a summary of the flavor description for the various combinations of flavor precursors and heme-containing protein.

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TABLE 7

Flavors generated by addition of precursors to LegH (1 %)

Precursor (concentration)						Flavor Description
	ribose (10 mM)	cysteine (10 mM)				some kind of cold-cut/sliced deli meat
	ribose (10 mM)	cysteine (10 mM)			IMP (2 mM)	bread crust with beef drippings, sweet, grassy, umami
	ribose (10 mM)	cysteine (10 mM)			lactic acid (1 mM)	bready, malty, browned, breadcrust
	ribose (10 mM)	cysteine (10 mM)			lysine (5 mM)	savory, beefy, little grassy, brothy, bread
	ribose (10 mM)	cysteine (10 mM)			alanine (5 mM)	savory, weak beefy, brothy, little metallic
	ribose (10 mM)	cysteine (10 mM)			I+G (2 mM)	savory, weak beefy, brothy, sweet
	ribose (10 mM)	cysteine (10 mM)			methionine	cooked potato
	ribose (10 mM)	cysteine (10 mM)		glutamic acid (5 mM)		little meaty, pretzel, brothy, savory, sweet, chocolate
glucose (10 mM)	ribose (10 mM)	cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)		slight beefy, browned, grasssy,
glucose (10 mM)	ribose (10 mM)	cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	IMP (2 mM)	bacon, very umami, savory, brothy, slight beef
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)		beef jerky, bloody, meaty, brothy
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	lactic acid (1 mM)	savory, beefy, bloody, meaty, savory, gravy

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glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	lysine (5 mM)	roast beef
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	alanine (5 mM)	boiled beef, sweet
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	I+G (2 mM)	beefy with a sulfury note
glucose (10 mM)		cysteine (10 mM)			I+G (2 mM)	sweet, malty, umami, meaty
glucose (10 mM)					I+G (2 mM)	savory, roast beef, grassy
glucose (10 mM)				glutamic acid (5 mM)		umami, savory, meaty, sweaty, fermented

Example 4: Type of sugar modulates flavor compounds created in the presence of Hemeprotein

The addition of different sugars to flavor reaction mixtures containing a hemeprotein and one or more flavor precursor compounds resulted in distinct differences in the flavor compounds generated and the overall flavor profile. LegH heme protein at 1% pre-cooked w/w/ was mixed with cysteine (10 mM) and glucose (20 mM) at pH 6 in phosphate buffer to form a flavor reaction mixture and heated to 150 C for 3 minutes; this reaction created flavor compounds known to be present in meat; *see* Table 8. Similarly, a flavor reaction mixture made when LegH heme protein at 1% was mixed with cysteine (10 mM) and ribose (20 mM) at pH 6 and heated to 150 C for 3 minutes created flavor compounds known to be in meat; *see* Table 8.

The characteristic flavor and fragrance components were mostly produced during the cooking process when the flavor precursor molecules reacted with the heme-protein. Gas chromatography–mass spectrometry (GCMS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to separate and identify different substances within a test sample. Samples were evaluated by GCMS to identify the flavor compounds generated after heating and also evaluated for their sensory profiles. Volatile chemicals were isolated from the head space around the flavor reactions. The profile of the volatile chemicals in the headspace around the flavor reaction mixtures is shown in Table 8. In particular, the use of ribose created some additional compounds as compared to glucose, as shown in Table 8.

Notably, the control mixtures of cysteine with ribose or glucose heated in the absence of the LegH heme-protein did not generate the same set of flavor compounds. The flavor reaction mixtures containing LegH also were evaluated by a blinded trained sensory panel, which described the samples with ribose as having beefy, savory, brothy, and gravy-like notes, and the samples with glucose as savory, bloody, metallic, raw meat, and bouillon-like.

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TABLE 8

Flavor compounds generated with cysteine, LegH, and either glucose or ribose in the flavor reaction mixture.

LegH 1%		
Compounds created	cysteine (10 mM), glucose (20 mM)	cysteine (10 mM), ribose (20 mM)
benzaldehyde	X	X
2-butanone	X	X
dimethyl trisulfide	X	X
2-pentyl-furan	X	X
2-methyl-propanal	X	X
thiazole	X	X
butyrolactone	X	X
2-acetylthiazole	X	X
pentanal	X	X
3-methyl-butanal	X	X
methyl-thiirane	X	X
nonanal	X	X
heptanal	X	X
2,3-butanedione	X	X
1,3,5-cycloheptatriene	X	X
propyl-cyclopropane	X	X
2-hexyl-furan	X	X
butanal	X	X
2-methyl-butanal	X	X
2-ethyl-furan		X
2-octanone	X	X
propanal	X	X

trichloromethane	X	
2-methyl-furan	X	X
furan	X	X
pyrazine	X	X
thiophene	X	X
1,3-dimethyl-benzene	X	X
octane		X
octanal	X	X
thiazole	X	X
2-pentanone		X
furfural	X	X
2-nonanone	X	X
(Z)-2-heptenal	X	X
(E)-2-heptenal	X	X
1-octene	X	X
formic acid, heptyl ester	X	X
2-pentyl-thiophene		X
1-octen-3-one	X	X
3-pentyl-furan	X	X
2-propenal		X
(E)-2-tridecen-1-ol		X
benzene		X
(E)-4-octene		X
1-penten-3-one		X
4-penten-2-one	X	X
2-methyl-thiazole		X
methyl-pyrazine		X
trans-2-(2-pentenyl)furan		X
3-ethylcyclopentanone		X

pyrrole	X	X
2-thiophenecarboxaldehyde		X
3-thiophenecarboxaldehyde		X

Example 5: Heme-protein in the presence of thiamine affects the production of certain flavor compounds

The addition of thiamine in a flavor reaction mixtures with a heme protein and other flavor precursors affected the formation of 5-Thiazoleethanol, 4-methyl-furan, 3,3'-dithiobis[2-methyl-thiazole, and 4-methylthiazole. These compounds are known to be present in meat and have beefy, meaty taste notes.

Flavor reaction mixtures at pH 6 containing LegH (1%), cysteine (10 mM), thiamine (1 mM), either glucose or ribose (20 mM), and with or without glutamic acid (10 mM) were prepared and subsequently heated to 150 C for 3 minutes. These flavor reaction samples then were evaluated by GCMS for the flavor compounds generated and evaluated by a trained panel for their sensory profiles. Volatile chemicals were isolated from the head space around the flavor reactions. GCMS showed 4-methyl-5-thiazoleethanol, 3,3'-dithiobis[2-methyl]-furan, and 4-methylthiazole compounds were created by a mixture of LegH with thiamine, a sugar (either glucose or ribose), and cysteine. The same flavor reaction mixtures without thiamine did not generate these compounds; additionally these compounds were not generated when heme-proteins were not present in the flavor reaction mixtures.

The flavor reaction samples also were evaluated by a blinded trained sensory panel, which described the samples with the addition of thiamine as more complex in taste and more beefy, meaty, and savory.

Example 6: Heme-proteins with nucleotides controls particular flavor compound production.

The addition of inosine monophosphate and guanosine monophosphate in mixes with heme protein and other precursors controlled the formation of flavor compounds (E)-4-octene, 2-ethyl-furan, 2-pentanone, 2,3-butanedione, 2-methyl-thiazole, , methyl-

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pyrazine, tridecane, (E)-2-octenal, 2-thiophenecarboxaldehyde, and 3-thiophenecarboxaldehyde. These compounds are known to be present in meat and have a beefy, meaty, buttery, and or savory flavor notes.

Reactions containing heme protein at 1% (LegH) with cysteine (10 mM), and glucose (20 mM), 1 mM IMP and 1 mM GMP, at pH 6.0 were prepared and heated to 150C for 3 minutes. Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors reacting heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction and identified using GCMS, creating a profile of the volatile chemicals in the headspace around the flavor reaction mixture. GCMS showed 4-octene, 2-ethyl furan, 2-pentanone, 2,3-butanedione, 2-methyl-thiazole, methyl-pyrazine, tridecane, 2-octenal, 2-thiophenecarboxaldehyde, 3-thiophenecarboxaldehyde compounds were created by a mixture of hemeprotein LegH with IMP, GMP, glucose, and cysteine. The same samples without IMP and GMP did not generate these compounds, additionally these compounds were also not created when heme-proteins were not present, just precursor molecules. Sensory evaluation by blinded trained panelist found the samples with the addition of inosine and guanosine as described as having more complexity in taste and more beefy, meaty, brothy and savory. Figure 2 shows the abundance of the novel flavor compounds created with heme protein at 1% was mixed in a reaction at pH 6, with cysteine (10 mM), and glucose (20 mM), IMP (1 mM) and GMP (1 mM), and detected by solid phase microextraction (SPME) and then detected by GCMS.

Example 7: Flavor generation with the addition of a particular organic acid

The addition of lactic acid in mixes with heme protein, ribose, and cysteine controlled the formation of the flavor compounds nonanal, thiazole, 2-acetylthiazole, and 8-methyl-1-undecene. These compounds are known to be present in meat.

Reactions containing heme protein at 1%, cysteine (10 mM), and ribose (20 mM), and lactic acid (1mM), pH 6.0, were prepared and heated to 150C for 3 minutes. Characteristic flavor and fragrance components were mostly produced during the cooking

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process where precursors reacting heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction and identified using GCMS, creating a profile of the generated compounds. Nonanal, thiazole, 2-acetylthiazole, and 8-methyl-1-undecene compounds were created by a mixture of LegH with lactic acid, ribose, and cysteine. The same samples without lactic acid did not generate these compounds, additionally these compounds were not created in the absence of heme-proteins.

Sensory evaluation by blinded trained panelist found the samples with the addition of lactic acid as described as beefy, savory, browned, bready, and having malty notes. The sample with everything but lactic acid rated lower in browned, bready and malty notes.

Example 8: Flavor generated with the addition of a particular amino acid.

The addition of lysine in mixes with heme protein ribose, and cysteine controlled the formation of flavor compounds dimethyl trisulfide, nonanal, 2-pentyl-thiophene, furfural, 2-nonenal, 1-octanol, 2-nonenal, thiazole, 2-acetylthiazole, phenylacetaldehyde, 2-acetylthiazole. These compounds are known to be present in meat and some have a beefy, meaty, and or savory flavor.

Reactions containing heme protein at 1%, cysteine (10 mM), and ribose (20 mM), and lysine (1mM), at pH 6.0, were prepared and heated to 150C for 3 minutes. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors could react with the heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction. Dimethyl trisulfide, nonanal, 2-pentyl-thiophene, furfural, 2-nonenal, 1-octanol, 2-nonenal, thiazole, 2-acetylthiazole, phenylacetaldehyde, 2-acetylthiazole compounds were created by a mixture of LegH with lactic acid, ribose, and cysteine. The same samples without lactic acid did not generate these compounds,

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additionally these compounds were not created when heme-proteins were not present, just precursor molecules. Sensory evaluation by blinded trained panelist found the samples with the addition of lysine as described as roast beefy, savory, and browned. The addition of lysine increased the roasted browned notes.

Example 9 - Flavor compound production by different Heme-proteins

The addition of different types of heme-proteins (LegH, Barley, *B. myoglobin*, or *A. aeolicus*) in flavor reaction mixtures containing one or more flavor precursor compounds results in many of the same key meat flavors, including but not limited to 2-pentyl-furan, 2,3-Butanedione, Thiophene, 2-methyl-thiazole, Pyrazine, Furan, Pyrrole, 2-methyl-furan and distinct differences in the flavor compounds, including but not limited to 2-pentyl-thiophene, Nonanal, 2-Nonanone, and 1-Octen-3-one. These differences in flavor compounds can change the overall taste profile. The different types of heme-protein were LegH, Barley, *B. myoglobin*, or *A. aeolicus* used at 1% w/w in a reaction mixed with cysteine (10 mM) and ribose (10 mM) at pH 6. The pre- reaction mixture was heated to 150 C for 3 minutes; this reaction created flavor compounds known to be present in meat; see Table 9. The characteristic flavor and fragrance components are mostly produced during the cooking process where the flavor precursor molecules react with the heme-protein. Samples were evaluated by GCMS to identify the flavor compounds generated after heating and also evaluated for their sensory profiles. Volatile chemicals were isolated from the head space around the flavor reactions. Table 9 shows the similarity and differences in volatile flavor compounds created by the different types of heme-proteins.

TABLE 9

Flavor compounds created by different heme-protein when heated with ribose and cysteine.

Name	LegH	Barley	*B. myoglobin*	*A. aeolicus*
Furan	x	x	x	x
Thiazole	x	x	x	x
benzaldehyde	x	x	x	x
2-acetylthiazole	x	x	x	x

2-methyl-propanal	x	x	x	x
furfural	x	x	x	x
2,3-butanedione	x	x	x	x
2-pentyl-furan	x	x	x	x
2-pentanone	x	x		
pyrazine	x	x	x	x
dimethyl trisulfide	x	x	x	x
3-methyl-butanal	x	x		x
2-methyl-thiazole	x	x	x	x
pentanal	x	x	x	x
1,3,5-cycloheptatriene	x	x	x	x
methacrolein	x	x	x	x
heptanal	x	x	x	x
2-methyl-butanal	x	x		x
isothiazole	x	x	x	x
thiophene	x	x	x	x
propanal	x	x	x	x
2-heptenal	x		x	x
methyl-pyrazine	x	x	x	x
1-octene	x		x	x
butanal	x	x	x	x
2-acetyl-propen-2-ol	x	x	x	x
pyrrole	x	x	x	x
2-methyl-furan	x	x	x	x
nonanal		x	x	x
2-propenal		x	x	x
2-decenal		x	x	x
2-nonanone		x		x
2-octanone		x	x	x
2-tridecen-1-ol,			x	x
2-octanone			x	
2-octenal			x	x
4-methyl-2-heptanone			x	x
octanal			x	x
2-undecenal				x
butyrolactone				x
1-octen-3-one				x
3-methylheptyl acetate				x

2-pentyl-thiophene				x

Example 10 - Generation of Meat flavors from different lipids

Several different samples including oils (canola oil or coconut oil), free fatty acids (FFA) (linoleic acid (C18:2), oleic acid (C18:1), stearic acid (C18:0), or myristic acid (C14:0)) and phospholipids (PL) (beef heart polar lipids extract, Biolipon95 (from Perimond), or NatCholinePC40 (from Perimond)) were tested for their ability to produce beefy flavor in the absence and in the presents of other precursors. Oils, FFAs, and PLs were added to 50 mM potassium phosphate buffer (PPB) pH 6.0 or a Maillard reaction mix (MRM) containing 50 mM potassium phosphate pH 6.0, 5 mM Cysteine, 10 mM Glucose, 0.1 mM Thiamine, and 0.1% (w/v) LegHemoglobin. Lipids in combination with MRM were designed to capture the cross reactions of lipid degradation and Maillard reaction productions while lipids in phosphate buffer functioned as a lipid control. The oils were added at 3% of the total 1 mL volume of solution while FFAs and PLs were added at 1% of the total 1 mL volumes. All samples were cooked at 150°C for 3 mins, cooled to 50°C and then analyzed using GCMS (SPME fiber sampling of headspace). After all samples were analyzed by GCMS the caps were removed and samples were smelled by a trained flavor scientist and aromas recorded.

Table 10

Legend showing components of each sample

Sample Name	Solution	Additives
MRM None	Maillard Reaction Mix	None
MRM_Linoelic Acid	Maillard Reaction Mix	1% linoleic acid
MRM_Oleic Acid	Maillard Reaction Mix	1% oleic acid
MRM_C14	Maillard Reaction Mix	1% C14:0 free fatty acid
MRM_C18	Maillard Reaction Mix	1% C18:0 free fatty acid
MRM_Canola	Maillard Reaction Mix	3% Canola Oil
MRM_Coconut	Maillard Reaction Mix	3% Coconut Oil
MRM_BeefHeart	Maillard Reaction Mix	1% Beef Heart Polar Lipids Extract
MRM_Biolipon95	Maillard Reaction Mix	1% Biolipon95 (emulsifier)

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MRM_NatCholinePC40	Maillard Reaction Mix	1% NatCholinePC40 (emulsifier)
KPhos6_Linoelic Acid	PPB, pH 6	1% linoelic acid
KPhos6_Oleic Acid	PPB, pH 6	1% oleic acid
KPhos6_C14	PPB, pH 6	1% C14:0 free fatty acid
KPhos6_C18	PPB, pH 6	1% C18:0 free fatty acid
KPhos6_Canola	PPB pH 6	3% Canola Oil
KPhos6_Coconut	PPB, pH 6	3% Coconut Oil
KPhos6_BeefHeart	PPB, pH 6	1% Beef Heart Polar Lipids Extract
KPhos6_Biolipon95	PPB, pH 6	1% Biolipon95 (emulsifier)
KPhos6_NatCholinePC40	PPB, pH 6	1% NatCholinePC40 (emulsifier)

Table 11 contains the aroma descriptions and Table 12 contains the GCMS data from the most interesting samples analyzed. Many of the lipids introduced a "fatty" aroma to MRM that was otherwise absent. The combinations of Linoleic Acid or NatCholinePC40 in MRM produced the greatest abundance of fatty compounds suggesting that these lipids may improve the flavor perception of beef tallow. Linoleic Acid and NatCholinePC40 also showed high abundance of earthy-mushroom aromas. The addition of lipids to MRM significantly increased the abundance of "nutty & roasted" aromas. Less desirable "green" aroma compounds were most prominent in samples with unsaturated free fatty acids (linoleic acid or oleic acid) or phospholipids. In general, the addition of lipids significantly increased the number of target beef compounds made.

TABLE 11

Aroma descriptions of each sample after it was cooked.

Sample Names	Aroma Descriptions
MRM_Only	brothy, malty, beef stew
KPhos6_BeefHeart	fatty, creamy, beef tallow, slight sweet, slight roasted nutty
MRM_BeefHeart	fatty, beef tallow, old meat, mushroom
KPhos6_Biolipon95	fatty, fresh
MRM_Biolipon95	fatty, brothy, hay, malty green

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KPhos6_NatCholinePC40	light fatty, fresh
MRM_NatCholinePC40	fatty, beef tallow, brothy
K-Phos6_C14	light/faint plastic/waxy
MRM_C14	brothy, beefy, minty, fresh
K-Phos6_C18	light/faint plastic/waxy
MRM_C18	beefy with cucumber &/or pepper aroma
K-Phos6_Canola	fresh, cucumber
MRM_Canola	fatty, brothy, oil, roasted nuts
K-Phos6_Coconut	nothing
MRM_Coconut	brothy, beefy, slight fatty, crackers
K-Phos6_Oleic Acid	fresh, cucumber, camphorous/minty-like
MRM_Oleic Acid	herbal, plastic, slight cheesy, brothy
K-Phos6_Linoelic Acid	light plastic
MRM_Linoelic Acid	fatty, light waxy, brothy, herbal

Table 12

List of aromatic compounds found in Beef by GCMS and a chart showing which were detected in each lipid plus MRM sample.

Compounds in Beef	MRM only	MRM_BeefHeart	MRM_NatCholinePC40	MRM_Linoleic acid
(s)-isopropyl lactate	N	N	N	N
1-ethyl-5-methylcyclopentene	Y	Y	Y	Y
1-heptanol	N	Y	N	N
1-hepten-3-ol	N	Y	Y	Y
1-heptene	N	Y	Y	Y
2-methyl-1-heptene	N	N	N	N
1-hexanol	N	Y	Y	Y
2-ethyl-1-hexanol	N	N	N	N
1-nonanol	N	N	Y	N
1-nonene	N	Y	Y	N
1-octanol	N	Y	Y	N
1-octen-3-ol	N	Y	Y	Y

1-octen-3-one	Y	Y	Y	Y
1-octene	N	N	N	N
1-pentanol	N	Y	Y	Y
1-penten-3-ol	N	Y	Y	N
1-propanol	N	N	N	N
8-methyl-1-undecene	N	Y	Y	Y
1,3-hexadiene	N	N	N	Y
3-ethyl-2-methyl-1,3-hexadiene	N	Y	Y	Y
1,3-octadiene	Y	N	N	Y
1,3,5-cycloheptatriene	N	N	N	N
2,3-dihydro-5,6-dimethyl-1,4-dioxin	N	N	N	N
1,7-octadien-3-ol	N	Y	N	N
1h-pyrrole-2-carboxaldehyde	N	N	N	N
2-methyl-1H-pyrrole	N	N	N	N
2-acetyl-2-thiazoline	Y	N	N	N
2-acetylthiazole	Y	Y	Y	Y
2-butanone	N	Y	Y	Y
2-butenal	N	Y	Y	Y
2-ethyl-2-butenal	N	N	N	Y
3-methyl-2-butenal	N	N	Y	Y
3-methyl-2-cyclohexen-1-one	N	N	N	N
2-decanone	Y	Y	Y	N
(E)-2-decenal	N	N	N	N
(Z)-2-decenal	Y	Y	Y	Y
2-furanmethanol	N	N	N	N
2-heptanone	Y	Y	Y	Y
6-methyl-2-heptanone	N	N	Y	N
(E)-2-heptenal	N	Y	Y	Y
(Z)-2-heptenal	N	N	N	Y
(E)-2-hexenal	N	Y	Y	Y
2-ethyl-2-hexenal	N	N	N	N
2-methyl-2-heptene	Y	N	N	N
2-n-heptylfuran	Y	N	N	N
2-n-octylfuran	Y	Y	Y	N

2-nonanone	N	Y	Y	N
(E)-2-nonenal	Y	Y	Y	Y
(Z)-2-nonenal	N	N	N	Y
2-octanone	Y	Y	Y	Y
(Z)-2-octen-1-ol	Y	Y	Y	Y
(E)-2-octenal	N	Y	Y	Y
2-pentanone	N	Y	Y	N
1-propoxy-2-propanol	N	N	N	N
1-(acetyloxy)-2-propanone	Y	N	N	N
1-hydroxy-2-propanone	Y	N	N	N
2-propenal	N	N	N	Y
2-thiophenecarboxaldehyde	Y	Y	Y	Y
2-undecenal	N	Y	Y	Y
2,3-butanedione	N	N	N	Y
2,3-pentanedione	N	N	N	N
(E,E)-2,4-decadienal	N	Y	Y	Y
2,4-decadienal	N	N	N	Y
(E,E)-2,4-heptadienal	N	Y	Y	Y
(E,E)-2,4-nonadienal	N	Y	Y	Y
2,6-dimethylpyrazine	N	N	N	N
(E,Z)-2,6-nonadienal	N	N	Y	N
5-ethyldihydro-2(3H)-furanone	N	Y	Y	Y
5-methyl-2(3H)-furanone	N	N	N	N
dihydro-5-pentyl-2(3H)-furanone	N	N	Y	Y
dihydro-5-propyl-2(3H)-furanone	N	N	N	N
2(5H)-furanone	N	N	N	N
tetrahydro-6-methyl-2H-pyran-2-one	N	N	N	N
3-ethylcyclopentanone	N	Y	Y	Y
3-hexanone	N	N	N	N
3-methyl-2-thiophenecarboxaldehyde	N	N	N	N
3-octanone	Y	Y	N	Y
3-octen-2-one	N	Y	Y	Y
3-thiophenecarboxaldehyde	N	Y	Y	Y

(E,E)-3,5-octadien-2-one	N	N	Y	Y
dihydro-2-methyl-3(2H)-furanone	N	N	N	N
4-cyanocyclohexene	N	N	N	N
4-cyclopentene-1,3-dione	N	N	Y	N
4-decyne	N	Y	N	N
(Z)-4-heptenal	N	Y	Y	Y
4-methyloctanoic acid	N	N	N	N
(E)-4-octene	N	N	N	N
2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one	Y	N	N	N
6-methyl-5-hepten-2-one	Y	N	N	N
acetaldehyde	N	N	N	Y
acetic acid	N	N	N	N
acetic acid ethenyl ester	Y	N	N	N
acetoin	Y	N	N	N
acetone	Y	N	N	Y
acetonitrile	N	N	N	Y
benzaldehyde	Y	Y	Y	Y
4-ethyl-benzaldehyde	N	Y	Y	N
benzene	Y	N	N	N
benzoic acid, hydrazide	Y	N	N	N
butanal	Y	N	N	Y
2-methyl-butanal	N	N	N	N
3-methyl-butanal	Y	N	N	N
butanoic acid	N	N	N	N
butyrolactone	Y	Y	N	Y
caprolactam	N	N	N	N
carbon disulfide	N	N	N	Y
1-ethyl-1-methyl-cyclopentane	Y	Y	Y	Y
propyl-cyclopropane	N	N	Y	Y
decanal	N	Y	Y	N
dihydro-3-(2H)-thiophenone	N	N	N	N
Dimethyl sulfide	Y	N	N	N
dimethyl sulfone	N	N	N	N
dimethyl trisulfide	Y	Y	N	N
ethanethiol	N	N	N	N

ethanol	N	N	N	Y
1-(1(H)-pyrrol-2-yl)-ethanone	N	N	N	N
1-(2-furanyl)-ethanone	N	N	N	N
ethosuximide	Y	N	N	N
formic acid, heptyl ester	Y	Y	N	N
furan	Y	N	N	Y
2-ethyl-furan	Y	N	N	N
2-hexyl-furan	Y	N	N	Y
2-methyl-furan	N	N	N	Y
2-pentyl-furan	N	Y	Y	Y
2-propyl-furan	N	N	Y	Y
3-methyl-furan	Y	N	N	N
3-pentyl-furan	Y	Y	Y	Y
furfural	N	Y	Y	Y
heptanal	N	Y	Y	Y
heptanoic acid	N	N	N	Y
2-methyl-hex-2-yn-4-one	N	N	N	N
hexanoic acid	N	N	N	Y
hydrogen sulfide	N	N	N	N
m-aminophenylacetylene	N	N	N	N
maleic anhydride	N	N	N	N
methacrolein	N	N	N	N
methanethiol	N	N	N	N
methyl ethanoate	N	N	N	N
methyl isobutyl ketone	Y	N	N	N
n-caproic acid vinyl ester	N	Y	Y	N
nonanal	N	Y	Y	Y
3-methyl-nonane	Y	N	N	N
nonanoic acid	Y	N	N	N
octanal	N	Y	Y	Y
octane	N	N	N	Y
octanoic acid	N	N	N	Y
oxalic acid, isobutyl pentyl ester	Y	N	N	N
p-cresol	N	N	N	N
pentanal	N	N	N	Y
pentanoic acid	Y	N	N	Y

4-ethyl-phenol	N	Y	Y	N
phenylacetaldehyde	Y	Y	Y	Y
(p-hydroxyphenyl)-phosphonic acid	Y	N	N	N
propanal	N	N	N	Y
2-methyl-propanal	N	N	N	N
propanoic acid	N	N	N	N
2-methyl-propanoic acid	Y	N	N	N
propanoic acid, ethenyl ester	N	N	N	N
pyrazine	N	Y	N	Y
2-ethyl-5-methyl-pyrazine	N	N	N	N
2-ethyl-6-methyl-pyrazine	N	N	N	N
2,3-dimethyl-pyrazine	N	N	N	N
2,5-dimethyl-pyrazine	N	N	N	N
3-ethyl-2,5-dimethyl-pyrazine	Y	N	N	N
ethyl-pyrazine	N	N	N	N
methyl-pyrazine	N	N	N	N
trimethyl-pyrazine	Y	N	N	N
pyridine	Y	N	Y	N
pyrrole	Y	Y	Y	Y
styrene	Y	N	Y	N
thiazole	Y	Y	Y	Y
methyl-thiirane	N	N	N	N
thiophene	N	N	N	Y
2-hexyl-thiophene	Y	N	Y	N
2-pentyl-thiophene	N	Y	N	N
trans-2-(2-pentenyl)furan	N	Y	Y	N
trans-3-nonen-2-one	N	Y	Y	Y
undecanoic acid	N	N	N	N
Total # of Compounds Detected:	54	63	66	76

In samples having fatty or creamy aromas, 2,4-decadienal, (E,E)-2,4-nonadienal, (E,E)-2,4-heptadienal, and/or (E,E)-2,4-decadienal were detected in the KPhos6_BeefHeart, MRM_BeefHeart, MRM_BioLipon95, MRM_NatCholinePC40,

Kphos6_Canola, MRM_Canola, KPhos6_Oleic Acid, KPhos6_Linoleic acid and MRM_Linoleic acid samples. For (E,E)-2,4-decadienal, the strongest signal intensity was in the MRM_NatCholinePC40 sample, followed by the MRM_Linoleic acid, KPhos6_Linoleic acid, MRM_BeefHeart, MRM_BioLipon95, KPhos6_BeefHeart, MRM_Oleic Acid, and KPhos6_Oleic Acid samples. For (E,E)-2,4-heptadienal, the strongest signal intensity was in the MRM_NatCholinePC40 sample followed by the MRM_Canola sample. (E,E)-2,4-heptadienal also was detected in the MRM_BioLipon95, MRM_BeefHeart, and MRM_Linoleic acid samples. For (E,E)-2,4-nonadienal, the strongest signal intensity was in the MRM_Canola and MRM_Linoleic acid samples. (E,E)-2,4-nonadienal also was detected in the Kphos6_Canola, MRM_NatCholinePC40, MRM_BioLipon95, MRM_BeefHeart, and KPhos6_Linoleic acid samples. For 2,4-decadienal, the strongest signal intensity was in the MRM_Linoleic acid sample. 2,4-decadienal also was detected in KPhos6_Linoleic acid, MRM_Canola, and KPhos6_Oleic Acid samples.

In samples having earthy or mushroom aromas, 3-octen-2-one, 1-octen-3-one, 3-octanone, and/or 1-octen-3-ol were detected in the KPhos6_BeefHeart, MRM_BeefHeart, Kphos_BioLipon95, MRM_BioLipon95, Kphos_NatCholinePC40, MRM_NatCholinePC40, MRM_Canola, KPhos6_Oleic Acid, MRM_Oleic Acid, KPhos6_Linoleic acid, and MRM_Linoleic acid samples. For 1-octen-3-ol, the strongest signal intensity was in the MRM_Linoleic acid sample, followed by MRM_NatCholinePC40, KPhos6_Linoleic acid, MRM_BeefHeart, KPhos6_BeefHeart, MRM_Canola, MRM_BioLipon95, KPhos6_Oleic Acid, and MRM_Oleic Acid samples. 3-octanone was detected in the MRM_Oleic Acid, KPhos6_Linoleic acid, and MRM_Linoleic acid samples. For 1-octen-3-one, the strongest signal intensity was in the MRM_Linoleic acid and MRM_BeefHeart samples, followed by KPhos6_Linoleic acid, MRM_NatCholinePC40, KPhos6_BeefHeart, MRM_BioLipon95, MRM_Oleic Acid, and KPhos6_Oleic Acid samples. For 3-octen-2-one, the strongest signal intensity was in the KPhos6_Linoleic acid sample, followed by MRM_Linoleic acid, MRM_NatCholinePC40, KPhos6_BeefHeart, KPhos6_Oleic Acid, MRM_Oleic Acid, MRM_BeefHeart, MRM_BioLipon95, MRM_Canola, Kphos_BioLipon95, and

Kphos_NatCholinePC40. Pyrazine was detected in the MRM_Coconut, MRM_C18, MRM_C14, and MRM_BioLipon95 samples.

In samples having a nutty and roasted aroma, thiazole and 2-acetylthiazole were the most abundant compounds detected, along with pyrazine, methyl pyrazine, trimethyl pyrazine, and 3-ethyl-2,5-dimethylpyrazine. 2-acetylthiazole was detected in all samples with MRM and most abundant in samples with MRM_Beefheat, MRM_biolipon95, MRM_Canola, and MRM_coconut. Thiazole was created in samples with MRM-Coconut, MRM_BeefHeat, MRM_Biolipon95, MRM_C14, MRM_C18, MRM_Canola, MRM_Oleic acid and MRM_Linoleic acid and MRM_NatCholinePC40. Pyrazine was present in the largest amount in samples with MRM-Coconut, followed by samples MRM_BeefHeat, MRM_Biolipon95, MRM_C14, MRM_C18, MRM_Canola having roughly equal amount, MRM_Oleic acid and MRM_Linoleic acid sample had even less. Methyl-pyrazine was present in MRM_Biolipon95 and MRM_Coconut. 3-ethyl-2,5-dimethyl- pyrazine and trimethyl- pyrazine, were present only without phospholipids in the MRM.

In samples having green, vegetable, or grass aromas, 1-heptanol, 1-hepten-3-ol, 1-hexanol, (E)-2-heptenal, (Z)-2-heptenal, (E)-2-hexenal, 2-pentyl-furan, and/or heptanal were detected in the KPhos6_BeefHeart, MRM_BeefHeart, Kphos_BioLipon95, MRM_BioLipon95, Kphos_NatCholinePC40, MRM_NatCholinePC40, Kphos_C14, MRM_C14, Kphos_C18, MRM_C18, MRM_Canola, MRM_Coconut, KPhos6_Oleic Acid, MRM_Oleic Acid, KPhos6_Linoleic acid, and MRM_Linoleic acid samples. For 2-pentyl-furan, the strongest signal intensity was in the KPhos6_BeefHeart sample, followed by the KPhos6_Linoleic acid, MRM_BioLipon95, MRM_Linoleic acid, MRM_BeefHeart, MRM_Oleic Acid, MRM_NatCholinePC40, MRM_Canola, KPhos6_Oleic Acid, and Kphos_NatCholinePC40 samples. For (E)-2-heptenal, the strongest signal intensity was in the MRM_BeefHeart, MRM_Canola, MRM_Oleic Acid, and KPhos6_Linoleic acid samples, followed by the KPhos6_Oleic Acid, MRM_BioLipon95, KPhos6_BeefHeart, MRM_Linoleic acid, MRM_NatCholinePC40, Kphos_BioLipon95, and Kphos_NatCholinePC40 samples. For (Z)-2-heptenal, the strongest signal intensity was in the MRM_Linoleic acid sample. MRM_Linoleic acid

also was detected in the KPhos6_Linoleic acid sample. For heptanal, the strongest signal intensity was in the MRM_Oleic Acid sample, followed by the KPhos6_Oleic Acid, MRM_C14, MRM_C18, MRM_Canola, MRM_BeefHeart, MRM_NatCholinePC40, MRM_Linoleic acid, and KPhos6_BeefHeart samples. For , (E)-2-hexenal, the strongest signal intensity was in the MRM_Linoleic acid sample, followed by the MRM_NatCholinePC40, KPhos6_Linoleic acid, and MRM_Oleic Acid samples.

Example 11 - Creation of beefy flavors using complex precursor mixtures

A formulation was prepared (the "magic mix," see Table 13 containing the estimated concentrations of amino acids, sugars, and other small molecules in beef based on their values reported in literature. The magic mix was tested for its ability to produce beefy flavors in the presence of LegHemoglobin (LegH). The magic mix and 1% w/v LegH were added to the meat replica, pH 6.0 (see Table 4) and baked in a convection oven for 7 minutes at 160°C. A control sample was prepared by adding 1% w/v LegH to the meat replica, pH 6.0 and baking in a convection oven for 7 minutes at 160°C.

The meat replica sample containing only LegH, was compared to the meat replica sample containing the magic mix and LegH by a sensory panel and GCMS analysis. Five tasters rated the flavored meat replicas for beefiness, bitterness, and levels of savory flavors, and off flavors. Each property was rated on a 7 point scale in which 7 was the highest amount of the specified property (e.g., a standard 80:20 ground beef would be rated 7 on the beefy scale). The Magic Mix flavor was rated one point higher in beefy character than the LegH only sample (FIG. 1).

To determine which chemical products were produced upon heating, a solution of Magic Mix was prepared with 1% w/v LegH at pH 6.0. The samples were cooked with shaking at 150°C for three minutes, then Solid Phase Micro Extraction (SPME) was performed for twelve minutes at 50°C to extract the volatile compounds above the headspace of the reaction. A search algorithm was used to analyze the retention time and mass fingerprint information of the volatile compounds and assign chemical names to peaks. Table 14 shows the compounds identified in both the Magic Mix + LegH (MM, average of two samples) and in the LegH alone in buffer (LegH, average of five samples)

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samples. The compounds in Table 14 are listed in order of the retention time (R.T., in seconds), and are designated as having a zero peak area (0), or a small (S), medium (M), or large (L) average peak area. Hundreds of compounds were identified between the samples, many of which are characteristic of beefy aroma, including but not limited to 1,3-bis(1,1-dimethylethyl)-benzene, 2-methyl 3-furanthiol, and Bis(2-methyl-4,5-dihydro-3-furyl) disulfide, which increased in the samples containing the Magic Mix and LegH.

Table 13

Chemical entities added to the Magic Mix

Chemical entity	mM
Alanine	5.6
Arginine	0.6
Asparagine	0.8
Aspartate	0.8
Cysteine	0.8
Glutamic acid	3.4
Glutamine	0.7
Glycine	1.3
Histidine	0.6
Isoleucine	0.8
Leucine	0.8
Lysine	0.7
Methionine	0.7
Phenylalanine	0.6
Proline	0.9
Threonine	0.8
Tryptophan	0.5
Tyrosine	0.6

Valine	0.9
glucose	5.6
Ribose	6.7
Maltodextrin	5.0
Thiamine	0.5
GMP	0.24
IMP	0.6
Lactic acid	1.0
creatine	1.0
NaCl	10
KCl	10
Kphos pH 6.0	10

TABLE 14

Compounds identified with GC-MS analysis in samples with MM and LegH, or LegH alone (average of five samples)

R.T.(s)	Name	MM with LegH	LegH alone
248	acetaldehyde	L	S
256.3	carbon disulfide	L	S
264.3	dimethyl sulfide	S	0
265	oxalic acid, isobutyl pentyl ester	M	0
268.1	2,3,4-trimethyl-pentane	M	0
269.2	methanethiol	S	0
283.4	propanal	M	0
285.4	octane	M	0
287.1	furan	M	0
295.3	2-methyl-propanal	L	S
297.6	acetone	L	S
319.3	2-propenal	M	S
338.1	2-methyl-furan	M	S
342.1	butanal	L	S

344.2	2,4-dimethyl-1-heptene	M	0
346.3	methacrolein	M	0
357.4	methyl-thiirane	L	0
360.2	3-methyl-furan	S	0
363.7	butanone	L	S
368.9	2,3-dihydro-5-methyl-furan	M	S
376.4	2-methyl-butanal	L	M
381.1	3-methyl-butanal	L	M
390.6	isopropyl alcohol	0	S
399.6	ethanol	L	M
406.2	2-propenoic acid, methyl ester	M	0
408.2	benzene	S	0
414.4	methyl vinyl ketone	M	0
416.4	2,2,4,6,6-pentamethyl-heptane	M	0
422.6	2-ethyl-furan	S	0
438.4	2-ethylacrolein	M	0
449.9	2-pentanone	S	0
453.2	pentanal/2,3-butanedione	L	0
453.8	2,3-butanedione	L	M
472.8	4,7-dimethyl-undecane	M	S
485.9	2-methyl-pentanal	M	0
492.6	2-methyl-1-penten-1-one	S	0
496.6	(E)-3-penten-2-one	M	0
508.6	1-penten-3-one	M	0
510.6	trichloromethane	M	M
520.4	p-dithiane-2,5-diol	M	0
525.5	3-methyl-pentanal	M	0
535.1	(E)-5-decene	M	0
536.5	toluene	M	S
537.9	2-butenal	M	S
543.8	4-penten-2-one	M	0
550.8	methyl thiolacetate	M	0
683.7	p-xylene	S	0
727.4	dimethyl selenone	M	0
738.3	methyl isopropyl disulphide	M	0
755	2-heptanone	M	0
758.7	heptanal	L	0
781.9	1,3-diisopropoxy-1,3-dimethyl-1,3-	S	M

	disilacyclobutane		
789.4	3-methyl-2-butenal	M	0
793.4	4-methyl-2-heptanone	M	0
810.4	pyrazine	M	0
818.8	isothiazole	S	0
827.1	acetyl valeryl	M	0
831.8	2-pentyl-furan	L	0
851	2-methyl-thiazole	S	0
853.3	isothiocyanato-methane	S	0
870.9	thiazole	L	0
879.2	styrene	M	0
890.7	1-(methylthio)-propane	M	0
895.6	methyl-pyrazine	M	0
910.5	thiocyanic acid, methyl ester	S	0
918.6	4-methylthiazole	M	0
921.4	2-octanone	M	0
923.9	2-methyl-cyclopentanone	M	0
927.9	octanal	L	S
934.3	tridecane	M	0
948.8	trans-2-(2-pentenyl)furan	S	0
961.9	1-hydroxy-2-propanone	M	0
974.5	(E)-2-heptenal	M	0
987.4	5-methyl-1-undecene	M	0
993.8	2-hexyl-furan	M	0
1007.8	7-methyl-(E)-5-undecene	M	0
1024.1	2-methyl-5-(methylthio)-furan,	S	0
1058.6	2-butyl-1-decene	M	0
1079.3	dimethyl trisulfide	L	S
1085.3	2-nonanone	M	0
1093.2	nonanal	L	M
1142.3	1,3-bis(1,1-dimethylethyl)-benzene	M	0
1149.6	(E)-2-octenal	M	0
1164.5	1-heptanol	M	0
1193.5	methional	L	0
1198.8	acetic acid	M	S
1207.2	furfural	M	0
1242.1	2-decanone	M	0
1250.8	decanal	M	0

1265.2	1-decen-3-one	M	0
1283.3	pyrrole	M	0
1292.6	5-ethenyl-4-methyl-thiazole	M	0
1294.3	benzaldehyde	L	M
1303.7	2-n-octylfuran	M	0
1305.6	(E)-2-nonenal	M	0
1341.4	1-octanol	M	0
1361.1	2-methyl-1(H)-pyrrole	S	0
1391.7	2-undecanone	M	0
1401.2	(E)-2-octen-1-ol	M	0
1448	butyrolactone	S	S
1456.3	(E)-2-decenal	M	0
1462.4	phenylacetaldehyde	L	S
1466.3	2-acetylthiazole	L	0
1471.3	acetophenone	M	S
1475.4	1-nonanol	M	0
1487	methyl (methylthio)methyl disulfide	M	0
1497.1	5-(2-chloroethyl)-4-methylthiazole	L	0
1497.5	1-(ethylthio)-2-(methylthio)-buta-1,3-diene	L	S
1512	3-thiophenecarboxaldehyde	M	0
1518.8	2-nonen-4-one	M	0
1531.7	2-thiophenecarboxaldehyde	S	0
1543.9	dodecanal	M	0
1551.6	4-ethyl-2-methyl-pyrrole	S	0
1558.2	3-(methylthio)-propanenitrile	S	0
1561.2	3-decen-2-one	M	0
1613.1	bis(2-methyl-4,5-dihydro-3-furyl) disulfide	M	0
1615.6	1,10-undecadiene	M	0
1619.5	2-undecenal	S	0
1668.9	2-phenylpropenal	M	0
1692.3	(Z)-3-decen-1-ol, acetate	M	0
1733.1	3-phenyl-furan	S	0
1739.7	4-nitrophenyl 2-thiophenecarboxylic acid ester	S	0
1741.2	5-formyl-4-methylthiazole	M	0
1749.7	pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester	M	0
1765.5	benzyl alcohol	S	0

1774.2	pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester	S	0
1796.9	dodecanal	M	0
1806.1	(1-ethyl-1-propenyl)-benzene	S	0
1825.6	1-undecanol	M	S
1827.9	2-methyl-3-furanthiol	M	0
1828.3	2-methyl-3-(methylthio) furan	M	0
1836.1	4-chloro-2,6-bis(1,1-dimethylethyl)-phenol	S	0
1844.1	phenol	S	S
1845.3	[(methylsulfonyl)methyl]-benzene	S	0
1850.3	(e)-2-tridecen-1-ol	M	0
1859.9	1-heptyl-1,2,3,4-tetrahydro-4-methyl-naphthalene	S	0
1863.2	2,4-decadienal	S	0
1905.1	3,3'-dithiobis[2-methyl]-furan	M	0
1906.9	3,5-di-tert-butylbenzoic acid	S	0
1909.6	4-ethoxy-benzoic acid, ethyl ester	S	0
1921.5	3-(phenylmethyl)-2,5-piperazinedione	S	0
1944.5	9-octadecenal	M	0
1959.7	3,5-bis(1,1-dimethylethyl)-phenol	M	S
1968.4	4-methyl-5-thiazoleethanol	M	S
2007.8	1,1'-(1,2-cyclobutanediyl)bis-cis-benzene	S	0
2019.8	benzoic acid	S	S
2026.4	4-quinolinecarboxaldehyde	S	0
2027.8	m-aminophenylacetylene	M	0

Example 12 – Ferrous Chlorin Catalyzes Production of Meat-like flavor compounds

Fresh green spinach (10 lb) was added to 500 mL water and finely ground in a Vitamix blender to yield 2 L of green suspension. Acetone (8L) was added with mixing and the material was allowed to extract for 1 hour. The material was filtered through Whatman filter paper and the acetone was removed on a rotary evaporator (Buchi). To the residual green suspension (500 mL) was added 2 mL of 10 M HCl, causing the suspension to turn brown. To this was added 1g of $FeCl_2.4H_2O$ in 10 mL H_20. The

solution was shaken then left at 4°C for 16 hours. This suspension was extracted with diethyl ether (3x50mL) to give a bright green organic phase, the combined organics were washed with saturated sodium chloride solution, dried over sodium sulfate, filtered and evaporated to leave a black paste (1.1g). The pellet was dissolved in chloroform for fractionation.

Chlorophyll and Ferrous chlorin crude fractions were stored at -20°C. Crude extracts were fractionated by reverse-phase high-pressure liquid chromatography (RP-HPLC). HPLC conditions are outlined in Table 15. Both chlorophyll and ferrous chlorophyll were eluted from the column with a peak retention time of 7.6 minutes. Eluted material was collected from 7.3 – 8.0 minutes. Collected fractions were pooled and stored on ice. Collected fractions were re-chromatographed and showed a single peak with retention time 7.6 minutes. The desired fractions were pooled, then 10% sunflower oil was added, methanol was removed on a rotary evaporator (Buchi).

TABLE 15

HPLC conditions for purification of chlorophyll and ferrous chlorin from crude extract.

Sample:	Chlorophyll or Fe-chlorin (~2mg/mL in $CHCl_3$)
System:	Agilent 1100 with Chemstation
Column:	Zorbax Bonus-RP (4.6 x 250 mm, 5uM)
Mobile phase:	acetonitrile, methanol, ethyl acetate (60:20:20) isocratic flow
Temperature:	30°C
Flow Rate:	1.0 mL per minute
Injection volume:	0.05 mL

Preparation of Flavor Reaction Containing Ferrous Chlorin or Leghemoglobin

A solution of ferrous chlorophyll was mixed with the Magic Mix (Table 13) to a final concentration of 0.35% ferrous chlorin, 1% glycerol, 0.005% tween-20, 5% sunflower oil, 100 mM NaCl, 20 mM phosphate at pH 6. Leghemoglobin (0.35%) at pH 6 in phosphate buffer (20 mM), 100 mM NaCl, was mixed with the Magic Mix (Table

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13), 1% glycerol, and 0.005% tween-20. The flavor reaction mixtures were heated to 150°C for 3 minutes; this reaction created flavor compounds known to be present in meat, created by hemoglobin and also created by ferrous chlorin; *see* Table 16.

The characteristic flavor and fragrance components were mostly produced during the cooking process when the flavor precursor molecules reacted with the heme-protein or the ferrous chlorophyll. Samples were evaluated by GCMS to identify the flavor compounds generated after heating. Volatile chemicals were isolated from the headspace around the flavor reactions. The profile of the volatile chemicals in the headspace around the flavor reaction mixtures that were similar between heme-protein and ferrous chlorin are shown in Table 16. Notabily, many of the compounds created by the ferrous chlorin are important in the flavor of meat.

TABLE 16

Flavor Compounds created by both Ferrous Chlorin and LegH with Magic Mix.

1-heptanol	acetone
1-hexanol	acetonitrile
1-octanol	benzaldehyde
1-octen-3-ol	butanal
1-octen-3-one	2-methyl-butanal
1-pentanol	dimethyl trisulfide
2-acetylthiazole	ethyl acetate
2-butenal	furan
3-methyl-2-butenal,	2-ethyl-furan
(Z)-2-decenal	2-hexyl furan
6-methyl-2-heptanone	2-pentyl-furan
(E)-2-heptenal	furfural
(E)-2-hexenal	heptanal
2-methyl-3-furanthiol	aminophenylacetylene
(E)-2-nonenal	methacrolein
(E)-2-octenal	methional
2-pentanone	octanal
1-hydroxy-2-propanone	octane
2-thiophenecarboxaldehyde	oxalic acid, diallyl ester
2-undecenal	2,3-butanedione

3-methyl-3-buten-2-one	2-methyl-propanal
3-thiophenecarboxaldehyde	pyrazine
(E)-4-octene,	2,3-dimethyl-pyrazine
methyl-pyrazine	2,5-dimethyl-pyrazine
thiazole	

Example 13 - Flavor creation by immobilized Hemin

Preparation of hemin linked CM Sepharose.

200 mg of bovine hemin (Sigma Aldrich) was loaded into a scintillation vial. A small magnetic stir bar, 800 μL acetonitrile, 64 μL 4-methylmorpholine, and 71 mg of N-hydroxysuccinimide were added in that order. The vial was placed in an ice bath and chilled then 118 mg of N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride was added with stirring, followed by 845 μL of Jeffamine ED900. This was stirred while allowing the black mixture to warm to ambient temperature. Chloroform (10 mL) was added to the mixture followed by water (4 mL). A flashlight was used to distinguish between organic and aqueous layers since both were black and the organic layer was pipetted off and concentrated to a dark black oil. The oil was dissolved in a 4:1 mixture of acetonitrile and ethanol to make an approximately 10% strength solution that was inky black in color.

2 mL of water swelled and equilibrated CM Sepharose was equilibrated in a BioRad minicolumn with 3 volumes of acetonitrile. The resin was resuspended in 1 mL acetonitrile and pipetted into a scintillation vial. This was followed with 44 microliters 4-methylmorpholine, 23 mg N-hydroxysuccinimide, and 39 mg of solid N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride. The mixture was vortexed vigorously and then shaken for three hours. To this white solid was added 570 microliters of inky black 20% strength hemin coupled diamine. The black solid was vortexed and shaken for an hour. The slurry strongly resembled Turkish coffee. The mixture was poured into a BioRad minicolumn and filtered, washed with acetonitrile until what came out no longer resembled espresso, then switched to deionized water, and finally 20 mM pH 9 sodium carbonate buffer. The black solid was washed until the effluent ran clear and then resuspended in 2 mL of buffer for storage until use.

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Flavor reaction

The flavor reaction was created with heme protein (equine myoglobin -Sigma) at 0.35% in a phosphate buffer (20 mM) at pH 6.0 with 100 mM NaCl , this was mixed with Magic Mix (Table 13). Another flavor reaction was created with Immobilized Hemin at 0.35% in a phosphate buffer (20 mM) at pH 6.0 with 100 mM NaCl, this was mixed with Magic Mix (Table 13). The flavor reaction mixtures were heated to 150°C for 3 minutes; this reaction created flavor compounds known to be present in meat.

The characteristic flavor and fragrance components were mostly produced during the cooking process when the flavor precursor molecules reacted with the Heme-protein or the immobilized Hemin. Samples were evaluated by GCMS to identify the flavor compounds generated after heating. Volatile chemicals were isolated from the headspace around the flavor reactions. As can be seen in Table 17, immobilized hemin catalyzed production of compounds similar to those whose production was catalyzed by myoglobin free in solution. Notably, the profiles of flavor compounds, measured by GCMS, produced by cooking mixtures containing the immobilized hemin and the heme-protein, respectively, were very similar.

TABLE 17

Flavor compounds produced by cooking mixtures containing either myoglobin free in solution or hemin coupled to a solid support

Flavor compound	myoglobin	hemin-linker-resin
2-methyl-5-(methylthio)-thiophene	Low	
dihydro-5-propyl-2(3H)-furanone	Low	
octane	Low	
pyrrole	Low	Low
methanethiol	Low	Low
2-thiophenecarboxaldehyde	Low	Low
methyl-pyrazine	Low	Low
1-hydroxy-2-propanone	Low	Low
propanal	Low	Low

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thiophene	Low	medium
pyridine	Low	Low
2-methyl-furan	Low	medium
oxalic acid, butyl propyl ester	Low	Low
pyrazine	medium	Low
oxalic acid, diallyl ester	medium	medium
2-butenal	medium	large
furfural	medium	medium
nonanal	medium	medium
2-ethyl-furan	medium	Low
ethanol	medium	very large
tert-butanol	medium	
3,3'-dithiobis[2-methyl]-furan	medium	medium
m-aminophenylacetylene	medium	medium
2,5-dihydro-3,4-dimethyl-furan	medium	medium
2-acetylthiazole	medium	medium
cyclohexane	medium	
ethyl tert-butyl ether	medium	
carbon disulfide	medium	medium
thiazole	medium	medium
acetonitrile	medium	large
2-pentyl-furan	medium	Low
3-thiophenecarboxaldehyde	medium	medium
2-methyl-butanal	medium	medium
thiazole	medium	large
2-methyl-3-furanthiol	larege	large
2-propenal	large	large
3-methyl-2-butenal	large	medium
2-methyl-3-(methylthio) furan	large	large
ethyl acetate	large	medium
methacrolein	large	medium
methyl-thiirane	large	large
methional	large	large
methyl alcohol	large	medium
2-butanone	large	Low
2,3-butanedione	large	medium
acetone	large	large
furan	large	medium

benzaldehyde	large	medium
methyl thiolacetate	large	medium
acetaldehyde	very large	very large
2-methyl-propanal	very large	very large
dimethyl trisulfide	very large	very large
3-methyl-butanal	very large	very large
propyl-cyclopropane		medium
(E)-2-octenal		medium
2-n-propylaziridine		medium
thiirane		medium
ethyl formate		medium
methyl vinyl ketone		medium
2-propenoic acid, ethyl ester		medium
1-nonanol		large
1-octene		large
1-heptanol		large
1-dodecene		large
phorone		very large

Example 14. The combination of precursors with Heme protein drives flavor reactions.

Three samples were compared: precursor mix alone, 1% heme protein alone, and precursor mix with 1% heme. The precursor mix was made of glucose (20 mM), ribose (20 mM), cysteine (10 mM), thiamine (1 mM), and glutamic acid (1 mM). Reactions were all at pH 6.0, prepared and heated to 150°C for 3 minutes. These three samples were run in duplicate. These samples were evaluated by GCMS for the flavor compounds generated. Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors could react with the heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction. The flavor compounds created in each sample is indicated in Table 18. As shown most of the flavor molecules were created on when the precursors are combined with the heme protein.

Table 18

Flavor molecules created by the combination of LegH and precursor mix.

Compound	Precursor mix	LegH	Precursor mix + Leg H
carbon disulfide	medium	medium	high
isopropyl alcohol	medium	medium	low
2-methyl-furan	low		low
butanal	low		medium
thiophene	low		low
2,3-butanedione	low	low	high
furan	low		medium
2,4-dimethyl-1-heptene		high	high
acetone		high	high
dimethyl trisulfide		medium	medium
2-methyl-heptane		medium	medium
2-pentanone		medium	
pentanal		medium	medium
2-pentyl-furan		medium	medium
2-methyl-propanal		low	high
2-acetatyl-1-propene		low	low
2-methyl-butanal		low	medium
1,3-dimethyl-benzene		low	low
octane		low	low
benzene		low	low
benzaldehyde			very high
2-butanone			very high
furfural			very high
thiazole			high
nonanal			high
thiazole			high
2-acetylthiazole			medium
3-methyl-butanal			medium
(Z)-2-heptenal			medium
heptanal			medium
methyl-thiirane			medium
3-ethyl-pentane			medium
phenylacetaldehyde			medium
2-hexyl-furan			medium

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2-nonanone			medium
propanal			medium
pyrazine			medium
(Z)-2-heptenal			medium
2-methyl-1-heptene			medium
2-ethyl-furan			medium
octanal			medium
(E)-4-octene			low
(E)-2-octenal			low
2-methyl-thiazole			low
2-propenal			low
1-octen-3-one			low
1-octene			low
2-octanone			low
dimethyl sulfide			low
3-pentyl-furan			low
2-n-octylfuran			low
2-pentyl-thiophene			low

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A food product comprising:

a) a highly conjugated heterocyclic ring complexed to iron ion; and

b) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone.

2. The food product of claim 1, wherein said highly conjugated heterocyclic ring complexed to an iron ion is selected from the group consisting of a heme moiety, and a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochorophyll, a corphin, a chlorophyllin, a bacteriochlorin, and an isobacteriochlorin moiety complexed to iron ion.

3. The food product of claim 2, wherein said heme moiety is a heme-containing protein, a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support.

4. The food product of claim 2, wherein said highly conjugated heterocyclic ring complexed to iron is ferrous chlorin.

5. A food product comprising:

a) a heme-containing protein; and

b) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a hydrolyzed protein, a malt extract, a yeast extract, and a peptone.

6. The food product of any one of claims 1-5, wherein said food product comprises two to fifty flavor precursors.

7. The food product of any one of claims 1-5, wherein said food product comprises two to forty flavor precursors.

8. The food product of any one of claims 1-5, wherein said food product comprises two to thirty-five flavor precursors.

9. The food product of any one of claims 1-5, wherein said food product comprises two to ten flavor precursors.

10. The food product of any one of claims 1-5, wherein said food product comprises two to six flavor precursors.

11. The food product of any one of claims 1-10, wherein said one or more flavor precursor molecules are selected from the group consisting of glucose, ribose, sucrose, maltose, cysteine, a cysteine derivative, thiamine, lysine, a lysine derivative, glutamic acid, a glutamic acid derivative, IMP, GMP, lactic acid, maltodextrin, creatine, alanine, arginine, asparagine, aspartate, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, linoleic acid, lecithin, and mixtures thereof.

12. The food product of any one of claims 3-11, wherein the heme-containing protein is a plant heme-containing protein.

13. The food product of any one of claims 3-11, wherein the heme-containing protein is a mammalian heme-containing protein.

14. The food product of any one of claims 3-11, wherein the heme-containing protein is a yeast or filamentous fungus heme-containing protein.

15. The food product of any one of claims 3-11, wherein the heme-containing protein is a bacterial or archeal heme-containing protein.

16. The food product of any one of claims 3-12, wherein the heme-containing protein is a non-symbiotic hemoglobin.

17. The food product of any one of claims 3-12, wherein the heme-containing protein is a leghemoglobin.

18. The food product of claim 17, wherein the leghemoglobin is a from soybean, alfalfa, lupin, fava bean, pea, cow pea, or lupine.

19. The food product of any one of claims 12-18, wherein the heme-containing protein is recombinantly produced, chemically synthesized, or transcribed and translated *in vitro*.

20. The food product of any one of claims 3-19, wherein the heme-containing protein comprises an amino acid sequence having at least 80% sequence identity to a polypeptide set forth in SEQ ID NOs:1-26.

21. The food product of any one of claims 1-20, wherein said heme-containing protein is isolated and purified.

22. The food product of any one of claim 1-21, further comprising a food-grade oil, a seasoning agent, a flavoring agent, a protein, a protein concentrate, an emulsifier, a gelling agent, or a fiber.

23. The food product of any one of claims 1-18, wherein said food product is a meat substitute.

24. The food product of any one of claim 1-19, wherein said food product is a soup base, stew base, snack food, bouillon powder, bouillon cube, or a flavor packet.

25. The food product of any one of claims 1-20, wherein said food product is free of animal products.

26. The food product of any one of claims 1-21, wherein said food product is a frozen food product.

27. The food product of any one of claims 1-22, wherein said food product is sealed within a packet or shaker.

28. A method for producing a flavor compound, said method comprising:

a) combining a highly conjugated heterocyclic ring complexed to iron ion with one or more flavor precursor molecules to form a mixture, said one or more flavor precursor molecules selected from the group consisting of glucose, ribose, fructose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, cottonseed oil, soybean oil, corn oil, palm fruit oil, palm seed oil, rice bran oil, safflower oil, olive oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and

b) heating the mixture to form one or more flavor compounds selected from the group consisting of phenylacetaldehyde, 1-octen-3-one, 2-n-heptylfuran, 2-thiophenecarboxaldehyde, 3-thiophenecarboxaldehyde, butyrolactone, 2-undecenal, methyl-pyrazine, furfural, 2-decanone, pyrrole, 1-octen-3-ol, 2-acetylthiazole, 2-(E)-octenal, decanal, benzaldehyde, (E)-2-nonenal, pyrazine, 1-hexanol, 1-heptanol, dimethyl trisulfide, 2-nonanone, 2-pentanone, 2-heptanone, 2,3-butanedione, heptanal, nonanal, 2-octanone, 1-octanol, 3-ethylcyclopentanone, 3-octen-2-one, (E,E)-2,4-heptadienal, (Z)-2-heptenal, 6-methyl-2-heptanone, (Z)-4-heptenal, (E,Z)-2,6-nonadienal, 3-methyl-2-butenal, 2-pentyl-furan, thiazole, (E,E)-2,4-decadienal, hexanoic acid, 1-ethyl-5-methylcyclopentene, (E,E)-2,4-nonadienal, (Z)-2-decenal, dihydro-5-pentyl-2(3H)-furanone, trans-3-nonen-2-one, (E,E)-3,5-octadien-2-one, (Z)-2-octen-1-ol, 5-ethyldihydro-2(3H)-furanone, 2-butenal, 1-penten-3-ol, (E)-2-hexenal, formic acid, heptyl ester, 2-pentyl-thiophene, (Z)-2-nonenal, 2-hexyl-thiophene, (E)-2-decenal, 2-ethyl-5-methyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine, 2-ethyl-1-hexanol, thiophene, 2-methyl-furan, pyridine, butanal, 2-ethyl-furan, 3-methyl-, butanal, 2-methyl-, butanal,

methacrolein, 2-methyl-propanal, propanal, acetaldehyde, 2-propyl-furan, dihydro-5-propyl-2(3H)-furanone, 1,3-hexadiene, 4-decyne, pentanal, 1-propanol, heptanoic acid, ethanethiol, 1-butanol, 1-penten-3-one, dimethyl sulfide, 2-ethyl furan, 2-pentyl-thiophene, 2-Propenal, 2-Tridecen-1-ol, 4-Octene, 2-methyl thiazole,, methyl-pyrazine, 2-butanone, 2-pentyl-furan, 2-methyl-propanal, butyrolactone,, 3-methyl-butanal, methyl-thiirane, 2-hexyl-furan, butanal,, 2-methyl-butanal, trichloromethane, 2-methyl-furan, furan, octanal, 2-Heptenal, 1-Octene, heptyl ester formic acid, 3-pentyl-furan, and 4-penten-2-one.

29. The method of claim 28, wherein said highly conjugated heterocyclic ring complexed to an iron ion is selected from the group consisting of a heme moiety, and a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochorophyll, a corphin, a chlorophyllin, a bacteriochlorin, or an isobacteriochlorin moiety complexed to iron ion.

30. The method of claim 29, wherein said heme moiety is a heme-containing protein, a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support.

31. The method of claim 30, wherein said heme moiety is a heme-containing protein.

32. The method of claim 29, wherein said highly conjugated heterocyclic ring complexed to an iron ion is ferrous chlorin.

33. The method of any one of claims 28-32, said method comprising combining cysteine, ribose, lactic acid, lysine, and/or thiamine with said highly conjugated heterocyclic ring complexed to iron.

34. A method for producing a flavor compound, said method comprising:

a) combining a heme-containing protein, cysteine, and a sugar, and one or more optional flavor precursor molecules to form a mixture, said one or more optional flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, cottonseed oil, soybean oil, corn oil, palm fruit oil, palm seed oil, rice bran oil, safflower oil, olive oil, canola oil, coconut oil, mango oil, a free fatty acid, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and

b) heating the mixture to form one or more flavor compounds set forth in Table 3, 8, 9, 12, 14, 16, or 17.

35. A method for imparting a meat-like flavor to a food product comprising contacting said food product with a flavoring composition, said flavoring composition comprising i) a heme-containing protein; and ii) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, cottonseed oil, soybean oil, corn oil, palm fruit oil, palm seed oil, rice bran oil, safflower oil, olive oil, sunflower oil, canola oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a hydrolyzed protein, a malt extract, a yeast extract, and a

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peptone; wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product.

36. The method of claim 35, wherein said meat-like flavor is a beef-like, chicken-like, pork-like, lamb-like flavor, or turkey-like.

37. The method of claim 35 or claim 36, wherein said flavoring composition further comprises a seasoning agent, a flavoring agent, a protein, a protein concentrate, or an emulsifier.

38. The method of any one of claims 35-37, wherein said flavoring composition is sealed within a packet or shaker.

39. A method of making a food product, said method comprising combining an isolated heme-containing protein and one or more flavor precursor molecules to form a mixture, said one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a hydrolyzed protein, a malt extract, a yeast extract, and a peptone; and heating the mixture.

40. A method for producing a flavor compound, said method comprising:

a) incubating a heme-moiety bound to a solid support with one or more flavor precursor molecules, said one or more flavor precursor molecules selected from the group consisting of cysteine, ribose, glucose, fructose, arabinose, glucose-6-phosphate, fructose

6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, cottonseed oil, soybean oil, corn oil, palm fruit oil, palm seed oil, rice bran oil, safflower oil, olive oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and

b) heating the heme-moiety bound to said solid support and one or more flavor precursors to form one or more flavor compounds selected from the group consisting of phenylacetaldehyde, 1-octen-3-one, 2-n-heptylfuran, 2-thiophenecarboxaldehyde, 3-thiophenecarboxaldehyde, butyrolactone, 2-undecenal, methyl-pyrazine, 2-furfural, decanone, pyrrole, 1-octen-3-ol, 2-acetylthiazole, (E)-2-octenal, decanal, benzaldehyde, (E)-2-nonenal, pyrazine, 1-hexanol, 1-heptanol, dimethyl trisulfide, 2-nonanone, 2-pentanone, 2-heptanone, 2,3-butanedione, heptanal, nonanal, 2-octanone, 1-octanol, 3-ethylcyclopentanone, 3-octen-2-one, (E,E)-2,4-heptadienal, (Z)-, 2-heptenal, 6-methyl-2-heptanone, (Z)-4-heptenal, (E,Z)-2,6-nonadienal, 3-methyl-2-butenal, 2-pentyl-furan, thiazole, (E,E)-2,4-decadienal, hexanoic acid, 1-ethyl-5-methylcyclopentene, (E,E)-2,4-nonadienal, (Z)-2-decenal, dihydro-5-pentyl-2(3H)-furanone, trans-3-nonen-2-one, (E,E)-3,5-octadien-2-one, (Z)-2-octen-1-ol, 5-ethyldihydro-2(3H)-furanone, 2-butenal, 1-penten-3-ol, (E)-2-hexenal, formic acid, heptyl ester, 2-pentyl-thiophene, (Z)-2-nonenal, 2-hexyl-thiophene, (E)-2-decenal, 2-ethyl-5-methyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine, 2-ethyl-1-hexanol, thiophene, 2-methyl-furan, pyridine, butanal, 2-ethyl-furan, 3-methyl-butanal, 2-methyl-butanal, methacrolein, 2-methyl-propanal, propanal, acetaldehyde, 2-propyl-furan, dihydro-5-propyl-2(3H)-furanone, 1,3-hexadiene, 4-decyne, pentanal, 1-propanol, heptanoic acid, ethanethiol, 1-butanol, 1-penten-3-one, dimethyl sulfide, 2-ethyl furan, 2-pentyl-thiophene, 2-propenal, 2-tridecen-1-ol, 4-octene, 2-methyl thiazole, methyl-pyrazine, 2-butanone, 2-pentyl-furan, 2-methyl-propanal,

butyrolactone, 3-methyl-butanal, methyl-thiirane, 2-hexyl-furan, butanal,, 2-methyl-butanal, trichloromethane, 2-methyl-furan, furan, octanal, 2-heptenal, 1-octene, heptyl ester formic acid, 3-pentyl-furan, and 4-penten-2-one.

FIGURE 1

SEQ ID NO:1 *Vigna radiata*

MTTTLERGFTEEQEALVVKSWNVMKKNSGELGLKFFLKIFEIAPSAQKLFSFLRDSTVP
LEQNPKLKPHAVSVFVMTCDSAVQLRKAGKVTVRESNLKKLGATHFRTGVANEHFEVTK
FALLETIKEAVPEMWSPAMKNAWGEAYDQLVDAIKYEMKPPSS

SEQ ID NO:2 *Methylacidiphilum infernorum*

MIDQKEKELIKESWKRIEPNKNEIGLLFYANLFKEEPTVSVLFQNPISSQSRKLMQVLG
ILVQGIDNLEGLIPTLQDLGRRHKQYGVVDSHYPLVGDCLLKSIQEYLGQGFTEEAKAA
WTKVYGIAAQVMTAE

SEQ ID NO:3 *Aquifex aeolicus*

MLSEETIRVIKSTVPLLKEHGTEITARMYELLFSKYPKTKELFAGASEEQPKKLANAII
AYATYIDRLEELDNAISTIARSHVRRNVKPEHYPLVKECLLQAIEEVLNPGEEVLKAWE
EAYDFLAKTLITLEKKLYSQP

SEQ ID NO:4 *Glycine max*

MGAFTEKQEALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLSNGVDPSNPKL
TGHAEKLFGLVRDSAGQLKANGTVVADAALGSIHAQKAITDPQFVVVKEALLKTIKEAV
GDKWSDELSSAWEVAYDELAAAIKKAF

SEQ ID NO:5 *Hordeum vulgare*

MSAAEGAVVFSEEKEALVLKSWAIMKKDSANLGLRFFLKIFEIAPSARQMFPFLRDSDV
PLETNPKLKTHAVSVFVMTCEAAAQLRKAGKITVRETTLKRLGGTHLKYGVADGHFEVT
RFALLETIKEALPADMWGPEMRNAWGEAYDQLVAAIKQEMKPAE

Magnaporthe oryzae, (SEQ ID NO:6)

```
  1 mdgavrldwt gldltgheih dgvpiasrvq vmvsfplfkd qhiimsskes psrksstigq
 61 strngscqad tqkgqlppvg ekpkpvkenp mkklkemsqr plptqhgdgt yptekkltgi
121 gedlkhirgy dvktllamvk sklkgeklkd dktmlmervm qlvarlptes kkraeltdsl
181 inelwesldh pplnylgpeh syrtpdgsyn hpfnpqlgaa gsryarsvip tvtppgalpd
241 pglifdsimg rtpnsyrkhp nnvssilwyw atiiihdifw tdprdintnk sssyldlapl
301 ygnsqemqds irtfkdgrmk pdcyadkrla gmppgvsvll imfnrfhnhv aenlalineg
361 grfnkpsdll egeareaawk kydndlfqva rlvtsglyin itlvdyvrni vnlnrvdttw
421 tldprqdaga hvgtadgaer gtgnavsaef nlcyrwhsci sekdskfvea qfqnifgkpa
481 sevrpdemwk gfakmeqntp adpgqrtfgg fkrgpdgkfd dddlvrcise avedvagafg
541 arnvpqamkv vetmgiiqgr kwnvaglnef rkhfhlkpys tfedinsdpg vaealrrlyd
```

```
 601 hpdnvelypg lvaeedkqpm vpgvgiapty tisrvvlsda vclvrgdrfy ttdftprnlt
 661 nwgykevdyd lsvnhgcvfy klfirafpnh fkqnsvyahy pmvvpsenkr ilealgradl
 721 fdfeapkyip prvnitsygg aeyiletqek ykvtwheglg flmgegglkf mlsgddplha
 781 qqrkcmaaql ykdgwteavk afyagmmeel lvsksyflgn nkhrhvdiir dvgnmvhvhf
 841 asqvfglplk taknptgvft eqemygilaa ifttiffdld psksfplrtk trevcqklak
 901 lveanvklin kipwsrgmfv gkpakdepls iygktmikgl kahglsdydi awshvvptsg
 961 amvpnqaqvf aqavdyylsp agmhyipeih mvalqpstpe tdalllgyam egirlagtfg
1021 syreaavddv vkedngrqvp vkagdrvfvs fvdaardpkh fpdpevvnpr rpakkyihyg
1081 vgphaclgrd asqiaitemf rclfrrrnvr rvpgpqggelk kvprpggfyv ymredwgglf
1141 pfpvtmrvmw dde
```

Fusarium oxysporum (SEQ ID NO:7)

```
   1 mkgsatlafa lvqfsaasql vwpskwdeve dllymqggfn krgfadalrt cefgsnvpgt
  61 qntaewlrta fhdaithdak agtggldasi ywessrpenp gkafnntfgf fsgfhnprat
 121 asdltalgtv lavgacngpr ipfragrida ykagpagvpe pstnlkdtfa aftkagftke
 181 emtamvacgh aiggvhsvdf peivgikadp nndtnvpfqk dvssfhngiv teylagtskn
 241 plvasknatf hsdkrifdnd katmkklstk agfnsmcadi ltrmidtvpk svqltpvlea
 301 ydvrpyitel slnnknkihf tgsvrvritn nirdnndlai nliyvgrdgk kvtvptqqvt
 361 fqggtsfgag evfanfefdt tmdakngitk ffiqevkpst katvthdnqk tggykvddtv
 421 lyqlqqscav leklpnaplv vtamvrdara kdaltlrvah kkpvkgsivp rfqtaitnfk
 481 atgkkssgyt gfqaktmfee qstyfdivlg gspasgvqfl tsqampsqcs
```

Fusarium graminearum (SEQ ID NO:8)

```
   1 masatrqfar aatratrngf aiaprqvirq qgrryyssep aqksssawiw ltgaavagga
  61 gyyfygnsas satakvfnps kedyqkvyne iaarleekdd yddgsygpvl vrlawhasgt
 121 ydketgtggs ngatmrfape sdhganagla aardflqpvk ekfpwitysd lwilagvcai
 181 qemlgpaipy rpgrsdrdvs gctpdgrlpd askrqdhlrg ifgrmgfndq eivalsgaha
 241 lgrchtdrsg ysgpwtfspt vltndyfrll veekwqwkkw ngpaqyedks tkslmmlpsd
 301 ialiedkkfk pwvekyakdn daffkdfsnv vlrlfelgvp faqgtenqrw tfkpthqe
```

SEQ ID NO: 9 *Chlamydomonas eugametos*

MSLFAKLGGREAVEAAVDKFYNKIVADPTVSTYFSNTDMKVQRSKQFAFLAYALGGASE
WKGKDMRTAHKDLVPHLSDVHFQAVARHLSDTLTELGVPPEDITDAMAVVASTRTEVLN
MPQQ

SEQ ID NO:10 *Tetrahymena pyriformis*

MNKPQTIYEKLGGENAMKAAVPLFYKKVLADERVKHFFKNTDMDHQTKQQTDFLTMLLG
GPNHYKGKNMTEAHKGMNLQNLHFDAIIENLAATLKELGVTDAVINEAAKVIEHTRKDM
LGK

SEQ ID NO:11 *Paramecium caudatum*

MSLFEQLGGQAAVQAVTAQFYANIQADATVATFFNGIDMPNQTNKTAAFLCAALGGPNA
WTGRNLKEVHANMGVSNAQFTTVIGHLRSALTGAGVAAALVEQTVAVAETVRGDVVTV

SEQ ID NO:12 *Aspergillus niger*

MPLTPEQIKIIKATVPVLQEYGTKITTAFYMNMSTVHPELNAVFNTANQVKGHQARALA
GALFAYASHIDDLGALGPAVELICNKHASLYIQADEYKIVGKYLLEAMKEVLGDACTDD
ILDAWGAAYWALADIMINREAALYKQSQG

SEQ ID NO:13 *Zea mays*

MALAEADDGAVVFGEEQEALVLKSWAVMKKDAANLGLRFFLKVFEIAPSAEQMFSFLRD
SDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRETTLKRLGATHLRYGVADGHF
EVTGFALLETIKEALPADMWSLEMKKAWAEAYSQLVAAIKREMKPDA

SEQ ID NO:14 *Oryza sativa subsp. japonica*

MALVEGNNGVSGGAVSFSEEQEALVLKSWAIMKKDSANIGLRFFLKIFEVAPSASQMFS
FLRNSDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRDTTLKRLGATHFKYGVG
DAHFEVTRFALLETIKEAVPVDMWSPAMKSAWSEAYNQLVAAIKQEMKPAE

SEQ ID NO:15 *Arabidopsis thaliana*

MESEGKIVFTEEQEALVVKSWSVMKKNSAELGLKLFIKIFEIAPTTKKMFSFLRDSPIP
AEQNPKLKPHAMSVFVMCCESAVQLRKTGKVTVRETTLKRLGASHSKYGVVDEHFEVAK
YALLETIKEAVPEMWSPEMKVAWGQAYDHLVAAIKAEMNLSN

SEQ ID NO:16 *Pisum sativum*

MGFTDKQEALVNSSWESFKQNLSGNSILFYTIILEKAPAAKGLFSFLKDTAGVEDSPKL
QAHAEQVFGLVRDSAAQLRTKGEVVLGNATLGAIHVQRGVTDPHFVVVKEALLQTIKKA
SGNNWSEELNTAWEVAYDGLATAIKKAMT

SEQ ID NO:17 *Vigna unguiculata*

MVAFSDKQEALVNGAYEAFKANIPKYSVVFYTTILEKAPAAKNLFSFLANGVDATNPKL
TGHAEKLFGLVRDSAAQLRASGGVVADAALGAVHSQKAVNDAQFVVVKEALVKTLKEAV
GDKWSDELGTAVELAYDELAAAIKKAY

SEQ ID NO:18 Bos taurus

MGLSDGEWQLVLNAWGKVEADVAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGNTVLTALGGILKKKGHHEAEVKHLAESHANKHKIPVKYLEFISDAIIHVLHA
KHPSDFGADAQAAMSKALELFRNDMAAQYKVLGFHG

SEQ ID NO:19 *Sus scrofa*
MGLSDGEWQLVLNVWGKVEADVAGHGQEVLIRLFKGHPETLEKFDKFKHLKSEDEMKAS
EDLKKHGNTVLTALGGILKKKGHHEAELTPLAQSHATKHKIPVKYLEFISEAIIQVLQS
KH PGDFGADAQGAMSKALELFRNDMAAKYKELGFQG

SEQ ID NO: 20 *Equus caballus*

MGLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHVLHS
KH PGDFGADAQGAMTKALELFRNDIAAKYKELGFQG

SEQ ID NO: 21 Nicotiana benthamiana

MSSFTEEQEALVVKSWDSMKKNAGEWGLKLFLKIFEIAPSAKKLFSFLKDSNVPL
EQNAKLKPHSKSVFVMTCEAAVQLRKAGKVVVRDSTLKKLGATHFKYGVADE
HFEVTKFALLETIKEAVPEMWSVDMKNAWGEAFDQLVNAIKTEMK

SEQ ID NO: 22 Bacillus subtilis

MGQSFNAPYEAIGEELLSQLVDTFYERVASHPLLKPIFPSDLTETARKQKQFLTQY
LGGPPLYTEEHGHPMLRARHLPFPITNERADAWLSCMKDAMDHVGLEGEIREFL
FGRLELTARHMVNQTEAEDRSS

SEQ ID NO: 23 Corynebacterium glutamicum

MTTSENFYDSVGGEETFSLIVHRFYEQVPNDDILGPMYPPDDFEGAEQRLKMFLS
QYWGGPKDYQEQRGHPRLRMRHVNYPIGVTAAERWLQLMSNALDGVDLTAEQ
REAIWEHMVRAADMLINSNPDPHA

SEQ ID NO: 24 Synechocystis PCC6803

MSTLYEKLGGTTAVDLAVDKFYERVLQDDRIKHFFADVDMAKQRAHQKAFLTY
AFGGTDKYDGRYMREAHKELVENHGLNGEHFDAVAEDLLATLKEMGVPEDLIA
EVAAVAGAPAHKRDVLNQ

SEQ ID NO: 25 Synechococcus sp. PCC 7335

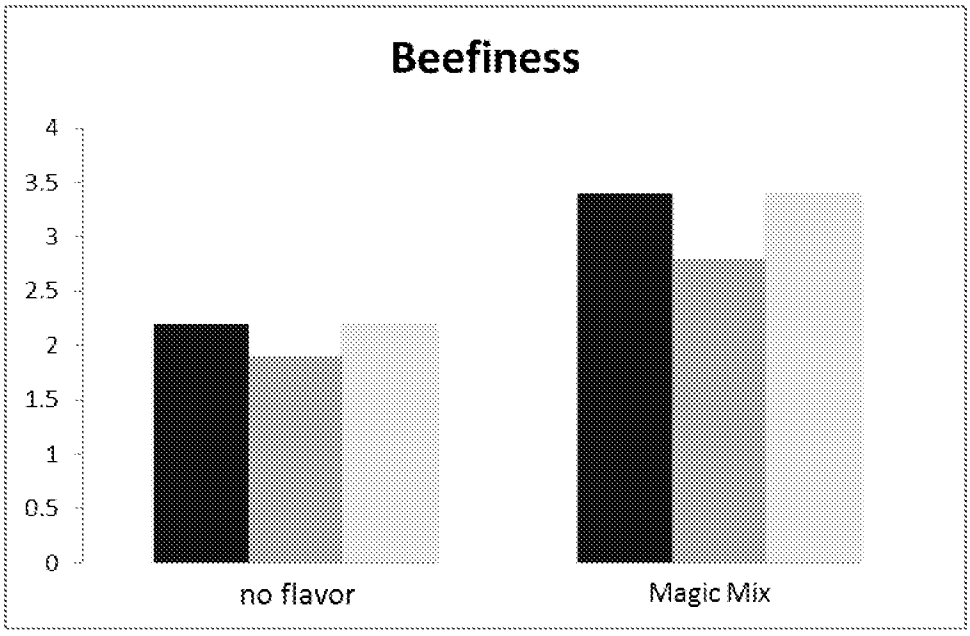
MDVALLEKSFEQISPRAIEFSASFYQNLFHHHPELKPLFAETSQTIQEKKLIFSLAAI
IENLRNPDILQPALKSLGARHAEVGTIKSHYPLVGQALIETFAEYLAADWTEQLA
TAWVEAYDVIASTMIEGADNPAAYLEPELTFYEWLDLYGEESPKVRNAIATLTH
FHYGEDPQDVQRDSRG

SEQ ID NO: 26 Nostoc commune

MSTLYDNIGGQPAIEQVVDELHKRIATDSLLAPVFAGTDMVKQRNHLVAFLAQIF
EGPKQYGGRPMDKTHAGLNLQQPHFDAIAKHLGERMAVRGVSAENTKAALDR
VTNMKGAILNK

SEQ ID NO: 27 Bacillus megaterium
MREKIHSPYELLGGEHTISKLVDAFYTRVGQHPELAPIFPDNLTETARKQKQFLTQ
YLGGPSLYTEEHGHPMLRARHLPFEITPSRAKAWLTCMHEAMDEINLEGPERDE
LYHRLILTAQHMINSPEQTDEKGFSH

FIGURE 2



US 20140220217A1

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(43) **Pub. Date: Aug. 7, 2014**

(54) **METHOD AND COMPOSITIONS FOR CONSUMABLES**

(71) Applicant: **MARAXI, INC.**, Menlo Park, CA (US)

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(73) Assignee: **MARAXI, INC.**, Menlo Park, CA (US)

(21) Appl. No.: **13/941,211**

(22) Filed: **Jul. 12, 2013**

Related U.S. Application Data

(63) Continuation-in-part of application No. PCT/US2012/046560, filed on Jul. 12, 2012.

(60) Provisional application No. 61/671,069, filed on Jul. 12, 2012, provisional application No. 61/572,205, filed on Jul. 12, 2011.

Publication Classification

(51) **Int. Cl.**

A23J 1/00	(2006.01)
A23J 1/14	(2006.01)
A23J 1/12	(2006.01)
A23J 1/06	(2006.01)

(52) **U.S. Cl.**

CPC ***A23J 1/00*** (2013.01); ***A23J 1/06*** (2013.01); ***A23J 1/14*** (2013.01); ***A23J 1/12*** (2013.01)

USPC **426/574**

(57) **ABSTRACT**

Disclosed herein are improved methods and compositions which more accurately replicate the characteristics that consumers value in the preparation and consumption of meat and which overcome the shortcomings and drawbacks of current meat substitutes.

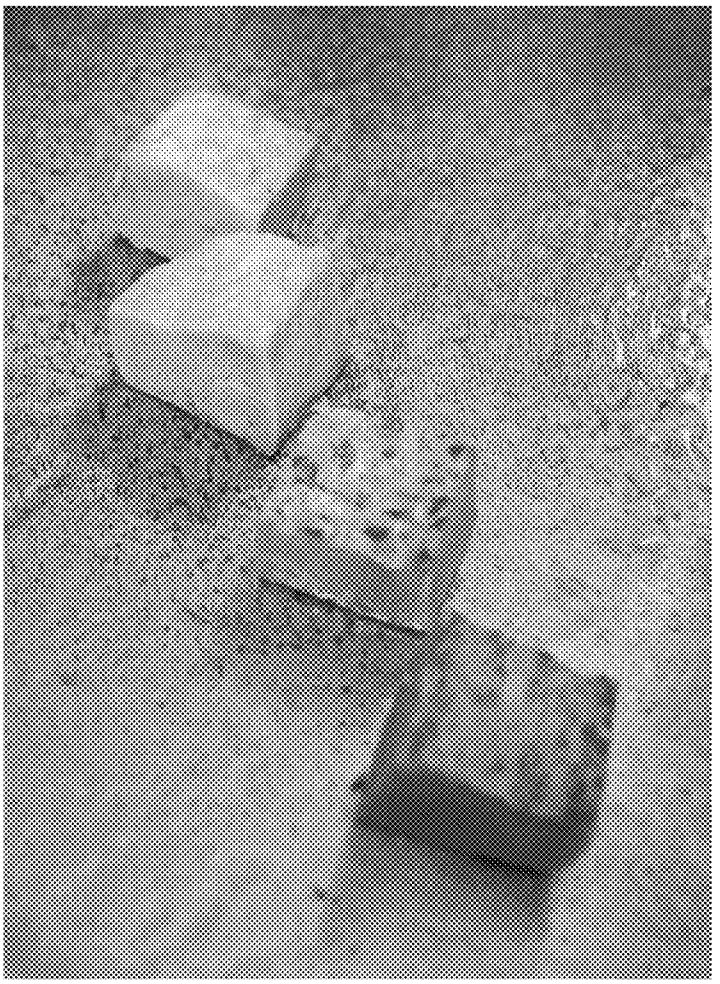


EXHIBIT 1B

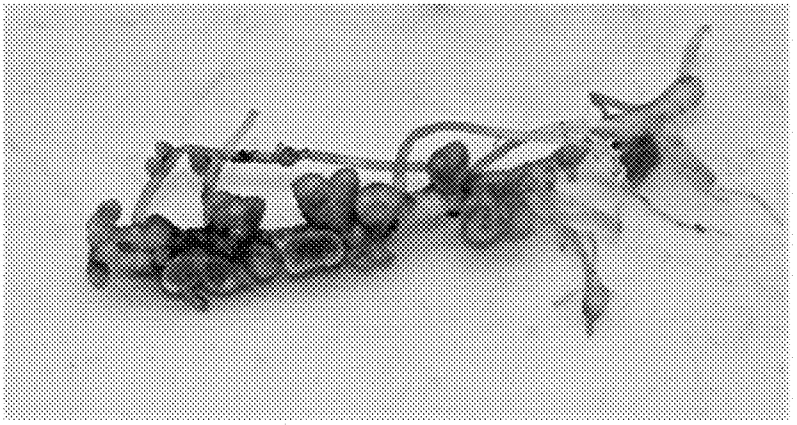


Figure 1

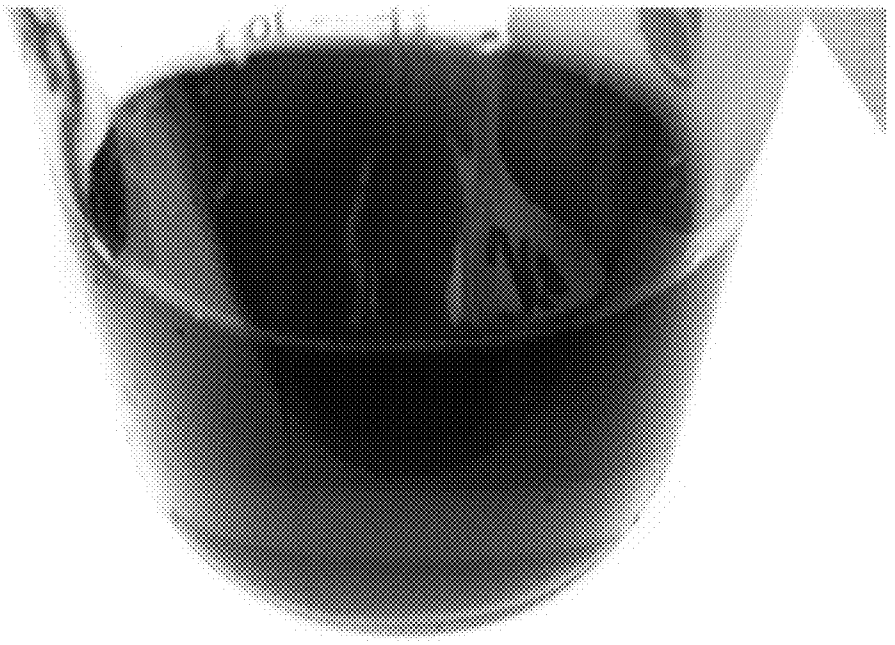


Figure 2

EXHIBIT 1B

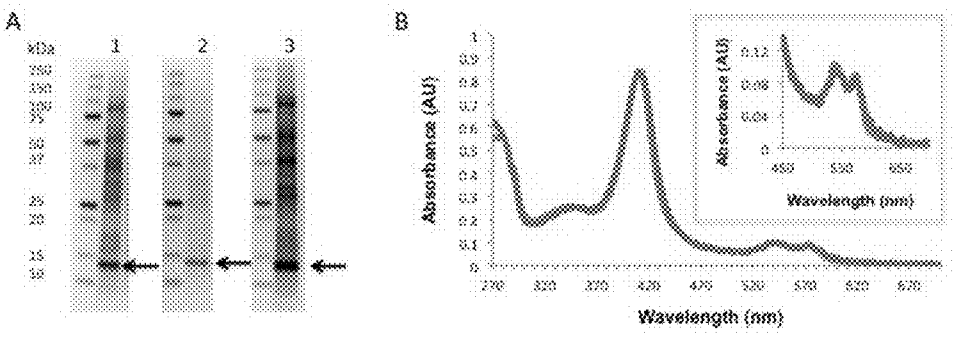


Figure 3

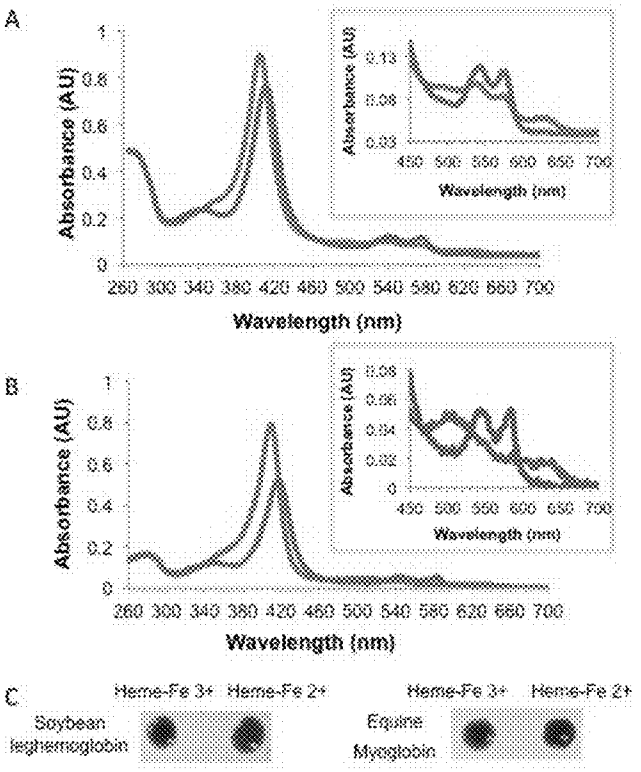


Figure 4

EXHIBIT 1B

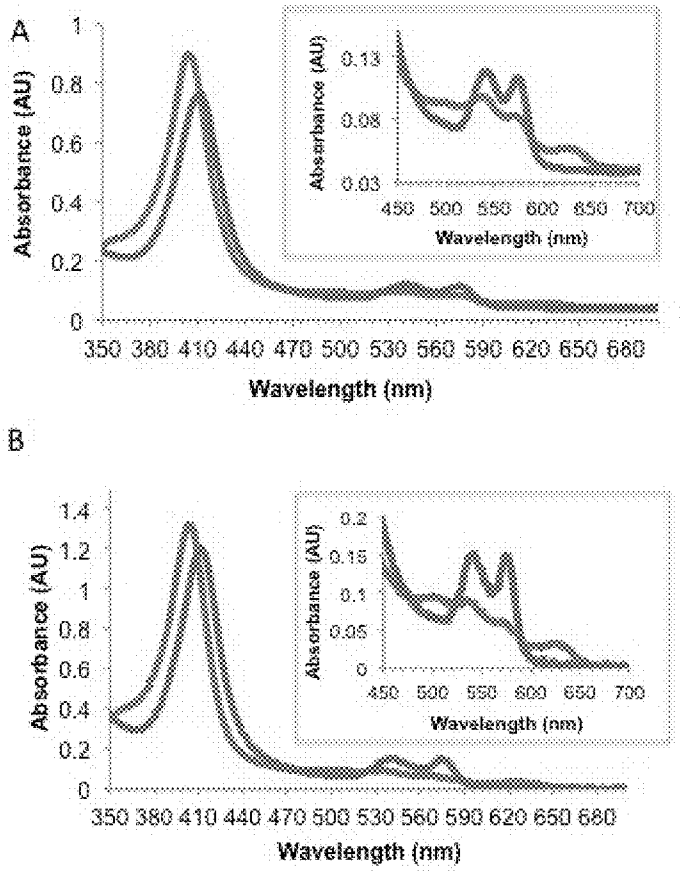


Figure 5

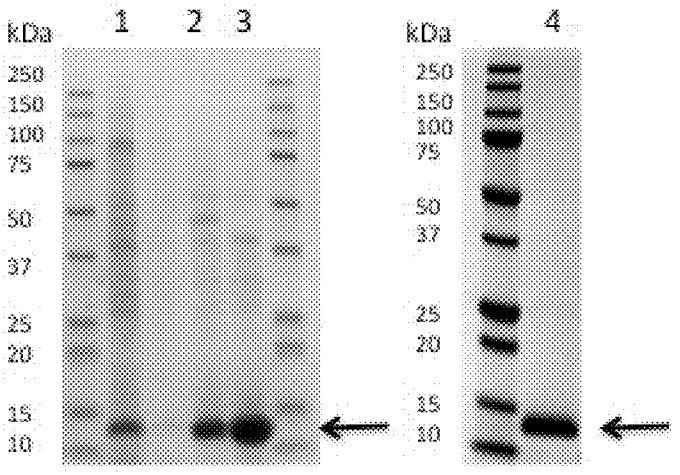


Figure 6

EXHIBIT 1B

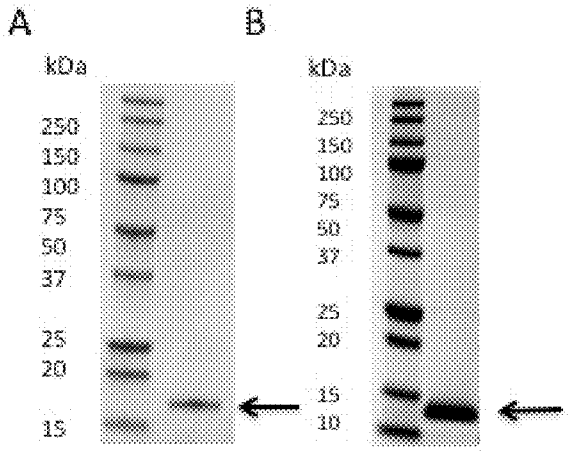


Figure 7

EXHIBIT 1B



Figure 8

EXHIBIT 1B



Figure 9

EXHIBIT 1B



Figure 10



Figure 11

EXHIBIT 1B

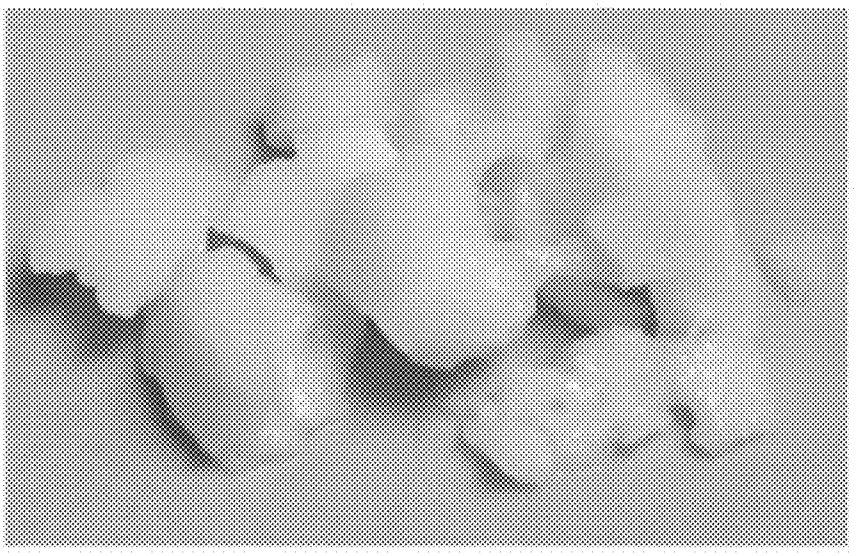


Figure 12

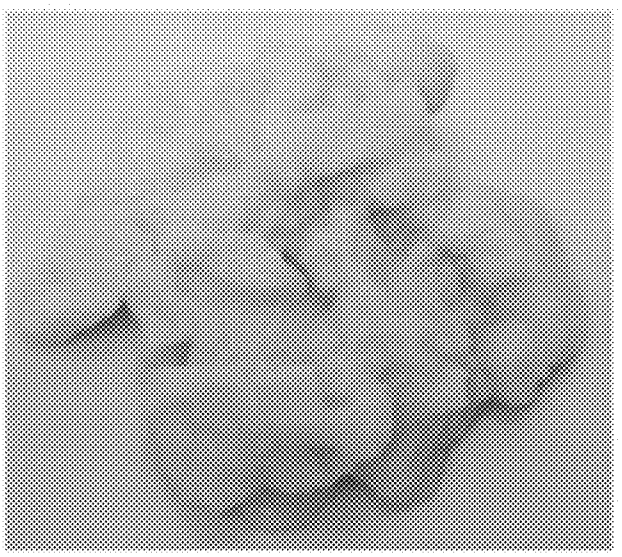


Figure 13

EXHIBIT 1B



Figure 14

EXHIBIT 1B

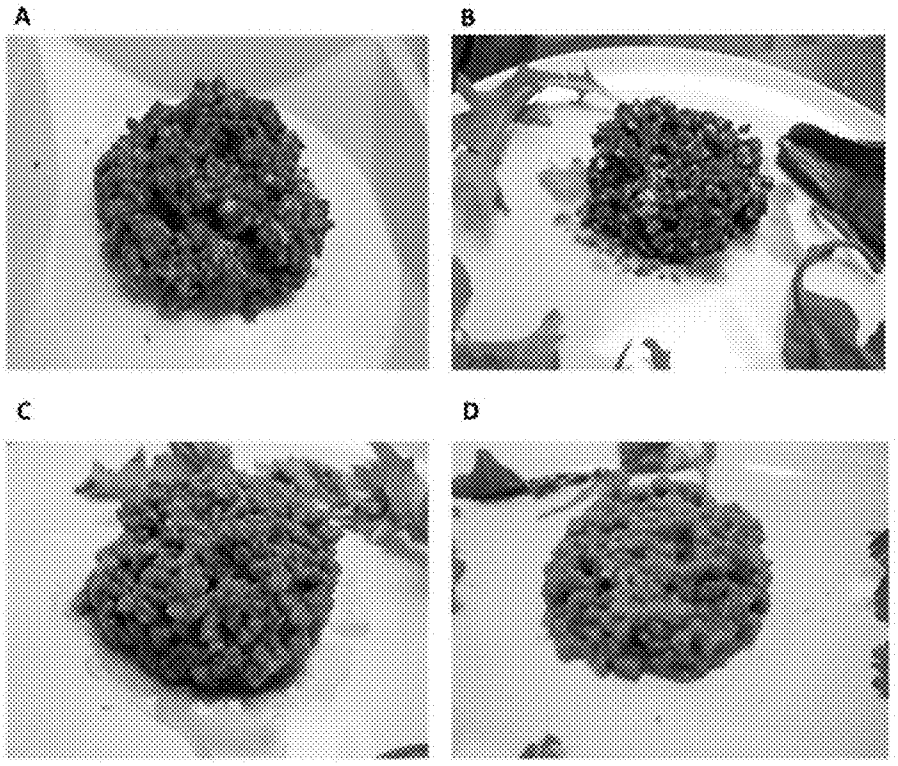


Figure 15

EXHIBIT 1B

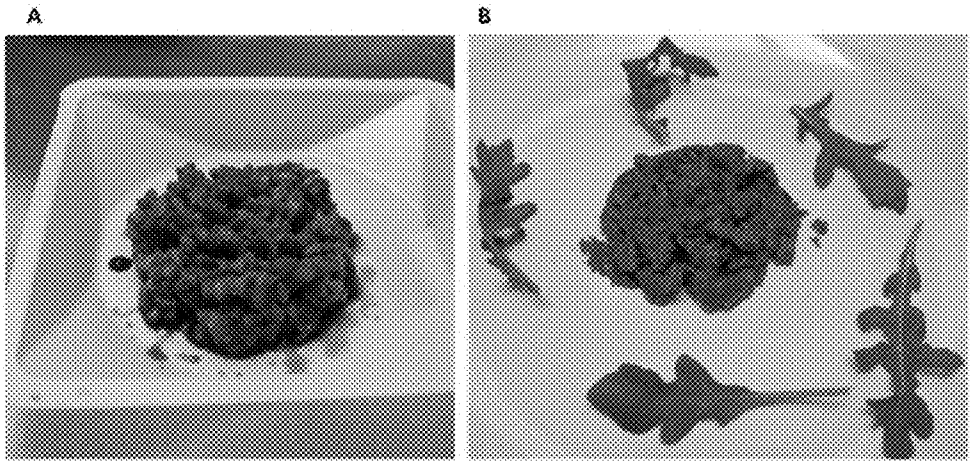


Figure 16

EXHIBIT 1B

METHOD AND COMPOSITIONS FOR CONSUMABLES

CROSS REFERENCE

[0001] This application is a continuation-in-part of International Application No. PCT/US2012/046560, filed Jul. 12, 2012, which claims the benefit of priority of U.S. Provisional Application 61/572,205, filed Jul. 12, 2011 and is a non-provisional of and claims the benefit of U.S. Provisional Application 61/671,069, filed Jul. 12, 2012, all of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] Animal farming has a profound negative environmental impact. Currently it is estimated that 30% of Earth's land surface is dedicated to animal farming and that livestock account for 20% of total terrestrial animal biomass. Due to this massive scale animal farming accounts for more than 18% of net greenhouse gas emissions. Animal farming may be the largest human source of water pollution, and animal farming is by far the world's largest threat to biodiversity. It has been estimated that if the worlds human population could shift from a meat containing diet to a diet free of animal products, 26% of Earth's land surface would be freed for other uses. Furthermore the shift to a vegetarian diet would massively reduce water and energy consumption.

[0003] The consumption of meat has a profound negative impact on human health. The heath benefits of a vegetarian diet are well established. If the human population would shift to a vegetarian diet the cost savings in health care would be significant.

[0004] Hunger is a worldwide problem, yet the world's 4 major commodity crops (soybeans, maize, wheat, and rice) already supply more than 100% of the human population's requirements for calories and protein, including every essential amino acid.

[0005] Plant based meat substitutes have largely failed to cause a shift to a vegetarian diet. The current state of the art for meat substitute compositions involves the extrusion of soy/grain mixture, resulting in products which largely fail to replicate the experience of cooking and eating meat. Common limitations of these products are a texture and mouthfeel that are more homogenous than that of equivalent meat products. Furthermore, as the products must largely be sold precooked, with artificial flavors and aromas built in, they fail to replicate aromas, flavors, and other key features associated with cooking meat. As a result, these products appeal mainly to a limited consumer base that is already committed to vegetarianism/veganism, but have failed to appeal to the larger consumer segment accustomed to eating meat.

[0006] Disclosed herein are improved methods and compositions which more accurately replicate the characteristics that consumers value in the preparation and consumption of meat and which overcome the shortcomings and drawbacks of current meat substitutes.

SUMMARY OF THE INVENTION

[0007] In some aspects, the invention provides a meat substitute composition comprising a protein content, wherein one or more isolated and purified proteins accounts for 10% or more of said protein content by weight, wherein said meat substitute composition accurately mimics the taste, texture, or color of a meat product derived from animal sources.

[0008] In one embodiment, the meat substitute composition accurately mimics the color of said meat product in its raw state and in a cooked state after cooking.

[0009] In another embodiment, the one or more isolated and purified proteins accounts for 25% or more of said protein content by weight.

[0010] In another embodiment, the one or more isolated and purified proteins accounts for 50% or more of said protein content by weight.

[0011] In another embodiment, the one or more isolated and purified proteins accounts for 75% or more of said protein content by weight.

[0012] In another embodiment, the one or more isolated and purified proteins accounts for 90% or more of said protein content by weight.

[0013] In another embodiment, gluten does not account for 10% or more of said protein content by weight.

[0014] In another embodiment, each of said isolated, purified proteins is separately isolated and purified.

[0015] In another embodiment, the meat substitute composition comprises 1-7 isolated and purified proteins.

[0016] In another embodiment, said 1-7 isolated and purified proteins are each isolated from different plant species.

[0017] In some embodiments the meat substitute comprises less than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 individual proteins isolated from one or more plant species.

[0018] In another embodiment, said protein content comprises no more than trace amounts of any other proteins derived from the one or more plant species.

[0019] In another embodiment, said one or more isolated and purified proteins are selected from the group consisting of leghemoglobin, non-symbiotic hemoglobin, hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

[0020] In another embodiment, said one or more isolated and purified proteins are not isolated from an animal.

[0021] In another embodiment, said one or more isolated and purified proteins are isolated from a single plant source.

[0022] In another embodiment, said one or more isolated and purified proteins are isolated from multiple plant sources.

[0023] In another embodiment, wherein said one or more isolated, purified proteins are isolated from a genetically modified organism.

[0024] In some embodiments, said genetically modified organism is a genetically modified bacteria or yeast organism.

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[0025] In some embodiments, said isolated, purified protein has been formed into fibers.

[0026] In particular embodiments, said fibers resemble skeletal muscle fibers.

[0027] In yet more particular embodiments, said fibers are asymmetric fibers.

[0028] In some embodiments, the meat substitute composition further comprises one or more isolated and purified iron-containing proteins.

[0029] In some embodiments, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

[0030] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSV-VFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAE KLFALVRDSAGQLKASGTVVADAALGS-VHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDE LSRAWEVAYDELAAAIKKA.

[0031] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 2. SEQ ID NO 2: MIDQKEKELI KESWKRIEPN KNEIGLLFYA NLFKEEPTVS VLFQN-PISSQ SRKLMQVLGI LVQGIDNLEG LIPTLQDLGR RHKQYGVVDS HYPLVGDCLL KSIQEYLGQG FTEEA-KAAWT KVYGIAAQVM TAE. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 80% homology to SEQ ID NO 2. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 90% homology to SEQ ID NO 2. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 98% homology to SEQ ID NO 2.

[0032] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 3. SEQ ID NO 3: MRKQPTVFEK LGGQAAMHAA VPLFYKKVLA DDRVKHYFKN TNMEHQAKQQ EDFLTMLLGG PNHYKGKNMA EAH-KGMNLQN SHFDAIIENL AATLKELGVS DQI-IGEAAKV IEHTRKDCLG K. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 80% homology to SEQ ID NO 3. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 90% homology to SEQ ID NO 3. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 98% homology to SEQ ID NO 3.

[0033] In some embodiments, the isolated and purified proteins are assembled into one or more gels.

[0034] In some embodiments, the meat substitute composition further comprises one or more fats.

[0035] In particular embodiments, said one or more fats are derived from a plant source.

[0036] In another aspect, the invention provides a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources.

[0037] In some embodiments, said indicator is a visual indicator that accurately mimics the color transition of a meat product during said cooking progression.

[0038] In one embodiment, said color transition is from red to brown.

[0039] In one embodiment, said color transition is from pink to white or tan.

[0040] In one embodiment, said visual indicator transitions from a translucent to opaque color during said cooking progression.

[0041] In some embodiments, the indicator is an olfactory indicator that indicates cooking progression.

[0042] In one embodiment, said olfactory indicator is one or more volatile odorants released during cooking.

[0043] In some embodiments, said indicator comprises one or more isolated, purified iron-containing proteins.

[0044] In particular embodiments, said one or more isolated, purified iron-containing proteins is in a reduced state before cooking.

[0045] In one embodiment, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

[0046] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSV-VFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAE KLFALVRDSAGQLKASGTVVADAALGS-VHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDE LSRAWEVAYDELAAAIKKA.

[0047] In some embodiments, said one or more isolated and purified iron-containing proteins are not isolated from an animal. In some embodiments compositions of the invention do not contain any proteins from an animal.

[0048] In particular embodiments, said one or more isolated and purified iron-containing proteins are isolated from one or more plant sources.

[0049] In yet more particular embodiments, said one or more isolated, purified proteins are isolated from the root nodules, roots, seeds, leaves, or stems of said one or more plant sources.

[0050] In other particular embodiments, said one or more plant sources are soy or pea plants.

[0051] In one embodiment, said one or more plant sources comprises one or more plants of the legume family.

[0052] In some embodiments, said one or more isolated and purified iron carrying proteins in a reduced or oxidized state has a similar UV-VIS profile to a myoglobin protein derived from an animal source when in an equivalent reduced or oxidized state.

[0053] In a particular embodiment, the difference between the peak absorbance wavelength of said one or more isolated and purified iron-containing proteins and the peak absorbance wavelength of myoglobin derived from an animal source is less than 5%.

[0054] In some embodiments, said one or more isolated, purified proteins are isolated from a genetically modified organism.

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[0055] In one embodiment, said genetically modified organism is a genetically modified bacteria or yeast organism.

[0056] In some embodiments, the meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[0057] In particular embodiments, the meat substitute product additionally contains less than 1% wheat gluten.

[0058] In a more particular embodiment, said meat substitute product contains no wheat gluten.

[0059] In other particular embodiments, said meat substitute product contains no soy protein isolate.

[0060] In other particular embodiments, said meat substitute product contains no soy protein concentrate.

[0061] In other particular embodiments, said meat substitute product contains no soy protein.

[0062] In a more particular embodiment, said meat substitute product contains less than 5% carbohydrates.

[0063] In other particular embodiments, said meat substitute product contains no tofu.

[0064] In some embodiments, said meat substitute product contains no tofu, and no wheat gluten.

[0065] In some embodiments, said meat substitute product contains no soy protein, and no wheat gluten.

[0066] In some embodiments, said meat substitute product contains no animal products and less than 5% carbohydrates.

[0067] In some embodiments, said meat substitute product contains less than 1% cellulose.

[0068] In some embodiments, said meat substitute product contains less than 5% insoluble carbohydrates.

[0069] In some embodiments, said meat substitute product contains no soy protein, and less than 1% cellulose.

[0070] In some embodiments, said meat substitute product contains no soy protein, and less than 5% insoluble carbohydrates.

[0071] In some embodiments, said meat substitute product contains no wheat gluten, and less than 1% cellulose.

[0072] In some embodiments, said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.

[0073] In another aspect, the invention provides a muscle tissue replica comprising a protein content, wherein said protein content comprises one or more isolated and purified proteins, wherein said muscle tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source.

[0074] In some embodiments, said one or more isolated and purified proteins accounts for at least 50% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 40% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 30% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 20% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 10% of said protein content by weight.

[0075] In some embodiments, said one or more isolated and purified proteins accounts for at least 50% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 40% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 30% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 20% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 10% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 5% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 1% of said composition content by weight.

[0076] In some embodiments, said protein content is derived from one or more non-animal sources.

[0077] In particular embodiments, said one or more non-animal sources is a plant source.

[0078] In other particular embodiments, said one or more non-animal sources is a genetically modified yeast or bacteria.

[0079] In some embodiments, each of said one or more isolated proteins is isolated and purified separately.

[0080] In some embodiments, said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

[0081] In one embodiment, said seed storage protein is moong bean 8S protein.

[0082] In some embodiments, said protein content is suspended in a gel.

[0083] In some embodiments, said protein content is in the form of a gel.

[0084] In one embodiment, said gel comprises an isolated, purified cross-linking enzyme.

[0085] In some embodiments, said isolated, purified cross-linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.

[0086] In a particular embodiment, said isolated, purified cross-linking enzyme is transglutaminase.

[0087] In some embodiments, said protein content has been assembled into fibers.

[0088] In particular embodiments, said fibers are arranged isotropically.

[0089] In one embodiment, said fibers are asymmetric fibers.

[0090] In some embodiments, the muscle tissue replica further comprises one or more isolated and purified iron-containing proteins.

[0091] In some embodiments, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-

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symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

[0092] In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAE KLFALVRDSAGQLKASGTVVADAALGSVHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDE LSRAWEVAYDELAAAIKKA. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 80% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 90% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 95% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 98% homology to SEQ ID NO 1.

[0093] In particular embodiments, the muscle tissue replica comprises a protein content, wherein (i) one isolated and purified protein that is not an iron-containing protein accounts for 40-95% of said protein content, (ii) one or more isolated and purified iron-containing proteins accounts for 1-20% of said protein content, and (iii) one or more cross-linking reagents accounts for 0.1-35% of said protein content.

[0094] In one embodiment, said protein content accounts for 5-50% of said replica by weight or by weight/volume.

[0095] In one embodiment, said one isolated and purified protein is moong bean 8S protein.

[0096] In one embodiment, said one or more isolated and purified iron-containing proteins is leghemoglobin.

[0097] In one embodiment, said one or more cross-linking reagents is transglutaminase.

[0098] In some embodiments, the muscle tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[0099] In particular embodiments, the muscle tissue replica additionally contains less than 1% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 5% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 10% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 0.1% wheat gluten.

[0100] In a more particular embodiment, said muscle tissue replica contains no wheat gluten.

[0101] In other particular embodiments, said muscle tissue replica contains no soy protein isolate.

[0102] In other particular embodiments, said muscle tissue replica contains no soy protein concentrate.

[0103] In other particular embodiments, said muscle tissue replica contains no soy protein.

[0104] In a more particular embodiment, said muscle tissue replica contains less than 5% carbohydrates.

[0105] In other particular embodiments, said muscle tissue replica contains no tofu.

[0106] In some embodiments, said muscle tissue replica contains no tofu, and no wheat gluten.

[0107] In some embodiments, said muscle tissue replica contains no soy protein, and no wheat gluten.

[0108] In some embodiments, said muscle tissue replica contains no animal products and less than 5% carbohydrates.

[0109] In some embodiments, said muscle tissue replica contains less than 1% cellulose.

[0110] In some embodiments, said muscle tissue replica contains less than 5% insoluble carbohydrates.

[0111] In some embodiments, said muscle tissue replica contains no soy protein, and less than 1% cellulose.

[0112] In some embodiments, said muscle tissue replica contains no soy protein, and less than 5% insoluble carbohydrates.

[0113] In some embodiments, said muscle tissue replica contains no wheat gluten, and less than 1% cellulose.

[0114] In some embodiments, said muscle tissue replica contains no wheat gluten, and less than 5% insoluble carbohydrates.

[0115] In some embodiments, the muscle tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[0116] In particular embodiments, the meat replica contains less than 1% wheat gluten. In particular embodiments, the meat replica contains less than 5% wheat gluten. In particular embodiments, the meat replica contains less than 10% wheat gluten. In particular embodiments, the meat replica contains less than 0.1% wheat gluten.

[0117] In a more particular embodiment, the meat replica contains no wheat gluten.

[0118] In other particular embodiments, the meat replica contains no soy protein isolate.

[0119] In other particular embodiments, the meat replica contains no soy protein concentrate.

[0120] In other particular embodiments, the meat replica contains no soy protein.

[0121] In a more particular embodiment, the meat replica contains less than 5% carbohydrates.

[0122] In other particular embodiments, the meat replica contains no tofu.

[0123] In some embodiments, the meat replica contains no tofu, and no wheat gluten.

[0124] In some embodiments, the meat replica contains no soy protein, and no wheat gluten.

[0125] In some embodiments, the meat replica contains no animal products and less than 5% carbohydrates.

[0126] In some embodiments, the meat replica contains less than 1% cellulose. In some embodiments, the meat replica contains less than 0.1% cellulose. In some embodiments, the meat replica contains less than 10% cellulose. In some embodiments, the meat replica contains less than 5% cellulose.

[0127] In some embodiments, the meat replica contains less than 5% insoluble carbohydrates.

[0128] In some embodiments, the meat replica contains no soy protein, and less than 1% cellulose.

[0129] In some embodiments, the meat replica contains no soy protein, and less than 5% insoluble carbohydrates.

[0130] In some embodiments, the meat replica contains no wheat gluten, and less than 1% cellulose.

[0131] In some embodiments, the meat replica contains no wheat gluten, and less than 5% insoluble carbohydrates.

[0132] In another aspect, the invention provides a fat tissue replica comprising a gelled emulsion, said gelled emulsion comprising a protein solution with fat droplets suspended therein.

[0133] In some embodiments, said fat droplets are derived from a non-animal source.

[0134] In some embodiments, said fat droplets are comprised of one or more plant oils.

[0135] In some embodiments, said one or more plant oils is selected from the group consisting of corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phosphatidic acids, lysophosphatidic acids, oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid.

[0136] In one embodiment, said one or more plant oils is rice bran oil or canola oil.

[0137] In some embodiments, said protein solution comprises one or more isolated, purified proteins.

[0138] In some embodiments, said one or more isolated, purified proteins accounts for 75% or more of the protein in said protein solution.

[0139] In some embodiments, said one or more isolated, purified proteins are derived from a non-animal source.

[0140] In some embodiments, said non-animal source is a plant source.

[0141] In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

[0142] In some embodiments, each of said one or more isolated proteins is isolated and purified separately.

[0143] In some embodiments, said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

[0144] In some embodiments, said one or more isolated, purified proteins is an albumin protein, a seed storage protein, or pea globulin protein.

[0145] In particular embodiments, said albumin protein is isolated pea albumin protein.

[0146] In some embodiments, said seed storage protein is moong bean 8S protein.

[0147] In some embodiments, said gelled emulsion comprises a protein solution comprising 1-3 isolated and purified proteins, wherein said solution accounts for 30-70% of the volume of said emulsion; a plant oil, wherein said plant oil accounts for 30-70% of the volume of said emulsion; and an isolated, purified cross-linking enzyme, wherein said cross-linking enzyme accounts for 0.5-5% of said emulsion by wt/volume; wherein said plant oil is emulsified in said protein solution, wherein said emulsion is formed into a gel by said cross-linking enzyme.

[0148] In other embodiments said gelled emulsion comprises a protein solution comprising 1-3 isolated and purified proteins, wherein said solution accounts for 1-30% of the volume of said emulsion; a plant oil, wherein said plant oil accounts for 70-99% of the volume of said emulsion; and an isolated, purified cross-linking enzyme, wherein said cross-linking enzyme accounts for 0.5-5% of said emulsion by wt/volume; wherein said plant oil is emulsified in said protein solution, wherein said emulsion is formed into a gel by said cross-linking enzyme.

[0149] In some embodiments, the fat replica further comprises a cross-linking enzyme.

[0150] In some embodiments, said cross-linking enzyme is transglutaminase.

[0151] In some embodiments, one of said 1-3 isolated and purified proteins is moong bean 8S protein, pea albumin protein, or pea globulin protein.

[0152] In particular embodiments, said plant oil is rice bran oil or canola oil.

[0153] In some embodiments, the fat tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[0154] In particular embodiments, the fat tissue replica additionally contains less than 1% wheat gluten.

[0155] In a more particular embodiment, said fat tissue replica contains no wheat gluten.

[0156] In other particular embodiments, said fat tissue replica contains no soy protein isolate.

[0157] In other particular embodiments, said fat tissue replica contains no soy protein concentrate.

[0158] In other particular embodiments, said fat tissue replica contains no soy protein.

[0159] In a more particular embodiment, said fat tissue replica contains less than 5% carbohydrates.

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[0160] In other particular embodiments, said fat tissue replica contains no tofu.

[0161] In some embodiments, said fat tissue replica contains no tofu, and no wheat gluten.

[0162] In some embodiments, said fat tissue replica contains no soy protein, and no wheat gluten.

[0163] In some embodiments, said fat tissue replica contains no animal products and less than 5% carbohydrates.

[0164] In some embodiments, said fat tissue replica contains less than 1% cellulose.

[0165] In some embodiments, said fat tissue replica contains less than 5% insoluble carbohydrates.

[0166] In some embodiments, said fat tissue replica contains no soy protein, and less than 1% cellulose.

[0167] In some embodiments, said fat tissue replica contains no soy protein, and less than 5% insoluble carbohydrates.

[0168] In some embodiments, said fat tissue replica contains no wheat gluten, and less than 1% cellulose.

[0169] In some embodiments, said fat tissue replica contains no wheat gluten, and less than 5% insoluble carbohydrates.

[0170] In another aspect, the invention provides a connective tissue replica, comprising a protein content comprising one or more isolated, purified proteins, wherein said protein content has been assembled into structures approximating the texture and visual appearance of connective tissue or skin.

[0171] In some embodiments, said protein content is derived from non-animal source.

[0172] In some embodiments, said non-animal source is a plant source.

[0173] In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

[0174] In some embodiments, said one or more isolated proteins account for 50% or more of said protein content by weight.

[0175] In some embodiments, said one isolated and purified protein accounts for 90% or more of said protein content by weight.

[0176] In some embodiments, each of said one or more isolated proteins is isolated and purified separately.

[0177] In some embodiments, said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

[0178] In some embodiments, said one or more isolated and purified proteins is a prolamin family protein.

[0179] In some embodiments, said one or more isolated and purified proteins is zein.

[0180] In some embodiments, said protein content is suspended in a gel.

[0181] In some embodiments, said gel comprises an isolated, purified cross-linking enzyme.

[0182] In some embodiments, said isolated, purified cross-linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.

[0183] In some embodiments, said isolated, purified cross-linking enzyme is transglutaminase.

[0184] In some embodiments, said protein content is formed into a fiber.

[0185] In some embodiments, said fiber is produced by an extrusion process.

[0186] In some embodiments, said fiber is stabilized by protein crosslinks.

[0187] In some embodiments, fiber contains an isolated, purified cross-linking enzyme.

[0188] In some embodiments, said isolated, purified cross-linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.

[0189] In some embodiments, said isolated, purified cross-linking enzyme is transglutaminase.

[0190] In another aspect, the invention provides a meat substitute product, comprising a muscle replica; a fat tissue replica; and a connective tissue replica; wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat.

[0191] In some embodiments, the meat substitute product comprises two or more of said muscle replica, fat tissue replica, and connective tissue replica.

[0192] In some embodiments of the meat substitute product, said muscle replica accounts for 40-90% of said product by weight, said fat tissue replica accounts for 1-60% of said product by weight, and said connective tissue replica accounts for 1-30% of said product by weight.

[0193] In some embodiments, the meat substitute product comprises 60-90% water; 5-30% protein content; and 1-20% of a fat or fat replica; wherein said protein content comprises one or more isolated, purified plant proteins.

[0194] In some embodiments, said protein content is derived from non-animal source.

[0195] In some embodiments, non-animal source is a plant source.

[0196] In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

[0197] In some embodiments, 50% or more of said protein content by weight are one or more isolated purified proteins.

[0198] In some embodiments, each of said one or more isolated proteins is isolated and purified separately from different plant species.

[0199] In some embodiments, one or more of said isolated proteins is selected from the group consisting of: hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyru-

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vate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

[0200] In some embodiments, the meat substitute product further comprises one or more isolated and purified iron-containing proteins.

[0201] In some embodiments, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins. In some embodiments, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAE KLFALVRDSAGQLKASGTVVADAALGSVHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDE LSRAWEVAYDELAAAIKKA

[0202] In some embodiments, the meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[0203] In particular embodiments, the meat substitute product additionally contains less than 1% wheat gluten.

[0204] In a more particular embodiment, said meat substitute product contains no wheat gluten.

[0205] In other particular embodiments, said meat substitute product contains no soy protein isolate.

[0206] In other particular embodiments, said meat substitute product contains no soy protein concentrate.

[0207] In other particular embodiments, said meat substitute product contains no soy protein.

[0208] In a more particular embodiment, said meat substitute product contains less than 5% carbohydrates.

[0209] In other particular embodiments, said meat substitute product contains no tofu.

[0210] In some embodiments, said meat substitute product contains no tofu, and no wheat gluten.

[0211] In some embodiments, said meat substitute product contains no soy protein, and no wheat gluten.

[0212] In some embodiments, said meat substitute product contains no animal products and less than 5% carbohydrates.

[0213] In some embodiments, said meat substitute product contains less than 1% cellulose.

[0214] In some embodiments, said meat substitute product contains less than 5% insoluble carbohydrates.

[0215] In some embodiments, said meat substitute product contains no soy protein, and less than 1% cellulose.

[0216] In some embodiments, said meat substitute product contains no soy protein, and less than 5% insoluble carbohydrates.

[0217] In some embodiments, said meat substitute product contains no wheat gluten, and less than 1% cellulose.

[0218] In some embodiments, said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.

[0219] In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by an animal.

[0220] In some embodiments, said one or more isolated, purified iron-containing proteins is derived from a non-animal source.

[0221] In some embodiments, said non-animal source is a plant source.

[0222] In some embodiments, said plant source comprises one or more plants of the legume family.

[0223] In some embodiments, said one or more plants of the legume family is a soy or pea plant.

[0224] In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

[0225] In some embodiments, said iron-containing protein is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

[0226] In one embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAE KLFALVRDSAGQLKASGTVVADAALGSVHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDE LSRAWEVAYDELAAAIKKA

[0227] In some embodiments, the food product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[0228] In particular embodiments, the food product additionally contains less than 1% wheat gluten.

[0229] In a more particular embodiment, said food product contains no wheat gluten.

[0230] In other particular embodiments, said food product contains no soy protein isolate.

[0231] In other particular embodiments, said food product contains no soy protein concentrate.

[0232] In other particular embodiments, said food product contains no soy protein.

[0233] In a more particular embodiment, said food product contains less than 5% carbohydrates.

[0234] In other particular embodiments, said food product contains no tofu.

[0235] In some embodiments, said food product contains no tofu, and no wheat gluten.

[0236] In some embodiments, said food product contains no soy protein, and no wheat gluten.

[0237] In some embodiments, said food product contains no animal products and less than 5% carbohydrates.

[0238] In some embodiments, said food product contains less than 1% cellulose.

[0239] In some embodiments, said food product contains less than 5% insoluble carbohydrates.

[0240] In some embodiments, said food product contains no soy protein, and less than 1% cellulose.

[0241] In some embodiments, said food product contains no soy protein, and less than 5% insoluble carbohydrates.

[0242] In some embodiments, said food product contains no wheat gluten, and less than 1% cellulose.

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[0243] In some embodiments, said food product contains no wheat gluten, and less than 5% insoluble carbohydrates.

[0244] In another aspect, the invention provides a method of making a meat substitute composition, comprising isolating and purifying one or more proteins; and assembling said one or more proteins into a physical organization that approximates the physical organization of meat.

[0245] In another aspect, the invention provides a method of making a muscle tissue replica, comprising isolating and purifying one or more proteins; and assembling said one or more proteins into a physical organization that approximates the physical organization of skeletal muscle.

[0246] In another aspect, the invention provides a method of making a fat tissue replica, comprising isolating and purifying one or more proteins; preparing a solution comprising one or more proteins; emulsifying one or more fats in said solution; and stabilizing said solution into a gelled emulsification with one or more cross-linking reagents.

[0247] In another aspect, the invention provides a method of making a connective tissue replica, comprising isolating and purifying one or more proteins; and precipitating said one or more proteins, wherein said precipitating results in said one or more proteins forming physical structures approximating the physical organization of connective tissue.

[0248] In some embodiments, said precipitating comprises solubilizing said one or more proteins in a first solution; and extruding said first solution into a second solution, wherein said one or more proteins is insoluble in said second solution, wherein said extruding induces precipitation of said one or more proteins.

[0249] In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by an animal.

[0250] In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by humans.

[0251] In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by an animal. In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by humans.

[0252] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0253] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0254] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0255] FIG. **1** depicts a portion of the root of a pea plant (*Pisum sativum*) with the root nodules sliced open to demonstrate the red color conferred by leghemoglobin contained therein. The sliced open rood nodule appears red.

[0256] FIG. **2** depicts leghemoglobin isolated from 1 oz of pea roots. The red color commonly attributed to meat is evident in the color photo.

[0257] FIG. **3** shows that leghemoglobins from different species are homologs and have similar color properties. In FIG. **3**, panel A shows an SDS_PAGE gels of lysed root-nodules of three legume plant species (1) Fava bean (2) English Pea (3) Soybean. Arrows mark respective leghemoglobins. Panel B shows the similarity of UV-VIS spectral profile of leghemoglobins from two different plant species (Favabean and Soybean).

[0258] FIG. **4** shows a comparison of reduced (heme iron 2+) and oxidized (heme iron 3+) soybean leghemoglobin (FIG. **4** panel A) and equine heart muscle myoglobin (FIG. **4** panel B) showing similarity of UV-VIS absorption profiles of two proteins. We purified soybean leghemoglobin from soybean root-nodules using here described protocol. Purified equine myoglobin was purchased from SigmaAldrich. Soybean leghemoglobin (FIG. **4** panel A) and equine myoglobin (FIG. **4** panel B) were reduced with 1 mm sodium hydrosulfite. Shown are UV-VIS absorption spectra of heme Fe3+ (blue line—the higher peak in FIGS. **4** and **5**) and heme Fe2+ (red line) of soybean leghemoglobin (FIG. **4** panel A) and equine myoglobin (FIG. **4** panel B). Insets show a zoom-in of UV-VIS spectra in 450 nm to 700 nm region. (FIG. **4** panel C) Images of 10 ul liquid droplet of a 40 mg/ml solution of soybean leghemoglobin in the heme-Fe3+ state (left droplet) showing characteristic rusty red color and a 40 mg/ml solution of soybean leghemoglobin solution in the heme-Fe2+ state (right droplet) showing characteristic red color of and (right image) corresponding samples of equine myoglobin.

[0259] FIG. **5** depicts examples of successful reduction of leghemoglobin heme iron with sodium hydrosulfite and titanium citrate. In FIG. **5** the UV-VIS spectrogram of purified soybean leghemoglobin in which the heme iron is in the oxidized (+3) state is represented by the blue curves in each panel (the blue curves have the higher peaks on the main graphs). The red curves in each panel represent the UV-VIS spectra of the same leghemoglobin species after reduction to the (+2) state (red lines) by addition of (Panel A) 1 mM sodium hydrosulfite or (Panel B) 0.24% (wt/v) titanium citrate in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride buffer. The Insets show a zoom-in of UV-VIS spectra in 450-700 nm region. For this example, leghemoglobin was purified from soybean root nodules using 60/90% ammonium sulfate fractionation and exchanged into 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride buffer. Sodium hydrosulfite stock solution was prepared by dissolving 100 mM sodium hydrosulfite in 1 mM sodium hydroxide in water. Titanium citrate stock solution was prepared from 20% (wt/v) Ti-chloride in hydrochloric acid by mixing it with 0.2M sodium citrate (1:10 v/v). pH was adjusted using sodium carbonate to pH 7.0.

[0260] FIG. **6** depicts an example of the leghemoglobin purification flow from soybean root nodules. The figure shows SDS-PAGE fractionation of different soybean leghemoglobin purification steps (Lane 1) Soybean root-nodule lysate; (Lane 2) Soybean root-nodule lysate purified by 60/90% (wt/v) ammonium sulfate fractionation. Shown is the protein content of 90% ammonium sulfate fractionated pro-

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tein pellet resuspended in 20 mm potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mM EDTA buffer; Proteins from 90% ammonium sulfate pellet were further purified by anion-exchange chromatography (FFQ GE Healthcare) in 20 mM potassium phosphate ph 7.4, 100 mM sodium chloride. Leghemoglobin collected in the flowthrough is shown in Lane 3. Anion-exchange flowthrough was fractionated using size-exclusion chromatography (Sephacryl S-100 GE Healthcare) and resulting leghemoglobin fraction is shown in Lane 4. Leghemoglobin content at different purification steps was determined by determining the fraction of leghemoglobin band on SDS-PAGE gel in a respective sample using ImageDoc analysis software (BioRad). Purity (partial abundance) of leghemoglobin at respective steps in the purification steps was: lysate: 32.7% (lane 1), 60/90% (wt/v) ammonium sulfate fractionation 78% (lane 2), anion-exchange chromatography ~83% (lane 3), and size-exclusion chromatography to ~95% (lane 4).

[0261] FIG. **7** shows stained SDS-PAGE gel analysis of (A) soybean leghemoglobin expressed and purified using recombinant protein technology and (B) soybean leghemoglobin purified from soybean root nodules. (A) Recombinant Soybean leghemoglobin A carrying His-tag and TEV protease His-tag removal site was expressed in *E. coli* BL21 strain and purified using His-tag affinity chromatography (Talon resin, CloneTech). The left lane contains molecular weight standards, the right lane contains purified recombinant soybean leghemoglobin A (arrow). Expected molecular weight of the recombinant soybean leghemoglobin A is 17.1 kDa. (B) SDS-PAGE gel of purified Soybean leghemoglobin from root nodules. The left lane contains molecular weight standards, the right lane contains purified soybean leghemoglobin A (arrow). Mass spectrometry analysis of purified material determined that all four soybean leghemoglobin isoforms are present, and are full length (data not shown). Expected molecular weights (MW) of soybean leghemoglobin isoforms range from MW15.4 to 15.8 kDa.

[0262] FIG. **8** shows an example of 6 cubes of a commercial meat analog (Quorn chicken analog), about 1 cm on a side, 4 of which (Left and lower right) have been soaked in a solution of about 10 mg/ml soybean leghemoglobin in 20 mM Potassium phosphate pH 7.4 and 100 mM NaCl; the remaining two (Upper right) were soaked in the same buffer without leghemoglobin. A deep pink color of the leghemoglobin-infused cubes is apparent in color photos contrasting the pale tan color of the un-infused cubes.

[0263] FIG. **9** shows the 4 leghemoglobin-infused cubes of Quorn chicken analog in the process of cooking in a pan at 350° C. The two lower cubes have been turned over to expose the grilled surface, which has turned brown. In the upper two cubes that the heated portion has turned grey-brown, while the cooler top surface retains its pink color. In some embodiments the consumable is injected with a heme containing solution, for instance a leghemoglobin solution, until the consumable is the color of uncooked meat.

[0264] FIG. **10** depicts 43 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 37 ml of leghemoglobin solution (46.5 mg/ml leghemoglobin and 20 mg/ml of other soybean root nodule protein) in 20 mM potassium phosphate, 100 mM NaCl, pH 7.3). 20 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly mixed, divided into two 50 ml Falcon tubes and incubated overnight at room temperature. Final protein concentrations were 65 mg/ml for moong bean protein, 18 mg/ml of leghemoglobin, 91 mg/ml total protein.

[0265] FIG. **11** depicts "White" muscle analog prepared by mixing 43 ml moong bean protein solution (150 mg/ml) with 45 ml of 11.7 mg/ml solution of leghemoglobin and 0.8% (wt/v) of transglutaminase solution. Final protein concentrations were 63 mg/ml for moong bean protein, 5.2 mg/ml of leghemoglobin, 68 mg/ml total protein.

[0266] FIG. **12** depicts a fat tissue analog based on moong beans and prepared in eppendorf tubes formed an opaque gel of off-white color, smooth uniform texture, with no visible discernible liquid that was not incorporated into the gel. The gel was freely standing, elastic and springy. The gel has a slight, pleasant aroma and a mild and pleasant flavor. The taste is mildly salty.

[0267] FIG. **13** depicts at tissue analog based on pea globulin and prepared in eppendorf tubes very similar to moong bean-based fat analog, except that it gave up a little of oil upon compression.

[0268] FIG. **14** shows connective-tissue analog strands that were created using a 1:3 ratio in 70% ethanol, loaded into a syringe with a 23 gauge needle (ID 0.337 mm). The solution was slowly extruded from the bottom of a 5 inch-high vessel into an excess of 5 M NaCl solution. The ethanol-zein solution being less dense than the NaCl solution, floated upward, drawing out a fibrous stand of solidifying zein. The NaCl was constantly stirred as the strands began to develop to assist in the strand lengthening. The strands bunch together and become a hard, dense mass.

[0269] FIG. **15** depicts a ground beef prototype patty was made by combining 62% (wt/wt) muscle analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "white muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (FIG. **15** panel A). A ground beef prototype patty was made by combining 62% muscle analog (62% "dark muscle analog" and 38% "white muscle analog), 29% fat tissue analog (from moong bean seed 8S protein and rice bran oil), 5% connective tissue analog (FIG. **15** panel B). A ground beef prototype patty was made by combining 71% (wt/wt) muscle tissue analog (composed of 60% "white" muscle analog, 40% "dark" muscle analog), 23% fat tissue (from pea seed globulin proteins and canola oil) (FIG. **15** panel C). A ground beef prototype patty was made by combining 67% "White" muscle analog, with 28% fat tissue analog (from pea globulins and rice bran oil), (FIG. **15**, panel D)

[0270] FIG. **16** depicts a ground beef patty analog was made by combining 62% (wt/wt) muscle tissue analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog. The panel on the left shows the patty before cooking and the panel on the right shows the same patty after cooking for about 2 minutes. Observers described the aroma of the cooking ground beef replica as distinctly "beefy".

DETAILED DESCRIPTION OF THE INVENTION

[0271] Methods and compositions for the production of consumables are described herein. The consumables can be for animal consumption. For example the consumable can be food fit for human consumption. The consumable can be approved by suitable regulatory authorities. The consumables can be sold in grocery stores or prepared in restaurants, schools, hospitals, military facilities, prisons, shelters, long-

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term care facilities, similar to already existing human foods. The consumables could also be food for domestic animals. For instance, dog food could be produced according to the present inventions. The consumables may also be food for wild animals. For instance, the consumables could be provided to non-domesticated predatory animals.

[0272] The consumables of the present invention can compete with, supplement or replace animal based foods. For instance the consumables can be meat replicas made entirely from plant sources. The consumables can be made to mimic the cut or appearance of meat as it is currently sold. For instance a consumable may be visually similar to or indistinguishable from ground beef or a particular cut of beef. Alternatively, the consumables can be made with a unique look or appearance. For instance the consumable could contain patterns or lettering that is based upon the structure of the consumable. In some instances the consumables look like traditional meat products after they are prepared. For example a consumable may be produced which is larger than a traditional cut of beef but which, after the consumable is sliced and cooked appears the same as a traditional cooked meet. In some embodiments the consumable may resemble a traditional meat shape in two dimensions, but not in a third. For example the consumable may resemble a cut of meat in two dimensions (for example when viewed from the top), but may be much longer (or thinker) than the traditional cut. So in some embodiments a composition that can be cut repeatedly into traditionally meat shaped products is provided.

[0273] The consumable may be made entirely from plant based sources. In some instances the consumable can be made from organic sources. The consumables may also be made from a combination of plant based sources and animal based sources. For instance, the consumable may be a ground beef product supplemented with plant based products of the invention.

[0274] The consumables can be made from local products. For instance the consumables can be made from plants grown within a certain radius of the eventual consumer. That radius could be 1, 10, 100, or 1000 miles for example. So, in some embodiments, the invention provides a method for producing a meat replica which does not contain products which have been shipped over 1, 10, 100, or 1000 miles prior to producing the meat replica.

[0275] The present invention provides methods for producing consistent properties from the consumables when they are produced from various sources. So, for example, a plant based meat replica produced from local plants in Iowa, USA, will have substantially similar taste, odor, and texture as a plant based meat replica produced from local plants in Lorraine, France. This consistency allows for methods for advertising locally grown foods with consistent properties. The consistency can arise from the concentration or purification of similar components at different locations. These components can be combined in predetermined ratios to insure consistency. In some embodiments a high degree of characteristic consistency is possible using components (e.g. isolated or concentrated proteins and fats) which come from the same plant species. In some embodiments a high degree of characteristic consistency is possible using components (e.g. isolated or concentrated proteins and fats) which come from the different plant species. In some embodiments the same proteins can be isolated from different plant species. In some embodiments the invention provides for a method comprising isolating similar plant constituents from plant sources in different locations, assembling in both locations compositions provided herein, and selling the compositions, wherein the compositions assembled and sold at different the geographic locations have consistent physical and chemical properties. In some embodiments the isolated constituents are from different plant populations in different locations. In some embodiments one or more of the isolated constituents are shipped to the separate geographic locations.

[0276] The consumables may require fewer resources to produce than consumables produced from domesticated animals. Accordingly, the present invention provides for meat replicates which require less water or energy to produce than meat. For example a consumable can require less than about 10, 50, 100, 200, 300, 500, or 1000 gallons of water per pound of consumable. For comparison beef can require over 2000 gallons of water per pound of meat.

[0277] The consumable may require less land are to produce than a meat product with similar protein content. For example the consumable may require 30% or less of the land area required to produce a meat product with similar protein content.

[0278] The consumable may have health benefits compared to an animal product it replaces in the diet. For example it may have less cholesterol or lower levels of saturated fats than comparable meat products.

[0279] The consumable may have animal welfare benefits compared to an animal product it replaces in the diet. For instance it may be produced without requiring confinement, forced feeding, premature weaning, disruption of maternal-offspring interactions, or slaughter of animals for their meat.

[0280] The consumable may have a smaller "carbon footprint" than the meat products they replace. For example the consumable may result in net greenhouse gas emissions of 1%, 5%, 10%, 25%, 50% or 75% of the greenhouse gas emissions attributable to the animal product it replaces.

[0281] The consumable may provide alternatives to animal products or combinations of animal products whose consumption is forbidden by religious beliefs. For example, the consumable may be a kosher pork chop.

[0282] The consumable can also be shipped in components and produced or assembled at a different location. When available local components can be used for production of the consumable. These can be supplemented with components which are not locally available. This allows for methods of producing consumables, for instance meat replicates, using less energy in shipment than is required for meat. For example, local water can be used in combination with a kit which provides other components of the consumable. Using local water will reduce shipping weight thereby reducing cost and environmental impact.

[0283] The consumables can be produced or assembled wholly or in part in areas where animal farming is not practical or is not allowed. The consumable can be produced or assembled within an urban environment. For example a kit may be provided to a user to enable the user to produce the consumable. The user could use local water or use plants from a rooftop garden, for instance in Shanghai. In another example, the consumables could be produced aboard a space craft, space station, or lunar base. Accordingly, the present invention provides methods and systems for the production of meat replicas for use in space travel or for training for the same. For instance the present invention could be used in earth based training for space travel. The consumables could

also be produced on an island or upon a manmade platform at sea where the keeping of livestock is difficult or prohibited.

[0284] The consumables are, in some embodiments, designed to replicate the experience of eating meat. The look, texture, and taste of the consumable can be such that it is similar or indistinguishable from meat. The invention therefore provides in certain embodiments methods for determining whether an animal or human can distinguish the consumable from meat.

[0285] One method to determine whether the consumable is comparable to meat is to a) define the properties of meat and b) determine whether the consumable has similar properties. Properties of meat that can be tested include mechanical properties such as hardness, cohesiveness, brittleness, chewiness, gumminess, viscosity, elasticity, and adhesiveness. Properties of meat that can be tested also include geometric properties such as particle size and shape, and particle shape and orientation. Additional properties can include moisture content and fat content. These properties can be described using terms such as "soft," "firm" or "hard" describe hardness; "crumbly," "crunchy," "brittle," "chewy," "tender," "tough," "short," "mealy," "pasty," or "gummy," to describe cohesiveness; "thin" or "viscous" to describe viscosity; "plastic" or "elastic" to describe elasticity; "sticky," "tacky" or "gooey" to describe adhesiveness; "gritty," "grainy" or "course" to describe particle shape and size; "fibrous," "cellular" or "crystalline" to describe particle shape and orientation, "dry," "moist," "wet," or "watery" to describe moisture content; or "oily" or "greasy" to describe fat content. So, in one embodiment a group of people can be asked to rate a certain meat, for instance ground beef, according to properties which describe the meat. These ratings can be used as an indication of the properties of the meat. The consumables of the present invention can then be compared to the meat to determine how similar the consumable is to the meat. In some instances the properties of the consumables are then altered to make the consumable more similar to the meat. So, in some embodiments, the consumable is rated similar to meat according to human evaluation. In some embodiments the consumable is indistinguishable from real meat to a human.

[0286] In some embodiments, subjects asked to identify the consumable identify it as a form of meat. In some embodiments one property of the compositions of the invention is that an animal, for example a human, will identify the composition as a meat. In some embodiments the human identifies the composition of the invention as having properties equivalent to meat. In some embodiments one or more properties of meat are equivalent according to a human's perception. Such properties include the properties that can be tested. In some embodiments a human identifies a consumable of the present invention as more meat like than meat substitutes found in the art.

[0287] In embodiments an experiment can demonstrate that consumable s acceptable to consumers. A panel can be used to screen a variety of consumables described herein. A number of human panelists can tested multiple consumable samples, namely, natural meats vs. the consumable compositions described herein. Variables such as fat content can be standardized for example to 20% fat using lean and fat meat mixes. Fat content can be determined using the Babcock for meat method (S. S. Nielson, Introduction to the Chemical Analysis of Foods (Jones & Bartlett Publishers, Boston, 1994)). Mixtures of ground beef and consumables of the invention prepared according to the procedure described herein can be formulated.

[0288] Panelists can be served samples in booths, under red lights or under white light, in an open consumer panel. Samples can be assigned random three-digit numbers and rotated in ballot position to prevent bias. Panelists can be asked to evaluate samples for tenderness, juiciness, texture, flavor, and overall acceptability using a hedonic scale from 1=dislike extremely, to 9=like extremely, with a median of 5=neither like nor, dislike. Panelists can be encouraged to rinse their mouths with water between samples, and given opportunity to comment on each sample.

[0289] The results of this experiment can indicate significant differences ($p<0.05$) or similarities between the traditional meats and the compositions of the invention.

[0290] These results will demonstrate that the compositions of the invention are judged as acceptably equivalent to real meat products. Additionally these results can demonstrate that compositions of the invention are preferred by panelist over other commercially available meat substitutes. So, in some embodiments the present invention provides for consumables that are significantly similar to traditional meats.

[0291] Consumables of the invention can also have similar physical characteristics as traditional meat. In one embodiment the force required to pierce a 1 inch thick structure (e.g. a patty) made of a consumable of the invention with a fixed diameter steel rod is not significantly different than the force required to pierce a 1 inch thick similar meat structure (e.g. a ground beef patty) with a similar fixed diameter steel rod. Accordingly, the invention provides for consumables with similar physical strength characteristics to meat.

[0292] In some embodiments composition of the invention have a similar cook loss characteristic as meat. In one embodiment a consumable of the invention with a similar fat and protein content as ground beef has the same reduction in size when cooked as real ground beef. Similar similarities in size loss profiles can be achieved for various compositions of consumables described herein matched to various meats.

[0293] In some embodiments the consumable is compared to real meat based upon olfactometer readings. In various embodiments the olfactometer can be used to assess odor concentration and odor thresholds, odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of appreciation, or relative intensity of odors. In some embodiments the olfactometer allows the training and automatic evaluation of expert panels. So in some embodiments the consumable is a product that causes similar or identical olfactometer readings. In some embodiments the similarity is sufficient to be beyond the detection threshold of human perception.

[0294] Gas chromatography-mass spectrometry (GCMS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to separate and identify different substances within a test sample. GCMS can, in some embodiments, be used to evaluate the properties of a consumable. For example volatile chemicals can be isolated from the head space around meat. These chemicals can be identified using GCMS. A profile of the volatile chemicals in the headspace around meat is thereby created. In some instances each peak of the GCMS can be further evaluated. For instance, a human could rate the experience of smelling the chemical responsible for a certain peak. This information could be used

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to further refine the profile. GCMS could then be used to evaluate the properties of the consumable. The GCMS profile could be used to refine the consumable.

[0295] Characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions molecules including amino acids, fats and sugars which are found in plants as well as meat. Therefore in some embodiments the consumable is tested for similarity to meat during or after cooking. In some embodiments human ratings, human evaluation, olfactometer readings, or GCMS measurements, or combinations thereof, are used to create an olfactory map of cooked meat. Similarly, an olfactory map of the consumable, for instance a meat replica, can be created. These maps can be compared to assess how similar the cooked consumable it so meat. In some embodiments the olfactory map of the consumable during or after cooking is similar to or indistinguishable from that of cooked or cooking meat. In some embodiments the similarity is sufficient to be beyond the detection threshold of human perception.

[0296] In one aspect, the invention provides a meat substitute product (alternatively referred to herein as "consumable") that is substantially or entirely composed of ingredients derived from non-animal sources, yet recapitulates key features associated with the cooking and consumption of an equivalent meat product derived from animals. The equivalent meat product can be a white meat or a dark meat. The equivalent meat product can be derived from any animal. Non-limiting examples of animals used to derive the equivalent meat product include farmed animals such as, e.g., cattle, sheep, pig, chicken, turkey, goose, duck, horse, dog or game animals (whether wild or farmed) such as, e.g., rabbit, deer, bison, buffalo, boar, snake, pheasant, quail, bear, elk, antelope, pigeon, dove, grouse, fox, wild pig, goat, kangaroo, emu, alligator, crocodile, turtle, groundhog, marmot, possum, partridge, squirrel, raccoon, whale, seal, ostrich, capybara, nutria, guinea pig, rat, mice, vole, any variety of insect or other arthropod, seafood such as, e.g, fish, crab, lobster, oyster, muscle, scallop, abalone, squid, octopus, sea urchin, tunicate and others. Many meat products are typically derived from skeletal muscle of an animal but it is understood that meat can also come from other muscles or organs of the animal. In some embodiments, the equivalent meat product is a cut of meat derived from skeletal muscle. In other embodiments, the equivalent meat product is an organ such as, e.g., a kidney, heart, liver, gallbladder, intestine, stomach, bone marrow, brain, thymus, lung, tongue. Accordingly, in some embodiments the compositions of the present invention are consumables similar to skeletal muscle or organs.

[0297] In some aspects, the present invention provides meat substitute products comprising one or more of a first composition comprising a muscle tissue replica, a second composition comprising an adipose tissue replica, and/or a third composition comprising a connective tissue replica, wherein the one or more compositions are combined in a manner that recapitulates the physical organization of meat. In other aspects, the present invention provides compositions for a muscle tissue replica (herein referred to as "muscle replica"), an adipose tissue replica (herein referred to as "fat replica"), and a connective tissue replica (herein referred to as "connective tissue replica"). In some embodiments, the compositions and meat substitute products are principally or entirely composed of ingredients derived from non-animal sources. In alternative embodiments, the muscle, fat, and/or connective tissue replica, or the meat substitute products comprising one or more of said replicas, are partially derived from animal sources but supplemented with ingredients derived from non-animal sources. In yet other alternative embodiments, the invention provides meat products substantially derived from animal sources but which are supplemented with one or more of a muscle tissue replica, a fat replica, and/or a connective tissue replica, wherein said replicas are derived substantially or entirely from non-animal sources. A non-limiting example of such a meat product is an ultra-lean ground beef product supplemented with a non-animal derived fat replica which improves texture and mouthfeel while preserving the health benefits of a consumable low in animal fat. Such alternative embodiments result in products with properties that more closely recapitulate key features associated with preparing and consuming meat but which are less costly and associated with a lesser environmental impact, less animal welfare impact, or improved health benefits for the consumer.

[0298] The physical organization of the meat substitute product can be manipulated by controlling the localization, organization, assembly, or orientation of the muscle, fat, and/or connective tissue replicas described herein. In some embodiments the product is designed in such a way that the replicas described herein are associated with one another as in meat. In some embodiments the consumable is designed so that after cooking the replicas described herein are associated with one another as in cooked meat. In some embodiments, one or more of the muscle, fat, and/or connective tissue replicas are combined in a manner that recapitulate the physical organization of different cuts or preparations of meat. In an example embodiment, the replicas are combined in a manner that approximates the physical organization of natural ground meat. In other embodiments, the replicas are combined in a manner that approximates different cuts of beef, such as, e.g., ribeye, filet mignon, London broil, among others.

Proteins and Protein Sources

[0299] In some embodiments, any of the meat substitute products, muscle tissue replica, fat replica, or connective tissue replica, comprise one or more isolated, purified proteins. In some embodiments, the meat substitute products are comprised of one or more of a muscle replica, a fat replica, and/or connective tissue replica which comprise one or more isolated, purified proteins. In other embodiments, the muscle replica, fat replica, and/or connective tissue replica comprises one or more isolated, purified proteins. In some embodiments, about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component is comprised of one or more isolated, purified proteins. For the purposes of this document, "purified protein" will refer to a preparation in which the cumulative abundance by mass of protein components other than the specified protein, which can be a single monomeric or multimeric protein species, is reduced by a factor of 2 or more, 3 or more, 5 or more, 10 or more, 20 or more, 50 or more, 100 or more or 1000 or more relative to the source material from which the specified protein was isolated.

[0300] In some embodiments, the one or more isolated, purified proteins are derived from non-animal sources. Non-limiting examples of non-animal sources include plants, funghi, bacteria, archaea, genetically modified organisms such as genetically modified bacteria or yeast, chemical or in vitro synthesis. In particular embodiments, the one or more isolated, purified proteins are derived from plant sources.

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Non-limiting examples of plant sources include grains such as, e.g., corn, maize, rice, wheat, barley, rye, triticale, teff, oilseeds including cottonseed, sunflower seed, safflower seed, rapeseed, leafy greens such as, e.g., lettuce, spinach, kale, collard greens, turnip greens, chard, mustard greens, dandelion greens, broccoli, cabbage, green matter not ordinarily consumed by humans, including biomass crops, including switchgrass, miscanthus, sorghum, other grasses, alfalfa, corn stover, green matter ordinarily discarded from harvested plants, sugar cane leaves, leaves of trees, root crops such as cassava, sweet potato, potato, carrots, beets, turnips, plants from the legume family, such as, e.g., clover, peas such as cowpeas, english peas, yellow peas, green peas, beans such as, e.g., soybeans, fava beans, lima beans, kidney beans, garbanzo beans, mung beans, pinto beans, lentils, lupins, mesquite, carob, soy, and peanuts, vetch (vicia), stylo (stylosanthes), arachis, indigofera, acacia, leucaena, cyamopsis, and sesbania. One of skill in the art will understand that proteins that can be isolated from any organism in the plant kingdom may be used in the present invention.

[0301] Proteins that are abundant in plants can be isolated in large quantities from one or more source plants and thus are an economical choice for use in any of the muscle, fat, connective tissue replicas, or meat substitute products. Accordingly, in some embodiments, the one or more isolated proteins comprises an abundant protein found in high levels in a plant and capable of being isolated and purified in large quantities. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70% of the total protein content of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5-40%, about 10-50%, about 20-60%, or about 30-70% of the total protein content of the source plant. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50% of the total weight of the dry matter of the source plant. In some embodiments, the abundant protein comprises about 0.5-5%, about 1-10%, about 5-20%, about 10-30%, about 15-40%, about 20-50% of the total weight of the dry matter of the source plant.

[0302] In particular embodiments, the one or more isolated proteins comprises an abundant protein that is found in high levels in the leaves of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80% of the total protein content of the leaves of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%, about 20%-60%, or about 30-70% of the total protein content of the leaves of the source plant. In particular embodiments, the one or more isolated proteins comprise ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco activase). Rubisco is a particularly useful protein for meat replicas because of its high solubility and an amino acid composition with close to the optimum proportions of essential amino acids for human nutrition. In particular embodiments, the one or more isolated proteins comprise ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase). In particular embodiments, the one or more isolated proteins comprise a vegetative storage protein (VSP).

[0303] In some embodiments, the one or more isolated proteins includes an abundant protein that is found in high levels in the seeds of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or 90% or more of the total protein content of the seeds of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%, about 20%-60%, or about 30-70% or >70% of the total protein content of the seeds of the source plant. Non-limiting examples of proteins found in high levels in the seeds of plants are seed storage proteins, e.g., albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins

[0304] In some embodiments, the one or more isolated proteins includes proteins that interact with lipids and help stabilize lipids in a structure. Without wishing to be bound by a particular theory, such proteins may improve the integration of lipids and/or fat replicas with other components of the meat substitute product, resulting in improved mouthfeel and texture of the final product. A non-limiting example of a lipid-interacting plant protein is the oleosin family of proteins. Oleosins are lipid-interacting proteins that are found in oil bodies of plants. Other non-limiting examples of plant proteins that can stabilize emulsions include seed storage proteins from Great Northern Beans, albumins from peas, globulins from peas, 8S globulins from Moong bean, 8S globulins from Kidney bean.

Muscle Replicas

[0305] A large number of meat products comprise a high proportion of skeletal muscle. Accordingly, the present invention provides a composition derived from non-animal sources which replicates or approximates key features of animal skeletal muscle. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources which replicates or approximates animal skeletal muscle. Such a composition will be labeled herein as "muscle replica". In some embodiments, the muscle replica and/or meat substitute product comprising the muscle replica are partially derived from animal sources. In some embodiments, the muscle replica and/or meat substitute product comprising the muscle replica are entirely derived from non-animal sources.

[0306] Many meat products comprise a high proportion of striated skeletal muscle in which individual muscle fibers are organized mainly in an isotropic fashion. Accordingly, in some embodiments the muscle replica comprises fibers that are to some extent organized isotropically. In some embodiments the fibers comprise a protein component. In some embodiments, the fibers comprise about 1%, about 2%, about 5%, about 10%, about 15%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99% or more of a protein component.

[0307] In some embodiments, the protein component comprises one or more isolated, purified proteins. For example the one or more isolated, purified protein can comprise the 8S globulin from Moong bean seeds, or the albumin or globulin fraction of pea seeds. These proteins provide examples of proteins with favorable properties for constructing meat replicas because of their ability to form gels with textures similar to animal muscle or fat tissue. Examples and embodiments of the one or more isolated, purified proteins are described

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herein. The list of potential candidates here is essentially open and may include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microorganisms, or synthesized in vitro. The proteins may be artificially designed to emulate physical properties of animal muscle tissue. The proteins may be artificially designed to emulate physical properties of animal muscle tissue. In some embodiments, one or more isolated, purified proteins accounts for about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component by weight.

[0308] Skeletal muscle of animals such as beef cattle typically contain substantial quantities of glycogen, which can comprise on the order of 1% of the mass of the muscle tissue at the time of slaughter. After slaughter, a fraction of this glycogen continues to be metabolized yielding products including lactic acid, which contributes to lowering the pH of the muscle tissue, a desirable quality in meat. Glycogen is a branched polymer of glucose linked together by alpha (1->4) glycosidic bonds in linear chains, with branch points comprising alpha (1->6) glycosidic bonds. Starches from plants, particularly amylopectins are also branched polymers of glucose linked together by alpha (1->4) glycosidic bonds in linear chains, with branch points comprising alpha (1->6) glycosidic bonds and can therefore be used as an analog of glycogen in constructing meat replicas. Thus in some embodiments, the muscle or meat replica includes a starch or pectin.

[0309] Additional components of animal muscle tissue include sodium, potassium, calcium, magnesium, other metal ions, lactic acid, other organic acids, free amino acids, peptides, nucleotides and sulfur compounds. Thus in some embodiments, the muscle replica can include sodium, potassium, calcium, magnesium, other metal ions, lactic acid, other organic acids, free amino acids, peptides, nucleotides and sulfur compounds. In some embodiments the concentration of sodium, potassium, calcium, magnesium, other metal ions, lactic acid, other organic acids, free amino acids, peptides, nucleotides and/or sulfur compounds in the muscle replica or consumable are within 10% of the concentrations found in a muscle or meat being replicated.

[0310] In another aspect, the invention provides methods for making a muscle replica. In some embodiments, the composition is formed into asymmetric fibers prior to incorporation into the consumable. In some embodiments these fibers replicate muscle fibers. In some embodiments the fibers are spun fibers. In other embodiments the fibers are extruded fibers. Accordingly, the present invention provides for methods for producing asymmetric or spun protein fibers. In some embodiments, the fibers are formed by extrusion of the protein component through an extruder. Methods of extrusion are well known in the art, and are described in U.S. Pat. No. 6,379,738, U.S. Pat. No. 3,693,533, US20120093994, which are herein incorporated by reference.

[0311] In some embodiments extrusion can be conducted using an MPF19 twin-screw extruder (APV Baker, Grand Rapids, Mich.) with a cooling die. The cooling die can cool the extrudate prior to return of the extrudate to atmospheric pressure, thus substantially inhibiting expansion or puffing of the final product. In the MPF19 apparatus, dry feed and liquid can be added separately and mixed in the barrel. Extrusion parameters can be, for example: screw speed of 200 rpm, product temperature at the die of 150 C., feed rate of 23 g/min, and water-flow rate of 11 g/min. Product temperature can be measured during extrusion by a thermocouple at the end of the extrusion barrel. Observations can be made on color, opacity, structure, and texture for each collected sample. Collected samples can be optionally dried at room temperature overnight, then ground to a fine powder (<60 mesh) using a Braun food grinder. The pH of samples can be measured in duplicate using 10% (w/v) slurries of powdered sample in distilled water.

[0312] Fat Replica

[0313] Animal fat is important for the experience of eating cooked meat. Accordingly, the present invention provides a composition derived from non-animal sources which recapitulates key features of animal fat. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources which recapitulates animal fat. Such a composition will be labeled herein as a "fat replica". In some embodiments, the fat replica and/or meat substitute product comprising the fat replica are partially derived from animal sources.

[0314] In some embodiments the meat substitute product has a fat component. In some embodiments the fat content of the consumable is 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, or 60% fat. In some embodiments, the fat replica comprises a gel with droplets of fat suspended therein. In some embodiments, the gel is a soft, elastic gel comprising proteins and optionally carbohydrates. In particular embodiments, the proteins used in the gel are plant or microbial proteins. In some embodiments, the proteins used in the fat replica might include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microorganisms, or synthesized in vitro. The proteins may be artificially designed to emulate physical properties of animal fat. The proteins may be artificially designed to emulate physical properties of animal fat.

[0315] The fat droplets used in some embodiments of the present invention can be from a variety of sources. In some embodiments, the sources are non-animal sources. In particular embodiments, the sources are plant sources. Non-limiting examples of oils include corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phosphatidic acids, lysophosphatidic acids, oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid,

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linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid.

[0316] In some embodiments, fat droplets are derived from pulp or seed oil. In other embodiments, the source may be yeast or mold. For instance, in one embodiment the fat droplets comprise triglycerides derived from *Mortierella isabellina.*

[0317] In some embodiments plant oils are modified to resemble animal fats. The plant oils can be modified with flavoring or other agents to recapitulate the taste and smell of meat during and after cooking. Accordingly, some aspects of the invention involve methods for testing the qualitative similarity between the cooking properties of animal fat and the cooking properties of plant oils in the consumable.

[0318] In some embodiments, the fat replica comprises a protein component comprising one or more isolated, purified proteins. The purified proteins contribute to the taste and texture of the meat replica. In some embodiments purified proteins can stabilize emulsified fats. In some embodiments the purified proteins can form gels upon denaturation or enzymatic crosslinking, which replicate the appearance and texture of animal fat. Examples and embodiments of the one or more isolated, purified proteins are described herein. In particular embodiments, the one or more isolated proteins comprise a protein isolated from the legume family of plants. Non-limiting examples of legume plants are described herein, although variations with other legumes are possible. In some embodiments, the legume plant is a pea plant. In some embodiments the isolated purified proteins stabilize emulsions. In some embodiments the isolated purified proteins form gels upon crosslinking or enzymatic crosslinking. In some embodiments, the isolated, purified proteins comprise seed storage proteins. In some embodiments, the isolated, purified proteins comprise albumin. In some embodiments, the isolated, purified proteins comprise globulin. In a particular embodiment, the isolated, purified protein is a purified pea albumin protein. In another particular embodiment, the isolated, purified protein is a purified pea globulin protein. In another particular embodiment the isolate purified protein is a Moong bean 8S globulin. In another particular embodiment, the isolated, purified protein is an oleosin. In another particular embodiment, the isolated, purified protein is a caloleosin. In another particular embodiment, the isolated, purified protein is Rubisco. In some embodiments, the protein component comprises about 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or more of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises about 0.1-5%, about 0.5-10%, about 1-20%, about 5-30%, about 10-50%, about 20-70%, or about 30-90% or more of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises a solution containing one or more isolated, purified proteins.

[0319] In some embodiments, the fat replica comprises cross-linking enzymes that catalyze reactions leading to covalent crosslinks between proteins. Cross-linking enzymes can be used to create or stabilize the desired structure and texture of the adipose tissue replica, to mimic the desired texture of an equivalent desired animal fat. Non-limiting examples of cross-linking enzymes include, e.g., transglutaminase, lysyl oxidases, or other amine oxidases (e.g. *Pichia pastoris* lysyl oxidase). In some embodiments, the cross-linking enzymes are isolated and purified from a non-animal source, examples and embodiments of which are described herein. In some embodiments, the fat replica comprises at least 0.0001%, or at least 0.001%, or at least 0.01%, or at least 0.1%, or at least 1% (wt/vol) of a cross-linking enzyme. In particular embodiments, the cross-linking enzyme is transglutaminase.

[0320] In another aspect, the invention provides methods for making a fat replica. In some embodiments, the fat droplets are suspended in a gel. In some embodiments the present invention provides for methods for producing droplets of fat suspended in the gel. The fat can isolated and homogenized. For example an organic solvent mixture can be used to help mix a lipid. The solvent can then be removed. At this point the lipid can be frozen, lyophilized, or stored. So in some aspects the invention provides for a method for isolating and storing a lipid which has been selected to have characteristics similar to animal fat. The lipid film or cake can then be hydrated. The hydration can utilize agitation or temperature changes. The hydration can occur in a precursor solution to a gel. After hydration the lipid suspension can be sonicated or extruded to further alter the properties of the lipid in the solution.

[0321] In some embodiments, the fat replica is assembled to approximate the organization adipose tissue in meat. In some embodiments some or all of the components of the fat replica are suspended in a gel. In various embodiments the gel can be a proteinaceous gel, a hydrogel, an organogel, or a xerogel. In some embodiments, the gel can be thickened to a desired consistency using an agent based on polysaccharides or proteins. For example fecula, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, alginin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, cellulose, methylcellulose, hydroxymethylcellulose, acadia gum, konjac, starch, pectin, amylopectin or proteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, of fungi can be used alone or in combination to thicken the gel, forming an architecture or structure for the consumable.

[0322] In particular embodiments, the fat replica is an emulsion comprising a solution of one or more proteins and one or more fats suspended therein as droplets. In some embodiments, the emulsion is stabilized by one or more cross-linking enzymes into a gel. In more particular embodiments, the one or more proteins in solution are isolated, purified proteins. In yet more particular embodiments, the isolated, purified proteins comprise a purified pea albumin enriched fraction. In other more particular embodiments, the isolated, purified proteins comprise a purified pea globulin enriched fraction. In other more particular embodiments, the isolated, purified proteins comprise a purified Moong bean 8S globulin enriched fraction. In yet more particular embodiments, the isolated, purified proteins comprise a Rubisco enriched fraction. In other particular embodiments, the one or more fats are derived from plant-based oils. In more particular embodiments, the one or more fats are derived from one or more of: corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter,

mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phosphatidic acids, lysophosphatidic acids, oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid. In yet even more particular embodiments, the one or more fats is a rice bran oil. In another particular embodiment, the one or more fats is a canola oil. In other particular embodiments, the cross-linking enzyme is transglutaminase, lysyl oxidase, or other amine oxidase. In yet even more particular embodiments, the cross-linking enzyme is transglutaminase. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of purified pea albumin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% canola oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of purified pea albumin emulsified with 40-80% rice bran oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% rice bran oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% canola oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel.

[0323] Connective Tissue Replica

[0324] Animal connective tissue provides key textural features that are an important component of the experience of eating meat. Accordingly, the present invention provides a composition derived from non-animal sources which recapitulates key features of animal connective tissue. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources which recapitulates important textural and visual features of animal connective tissue. Such a composition will be labeled herein as "connective tissue replica". In some embodiments, the connective tissue replica and/or meat substitute product comprising the connective tissue replica are partially derived from animal sources.

[0325] Animal connective tissue can generally be divided into fascia-type and cartilage-type tissue. Fascia-type tissue is highly fibrous, resistant against extension (has high elastic modulus), and has a high protein content, a moderate water content (ca. 50%), and low-to-none fat and polysaccharide content. Accordingly, the present invention provides a connective tissue replica that recapitulates key features of fascia type tissue. In some embodiments, the connective tissue replica comprises about 50% protein by total weight, about 50% by liquid weight, and has a low fat and polysaccharide component.

[0326] The protein content of most fascia-type connective tissue is comprised mainly of collagen. Collagen is characterized by a high fraction of proline and alanine, and also is assembled into characteristic elongated fibrils or rod-like, flexible structures. Prolamins are one family of proteins found in non-animal sources, such as plant sources. Prolamins are highly abundant in plants and are similar in amino acid composition to collagen. Among proteins we tested for this purpose, prolamins were particularly favorable because of their low cost and their ability to readily form fibers or sheets when spun or extruded. Non-limiting examples of prolamin family proteins include, e.g., zein (found in corn), these include hordein from barley, gliadin from wheat, secalin, extensins from rye, kafirin from sorghum, avenin from oats. In fascia-type connective tissue, the prolamin family of proteins, individually or combinations thereof, demonstrates suitability for the protein component because they are highly abundant, similar in global amino acid composition to collagen (high fraction of proline and alanine), and amenable to processing into films and fibers. In addition to zein (found in corn), these include hordein from barley, gliadin from wheat, secalin, extensins from rye, kafirin from sorghum, avenin from oats. Other proteins may be necessary to supplement prolamins in order to achieve targets specifications for physicochemical and nutritional properties. The list of potential candidates here is essentially open and may include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microorganisms, or synthesized in vitro. The proteins may be artificially designed to emulate physical properties of animal connective tissue. animal-derived or recombinant collagen, extensins (hydroxyproline-rich glycoproteins abundant in cell walls e.g. *Arabidopsis thaliana*, monomers of which are "collagen-like" rod-like flexible molecules). The proteins may be artificially designed to emulate physical properties of animal connective tissue.

[0327] Methods for forming fascia-type connective tissue will be as those practiced in the art with a bias towards methods producing fibrous or fibrous-like structures by biological, chemical, or physical means, individually or in combination, serially or in parallel, before final forming. These methods may include extrusion or spinning.

[0328] Cartilage-type tissue is macroscopically homogenous, resistant against compression, has higher water content (up to 80%), lower protein (collagen) content, and higher polysaccharide (proteoglycans) contents (ca. 10% each).

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[0329] Compositionally, cartilage-type connective tissue will be very similar to fascia-type tissue with the relative ratios of each adjusted to more closely mimic 'meat' connective tissue.

[0330] Methods for forming cartilage-type connective tissue will be similar to those for fascia-type connective tissue, but with a bias towards methods producing isotropically homogenous structures.

[0331] The fat can be suspended in a gel. In some embodiments the present invention provides for methods for producing droplets of fat suspended in the proteinaceous gel. The fat can be isolated from plant tissues and emulsified. The emulsification can utilize high-speed blending, homogenization, agitation or temperature changes. The lipid suspension can be sonicated or extruded to further alter the properties of the lipid in the solution. At this point, in some embodiments other components of the consumable are added to the solution followed by a gelling agent. In some embodiments crosslinking agents (e.g. transglutaminase or lysyl oxidase) are added to bind the components of the consumable. In other embodiments the gelling agent is added and the lipid/gel suspension is later combined with additional components of the consumable. In fascia-type connective tissue, the prolamin family of proteins, individually or combinations thereof, demonstrates suitability for the protein component because they are highly abundant, similar in global amino acid composition to collagen (high fraction of proline and alanine), and amenable to processing into films. In addition to zein (found in corn), these include hordein from barley, gliadin from wheat, secalin, extensions from rye, kafirin from sorghum, avenin from oats. Other proteins may be necessary to supplement prolamins in order to achieve targets specifications for physicochemical and nutritional properties. The list of potential candidates here is essentially open and may include any major seed storage proteins, animal-derived or recombinant collagen, extensins (hydroxyproline-rich glycoproteins abundant in cell walls e.g. *Arabidopsis thaliana*, monomers of which are "collagen-like" rod-like flexible molecules).

[0332] In some embodiments some or all of the components of the consumable are suspended in a gel. In various embodiments the gel can be a hydrogel, an organogel, or a xerogel, The gel can be made thick using an agent based on polysaccharides or proteins. For example fecula, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, alginin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, cellulose, methylcellulose, hydroxymethylcellulose, acadia gum, konjac, starch, pectin, amylopectin or proteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, of fungi can be used alone or in combination to thicken the gel, forming an architecture or structure for the consumable. Enzymes that catalyze reactions leading to covalent crosslinks between proteins can also be used alone or in combination to form an architecture or structure for the consumable. For example transclutaminase, lysyl oxidases, or other amine oxidases (e.g. *Pichia pastoris* lysyl oxidase (PPLO)) can be used alone or in combination to form an architecture or structure for the consumable. In some embodiments multiple gels with different components are combined to form the consumable. For example a gel containing a plant-based protein can be associated with a gel containing a plant-based fat. In some embodiments fibers or stings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

[0333] The compositions of the invention can be puffed or expanded by heating, such as frying, baking, microwave heating, heating in a forced air system, heating in an air tunnel, and the like, according to methods well known in the art.

[0334] In some embodiments multiple gels with different components are combined to form the consumable. For example a gel containing a plant-based protein can be associated with a gel containing a plant-based fat. In some embodiments fibers or strings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

[0335] In some embodiments the meat replica contains no animal products, less than 1% wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[0336] In some embodiments the meat replica contains no animal products, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[0337] In some embodiments the meat replica contains no animal products, no soy protein isolate, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[0338] In some embodiments the meat replica contains no animal products, no soy protein concentrate, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[0339] In some embodiments the meat replica contains no animal products, no soy protein, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[0340] In some embodiments the meat replica contains no animal products, no tofu, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[0341] In some embodiments the meat replica contains no animal products, no tofu, and no wheat gluten.

[0342] In some embodiments the meat replica contains no animal products, no soy protein, and no wheat gluten.

[0343] In some embodiments the meat replica contains no methylcellulose, no carrageenan, no caramel color, no Konjac flour, no gum Arabic, and no acacia gum.

[0344] In some embodiments the meat replica contains no animal products and less than 5% carbohydrates.

[0345] In some embodiments the meat replica contains no animal products, no soy protein, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum and less than 5% carbohydrates.

[0346] In some embodiments the meat replica contains no animal products, and less than 1% cellulose.

[0347] In some embodiments the meat replica contains no animal products, and less than 5% insoluble carbohydrates.

[0348] In some embodiments the meat replica contains no animal products, no soy protein, and less than 1% cellulose.

[0349] In some embodiments the meat replica contains no animal products, no soy protein, and less than 5% insoluble carbohydrates.

[0350] In some embodiments the meat replica contains no animal products, no wheat gluten, and less than 1% cellulose.

[0351] In some embodiments the meat replica contains no animal products, no wheat gluten, and less than 5% insoluble carbohydrates.

[0352] The percentage of different components may also be controlled. For example non-animal-based substitutes for muscle, fat tissue, connective tissue, and blood components can be combined in different ratios and physical organizations to best approximate the look and feel of meat. The various can also components can be arranged to insure consistency between bites of the consumable. The components can be arranged to insure that no waste is generated from the consumable. For example, while a traditional cut of meat may have portions that are not typically eaten, a meat replicate can improve upon meat by not including these inedible portions. Such an improvement allows for all of the product made or shipped to be consumed, which cuts down on waste and shipping costs. Alternatively, a meat replica may include inedible portions to mimic the experience of meat consumption. Such portions can include bone, cartilage, connective tissue, or other materials commonly referred to as gristle, or materials included simulating these components. In some embodiments the consumable may contain simulated inedible portions of meat products which are designed to serve secondary functions. For example a simulated bone can be designed to disperse heat during cooking, making the cooking of the consumable faster or more uniform than meat. In other embodiments a simulated bone may also serve to keep the consumable at a constant temperature during shipping. In other embodiments, the simulated inedible portions may be biodegradable.

[0353] In some embodiments the meat substitute compositions contains no animal protein, comprising between 10-30% protein, between 5-80% water, between 5-70% fat, comprising one or more isolated purified proteins. In particular embodiments, the meat substitute compositions comprise transglutaminase.

[0354] In some embodiments the consumable contains components to replicate the components of meat. The main component of meat is typically skeletal muscle. Skeletal muscle typically consists of roughly 75 percent water, 19 percent protein, 2.5 percent intramuscular fat, 1.2 percent carbohydrates and 2.3 percent other soluble non-protein substances. These include organic acids, sulfur compounds, nitrogenous compounds, such as amino acids and nucleotides, and inorganic substances such as minerals. Accordingly, some embodiments of the present invention provide for replicating approximations of this composition for the consumable. F or example, in some embodiments the consumable is a plant-based meat replica can comprise roughly 75% water, 19% protein, 2.5% fat, 1.2% carbohydrates; and 2.3 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 60-90% water, 10-30% protein, 1-20% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 60-90% water, 5-10% protein, 1-20% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 0-50% water, 5-30% protein, 20-80%% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments, the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin. Some meat also contains myoglobin, a heme protein, which accounts for most of the red color and iron content of some meat. In some embodiments, the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin. It is understood that these percentages can vary in meat and the meat replicas can be produced to approximate the natural variation in meat. Additionally, in some instances, the present invention provides for improved meat replicas, which comprise these components in typically unnatural percentages. For example a meat replica can be produced with a higher than typical average fat content. The percentages of these components may also be altered to increase other desirable properties.

[0355] In some instances a meat replica is designed so that, when cooked, the percentages of components are similar to cooked meat. So, in some embodiments, the uncooked consumable has different percentages of components than uncooked meat, but when cooked the consumable is similar to cooked meat. For example, a meat replica may be made with a higher than typical water content for raw meat, but when cooked in a microwave the resulting product has percentages of components similar to meat cooked over a fire.

[0356] In some embodiments the consumable is a meat replica with a lower that typical water content for meat. In some embodiments the inventions provides for methods for hydrating a meat replica to cause the meat replica to have a content similar to meat. For example a meat replica with a water content that would be low for meat, for example 1%, 10%, 20%, 30%, 40% or 50% water, is hydrated to roughly 75% water. Once hydrated, in some embodiments, the meat replica is then cooked for human consumption.

[0357] The consumable can have a protein component. In some embodiments the protein content of the consumable is 10%, 20%, 30%, or 40%. In some embodiments the protein content of the consumable is similar to meat. In some embodiments the protein content in the consumable is greater than that of meat. In some embodiments the consumable has less protein than meat.

[0358] The protein in the consumable can come from a variety or combination of sources. Non-animal sources can provide some or all of the protein in the consumable. Non-animal sources can include vegetables, fruits, nuts, grains, algae, bacteria, or fungi. The protein can be isolated or concentrated from one or more of these sources. In some embodiments the consumable is a meat replica comprising protein only obtained from non-animal sources.

[0359] In some embodiments protein is formed into asymmetric fibers for incorporation into the consumable. In some embodiments these fibers replicate muscle fibers. In some embodiments the protein are spun fibers. Accordingly, the present invention provides for methods for producing asymmetric or spun protein fibers. In some embodiments the consumable contains a protein or proteins that have all of the amino acids found in proteins that are essential for human nutrition. In some embodiments the proteins added to the consumable are supplemented with amino acids.

[0360] Indicators of Cooking Meat

[0361] The release of odorants upon cooking is an important aspect of meat consumption. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking beef. In some embodiments, the consumable when cooked generates an aroma recognizable by humans as typical of cooking pork. In some embodiments, the consumable is a meat replica entirely com-

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posed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking bacon. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking chicken. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking lamb. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking fish. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking turkey. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with an odorant that is released upon cooking. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with an odorant that is produced by chemical reactions that take place upon cooking. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing mixtures of proteins, peptides, amino acids, nucleotides, sugars and polysaccharides and fats in combinations and spatial arrangements that enable these compounds to undergo chemical reactions during cooking to produce odorants and flavor-producing compounds. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a volatile or labile odorant that is released upon cooking. In some embodiments the consumable is a method for preparing a meat replica where meat replicas principally or entirely composed of ingredients derived from non-animal sources are heated to release a volatile or labile odorant.

[0362] Odorants released during cooking of meat are generated by reactions that can involve as reactants fats, protein, amino acids, peptides, nucleotides, organic acids, sulfur compounds, sugars and other carbohydrates. In some embodiments the odorants that combine during the cooking of meat are identified and located near one another in the consumable, such that upon cooking of the consumable the odorants combine. So, in some embodiments, the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions involving amino acids, fats and sugars found in plants as well as meat. So, in some embodiments, the characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions involving one or more amino acids, fats, peptides, nucleotides, organic acids, sulfur compounds, sugars and other carbohydrates found in plants as well as meat.

[0363] Some reactions that generate odorants released during cooking of meat can be catalyzed by iron, in particular the heme iron of myoglobin. Thus in some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by iron. In some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by heme. In some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by the heme iron in leghemoglobin. In some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by the heme iron in a heme protein.

[0364] Evidence that the presence of leghemoglobin contributes favorably to aroma of meat replicas: A muscle replica comprising pea flour, sunflower oil, and glucose was heated for 10 minutes at 140 C in the presence of either reduced leghemoglobin (LHb) or a mixture of iron (Fe3+), sodium and EDTA (EFS) in sealed containers carrying solid phase microextraction (SPME) fibers. These fibers contain polydimethylsiloxane (PDMS) which adsorbs volatile compounds for analysis by GC-MS. Analysis of GC-MS data from multiple replicas reveal consistent differences between the LHb and EFS samples. Non-limiting examples of compounds found exclusively or more abundantly in the LHb samples are: 2-octanone, 2-methyl furan, which are often associated with the aroma of cooked meat, and many other unidentified compounds.

[0365] Color Indicators

[0366] The color of meat is an important part the experience of cooking and eating meat. For instance, cuts of beef are of a characteristic red color in a raw state and gradually transition to a brown color during cooking. As another example, white meats such as chicken or pork have a characteristic pink color in their raw state and gradually transition to a white or brownish color during cooking. The amount of the color transition is used to indicate the cooking progression of beef and titrate the cooking time and temperature to produce the desired state of done-ness. In some aspects, the invention provides a non-meat based meat substitute product that provides a visual indicator of cooking progression. In some embodiments, the visual indicator is a color indicator that undergoes a color transition during cooking. In particular embodiments, the color indicator recapitulates the color transition of a cut of meat as the meat progresses from a raw to a cooked state. In more particular embodiments, the color indicator colors the meat substitute product a red color before cooking to indicate a raw state and causes the meat substitute product to transition to a brown color during cooking progression. In other particular embodiments, the color indicator colors the meat substitute product a pink color before cooking to indicate a raw state and causes the meat substitute product to transition to a white or brown color during cooking progression.

[0367] The main determinant of the nutritional definition of the color of meat is the concentration of iron carrying proteins in the meat. In the skeletal muscle component of meat products, one of the main iron-carrying proteins is myoglobin. It is estimated that the white meat of chicken has under 0.05%; pork and veal have 0.1-0.3%; young beef has 0.4-1.0%; and old beef has 1.5-2.0%. So, in some embodiments, the consumable is a meat replica which comprises an iron-carrying protein. In some embodiments, the meat replica comprises about 0.05%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, or more than about 2% of an iron-carrying protein by dry weight or total weight. In some cases, the iron carrying protein has been isolated and purified from a source. In other cases, the iron carrying protein has not been isolated and purified. In some cases, the source of the iron-carrying protein is an animal source, or a non-animal source such as a plant, fungus, or genetically modified organ-

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isms such as, e.g., bacteria or yeast. In some cases, the iron-carrying protein is myoglobin. In some embodiments the consumable is a plant based meat replica that has animal myoglobin added. So, for example a replica of young beef can have about 0.4-1% myoglobin. In some cases, the iron-carrying protein is leghemoglobin. In some embodiments the consumable is a plant based meat replica that has leghemoglobin added. So, for example a replica of young beef can have about 0.4-1% leghemoglobin. In some cases, the iron-carrying protein is a cytochrome. In some embodiments the consumable is a plant based meat replica that has a cytochrome added. So, for example a replica of young beef can have about 0.4-1% of a cytochrome.

[0368] Another example of iron-carrying proteins is hemoglobin, the iron-containing oxygen-binding protein in the red blood cells of vertebrates. Hemoglobin is similar in color to myoglobin. In some embodiments the invention provides methods of saving and recycling blood from animal farming to supplement the color of a consumable. For example blood is saved from a slaughter house, hemoglobin from the blood is used to enhance the color of a consumable. In some aspects the consumable is a plant-based meat replica containing hemoglobin.

[0369] Additional iron containing proteins exist in nature. In some embodiments the consumable comprises an iron containing protein that is not myoglobin. In some embodiments the consumable does not contain myoglobin. In some embodiments the consumable does not contain hemoglobin. In some embodiments the consumable is a meat replica that comprises an iron containing protein other than myoglobin or hemoglobin.

[0370] Examples of iron containing proteins include hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins. In various embodiments these iron containing proteins are added to the consumable to alter the visual characteristics or iron content of the consumable. In some embodiments the consumable comprises a hemoprotein (e.g. hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins).

[0371] Leghemoglobin, similar in structure and physical properties to myoglobin, is readily available as an unused by-product of commodity legume crops (eg., soybean, pea). The leghemoglobin in the roots of these crops in the US exceeds the myoglobin content of all the red meat consumed in the US. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, including a muscle tissue replica, an adipose tissue replica, a connective tissue replica, and leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a heme protein. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a member of the globin protein family. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a high iron content from a heme protein. In some embodiments the iron content is similar to meat. In some embodiments the consumable has the distinctive red color of meat, such color provided by leghemoglobin.

[0372] Leghemoglobin is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.

[0373] A heme protein is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.

[0374] A heme protein from the group of: Hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.

Food Products Comprising Isolated, Purified Leghemoglobin

[0375] In some embodiments leghemoglobin is added to meat to enhance the properties of meat. For example, a leghemoglobin containing solution can be injected into raw or cooked meat. In another example a leghemoglobin solution is dripped over meat or a consumable of the invention to enhance appearance. In one embodiment advertising, photography, or videography of food products such as meat or a meat substitute is enhanced with leghemoglobin.

Sources of Leghemoglobin

[0376] In some embodiments the present invention provides methods for obtaining leghemoglobin from plants. Leghemoglobin can be obtained from a variety of plants. Various legumes species and their varieties, for example, Soybean, Fava bean, Lima bean, Cowpeas, English peas, Yellow peas, Lupine, Kidney bean, Garbanzo beans, Peanut, Alfalfa, Vetch hay, Clover, Lespedeza and Pinto bean, contain nitrogen-fixing root nodules in which leghemoglobin has a key role in controlling oxygen concentrations (for example root nodules from a pea plant, FIG. **1**). FIG. **2** shows 100 mls of leghemoglobin solution isolated from 30 grams of pea root nodules. Leghemoglobins from different species are homologs and have similar color properties (FIG. **3**). In FIG. **3**, panel A shows an SDS_PAGE gels of lysed root-nodules of three legume plant species (1) Fava bean (2) English Pea (3) Soybean. Arrows mark respective leghemoglobins. Note that leghemoglobin is the most abundant soluble protein in each lysate. Panel B shows the similarity of UV-VIS spectral profile of leghemoglobins from two different plant species (Fava-

bean and Soybean). We purified leghemoglobin from fava bean (green curve) and Soybean (red curve) root nodules using the protocol described elsewhere in the specification. UV-VIS spectra of both proteins shows that the heme iron is in the reduced (+2) state. Note that they are almost perfectly superimposed, consistent with their visually identical red color. The heme iron in the respective leghemoglobins was reduced to the +2 oxidation state by incubating Fava bean and Soybean leghemoglobin with 10 mM sodium hydrosulfite in 20 mM potassium-phosphate pH 7.4, 100 mM sodium chloride buffer. Sodium hydrosulfite was then removed from the leghemoglobin solution using gel-exclusion chromatography. Inset shows a zoom-in of UV-VIS spectra in 450 nm to 700 nm region. Some plant species express several leghemoglobin isoforms (for example soybean has four leghemoglobin isoforms). Minor variations in precise amino acid sequence can modify overall charge of the protein at a particular pH and can modify precise structural conformation of iron containing heme group in leghemoglobin. Differences in structural conformation of heme group of different leghemoglobins can influence oxidation and reduction rates of the heme iron. These differences may contribute to color and flavor generation properties of different leghemoglobins.

[0377] Leghemoglobin has a virtually identical absorbance spectrum and visual appearance to myoglobin from animal muscle. FIG. **4** shows a comparison of reduced (heme iron 2+) and oxidized (heme iron 3+) soybean leghemoglobin (FIG. **4** panel A) and equine heart muscle myoglobin (FIG. **4** panel B) showing similarity of UV-VIS absorption profiles of two proteins. We purified soybean leghemoglobin from soybean root-nodules using here described protocol. Purified equine myoglobin was purchased from SigmaAldrich. Soybean leghemoglobin (FIG. **4** panel A) and equine myoglobin (FIG. **4** panel B) were reduced with 1 mm sodium hydrosulfite. Shown are UV-VIS absorption spectra of heme Fe3+ (blue line) and heme Fe2+ (red line) of soybean leghemoglobin (FIG. **4** panel A) and equine myoglobin (FIG. **4** panel B). Insets show a zoom-in of UV-VIS spectra in 450 nm to 700 nm region. (FIG. **4** panel C) Images of 10 ul liquid droplet of a 40 mg/ml solution of soybean leghemoglobin in the heme-Fe3+ state (left droplet) showing characteristic rusty red color and a 40 mg/ml solution of soybean leghemoglobin solution in the heme-Fe2+ state (right droplet) showing characteristic red color of and (right image) corresponding samples of equine myoglobin.

[0378] In other embodiments, leghemoglobin can be sourced from non-plant sources, such as from organisms such as bacteria or yeast which have been genetically modified to express high levels of leghemoglobin.

[0379] The oxidation state of the iron ion in leghemoglobin is important for its color. Leghemoglobin with the heme iron in the +2 oxidation state appears vivid red in color, while leghemoglobin with the heme iron in the +3 oxidation state appears brownish red. Thus, in using leghemoglobin as a source of red color in a meat replica, it is desirable to reduce the heme iron from the +3 state to the +2 state. Heme iron in leghemoglobin can be switched from oxidized (+3) state to reduced (+2) state with reducing reagents. Examples of successful reduction of leghemoglobin heme iron with sodium hydrosulfite and titanium citrate are illustrated in FIG. **5**. In FIG. **5** the UV-VIS spectrogram of purified soybean leghemoglobin in which the heme iron is in the oxidized (+3) state is represented by the blue curves in each panel. The red curves in each panel represent the UV-VIS spectra of the same leghemoglobin species after reduction to the (+2) state (red lines) by addition of (Panel A) 1 mM sodium hydrosulfite or (Panel B) 0.24% (wt/v) titanium citrate in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride buffer. The Insets show a zoom-in of UV-VIS spectra in 450-700 nm region. For this example, leghemoglobin was purified from soybean root nodules using 60/90% ammonium sulfate fractionation and exchanged into 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride buffer. Sodium hydrosulfite stock solution was prepared by dissolving 100 mM sodium hydrosulfite in 1 mM sodium hydroxide in water. Titanium citrate stock solution was prepared from 20% (wt/v) Ti-chloride in hydrochloric acid by mixing it with 0.2M sodium citrate (1:10 v/v). pH was adjusted using sodium carbonate to pH 7.0.

[0380] Leghemoglobin can be purified from legume root nodules, such as the root nodules of peas or soybeans (FIG. **1** shows Leghemoglobin isolated from pea root nodules). Root nodules from soy beans were thoroughly cleaned to remove soil and extraneous root tissues prior to root nodule lysis in 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mm EDTA and 1 mM ascorbic acid. Root nodules were lysed by grinding root-nodules using a Vitamix blender. For some samples Polyvinylpyrrolidone polymer was added at 30% wt/v to aid in removal of plant phenolic small molecules that mediate oxidation of leghemoglobin heme-iron. Root nodule lysate was fractionated using ammonium sulfate in two steps, first ammonium sulfate was added to 60% wt/v. Pellet was discarded and supernatant brought to 90% wt/v. ammonium sulfate. Leghemoglobin was collected as a precipitated pellet in 90% ammonium sulfate fraction. Ammonium sulfate precipitated leghemoglobin was resuspended in 20 mM potassium phosphate, 1 mM EDTA, 50 mM sodium chloride and ammonium sulfate was removed using dialysis or size-exclusion chromatography in the same buffer. In some instances this was the last purification step, while in other instances leghemoglobin was further purified using anion-exchange chromatography (FFQ GE Healthcare), which was sometimes followed by size-exclusion chromatography (Sephacryl S-100, GE Healthcare). Soybean leghemoglobin from 90% ammonium sulfate pellet was loaded on anion exchange columns (FFQ or DEAE, GE Healthcare) in different buffers (20 mM potassium phosphate pH 7.4, containing 0 to 100 mM sodium chloride, 20 mM Tris pH 8 containing 0 to 100 mM sodium chloride, 20 mM sodium borax pH 9.8, 20 mM sodium chloride, 20 mM sodium carbonate pH 9, 20 mM sodium chloride) and purified either in flow-through or using sodium chloride (0-1M salt gradient). An example of the leghemoglobin purification flow from soybean root nodules is represented in FIG. **6**. The figure shows SDS-PAGE fractionation of different soybean leghemoglobin purification steps (Lane 1) Soybean root-nodule lysate; (Lane 2) Soybean root-nodule lysate purified by 60/90% (wt/v) ammonium sulfate fractionation. Shown is the protein content of 90% ammonium sulfate fractionated protein pellet resuspended in 20 mm potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mM EDTA buffer; Proteins from 90% ammonium sulfate pellet were further purified by anion-exchange chromatography (FFQ GE Healthcare) in 20 mM potassium phosphate ph 7.4, 100 mM sodium chloride. Leghemoglobin collected in the flowthrough is shown in Lane 3. Anion-exchange flowthrough was fractionated using size-exclusion chromatography (Sephacryl S-100 GE Healthcare) and resulting leghemoglobin fraction is shown in Lane 4. Leghemoglobin content at different purification steps was determined by

EXHIBIT 1B

determining the fraction of leghemoglobin band on SDS-PAGE gel in a respective sample using ImageDoc analysis software (BioRad). Purity (partial abundance) of leghemoglobin at respective steps in the purification steps was: lysate: 32.7% (lane 1), 60/90% (wt/v) ammonium sulfate fractionation 78% (lane 2), anion-exchange chromatography ~83% (lane 3), and size-exclusion chromatography to ~95% (lane 4).

[0381] Leghemoglobin can also be produced by genetically engineering a bacterium or fungus to produce it. One illustrative example is shown in FIG. **7**. FIG. **7** shows stained SDS-PAGE gel analysis of (A) soybean leghemoglobin expressed and purified using recombinant protein technology and (B) soybean leghemoglobin purified from soybean root nodules. (A) Recombinant Soybean leghemoglobin A carrying His-tag and TEV protease His-tag removal site was expressed in *E. coli* BL21 strain and purified using His-tag affinity chromatography (Talon resin, CloneTech). The left lane contains molecular weight standards, the right lane contains purified recombinant soybean leghemoglobin A (arrow). Expected molecular weight of the recombinant soybean leghemoglobin A is 17.1 kDa. (B) SDS-PAGE gel of purified Soybean leghemoglobin from root nodules. The left lane contains molecular weight standards, the right lane contains purified soybean leghemoglobin A (arrow). Mass spectrometry analysis of purified material determined that all four soybean leghemoglobin isoforms are present, and are full length (data not shown). Expected molecular weights (MW) of soybean leghemoglobin isoforms range from MW15.4 to 15.8 kDa.

[0382] Leghemoglobin purified from soybean and fava root nodules, respectively was tasted by a panel of volunteers and in each case described as tasting like blood.

[0383] Leghemoglobin can be isolated from the roots nodules of legumes such as soy beans, fava beans, cow peas, lima beans, garbanzo beans, peas, lupine, lotus japonicum or other legumes. The root nodule (for example root nodules from a pea plant) is obtained and homogenized in an aqueous solution, soluble proteins including leghemoglobin are recovered after insoluble matter is removed by precipitation or filtration. Leghemoglobin can be purified by selective precipitation and/or chromatography and/or the use of molecules with specific affinity for leghemoglobin. (FIG. **1**, showing 100 mls of solution of leghemoglobin isolated from 30 grams of pea root nodules).

[0384] Heme proteins, for example leghemoglobin, can be combined with other plant based meat replica components. In some embodiments the heme proteins are captured in a gel which contains other components, for example lipids and or proteins. In some aspects a multiple gels are combined with non-gel based heme proteins. In some embodiments the combination of the heme proteins and the other compounds of the consumable are done to insure that the heme proteins are able to diffuse through the consumable. In some embodiments the consumable is ed in a heme-protein containing solution, for instance a leghemoglobin solution. In some embodiments the consumable is soaked in a heme protein containing solution, for instance a leghemoglobin solution for 1, 5, 10, 15, 20 or 30 hours. In some embodiments the consumable is soaked in a heme containing solution, for instance a leghemoglobin solution for 1, 5, 10, 15, 30, or 45 minutes.

[0385] FIG. **8** shows an example of 6 cubes of a commercial meat analog (Quorn chicken analog), about 1 cm on a side, 4 of which (Left and lower right) have been soaked in a solution of about 10 mg/ml soybean leghemoglobin in 20 mM Potassium phosphate pH 7.4 and 100 mM NaCl; the remaining two (Upper right) were soaked in the same buffer without leghemoglobin. Note the deep pink color of the leghemoglobin-infused cubes in contrast to the pale tan color of the uninfused cubes.

[0386] FIG. **9** shows the 4 leghemoglobin-infused cubes of Quorn chicken analog in the process of cooking in a pan at 350° C. The two lower cubes have been turned over to expose the grilled surface, which has turned brown. Note in the upper two cubes that the heated portion has turned grey-brown, while the cooler top surface retains its pink color. In some embodiments the consumable is injected with a heme containing solution, for instance a leghemoglobin solution, until the consumable is the color of uncooked meat.

[0387] Given the usefulness of heme proteins for coloring consumables it will be useful to detect whether a product contains a particular heme protein. Accordingly the present invention includes in some embodiments methods to determine whether a product contains a heme protein. Methods for detecting proteins are well known in the art. For example an ELISA or proximity-ligation assay or luninex assay or western blot analysis can be performed to determine whether leghemoglobin is present in a food product such as meat or a meat replica. In one embodiment the detection methods are performed to determine whether meat has been altered with leghemoglobin.

EXAMPLES

[0388] An exemplary muscle replica composition comprising one or more isolated, purified plant proteins is described herein.

[0389] Protein Purification for Components of the Replica

[0390] Moong bean seeds, Green Pea dry seed were purchased as milled flour and used for purification of respective seed storage proteins. Rubsico was purified from fresh alfalfa plant. Protein composition at individual fractionation steps was monitored by SDS-PAGE and protein concentrations were measured by standard UV-VIS and Pierce assay methods.

[0391] Moong bean 8S globulins: Moong bean flour was resuspended in in 50 mM potassium phosphate buffer pH 7 and 0.5M NaCl at 1:4 (wt/v) ratio, and mixture was incubated for 1 hr. Unsoluble material was separated by centrifugation and proteins in the supernatant were fractionated by addition of ammonium sulfate in 2 steps: 50% (wt/v) followed by 90% (wt/v). Protein precipitated in 90% fraction contained the moong bean 8S globulins and was stored at −20 C until further use.

[0392] Pea-albumins: Green pea dry seed flour was resuspended at 1:10 (wt/v) ratio in 50 mM sodium acetate buffer pH 5 and incubated for 1 hr. Unsoluble material was separated by centrifugation and proteins in the supernatant were fractionated by ammonium sulfate precipitation in two steps: 50% (wt/v) followed by 90% (wt/v). Ammonium sulfate solutions were stirred for 1 hour and ammonium sulfate precipitated proteins removed by centrifugation. Proteins of interest precipitated in 90% (wt/v) ammonium sulfate. Pellet was stored at −20 C until further use.

[0393] Pea-globulins: Green pea dry seed flour was resuspended at 1:10 (wt/v) ratio in 20 mM potassium phosphate buffer pH 8, 0.4M sodium chloride and stirred for 1 hr. After centrifugation, the supernatant was subjected to ammonium sulfate fractionation. First, supernatant was brought to 50%

(wt/v) ammonium sulfate, and precipitated proteins removed. Second, 50% (wt/v) ammonium sulfate supernatant was brought to 80% (wt/v) ammonium sulfate saturation. The 80% (wt/v) ammonium sulfate pelleted protein contained globulins of interest. Pellet was stored at −20° C. until further use.

[0394] RuBisCO: RuBisCO was fractionated from alfalfa greens (or other green plants eg soybean plants, spinach etc) by first grinding leaves with 4 volumes of cold 50 mM KPhosphate buffer pH 7.4 buffer (with (in lab) or without (in field) 0.5M NaCl+2 mM DTT+1 mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (crude lysate) was used in further purification steps. Proteins in the crude lysate were fractionated by addition of ammonium sulfate to 30% (wt/v) saturation. The solution was stirred for 1 hr and then centrifuged. The pellet from this step was discarded and additional ammonium sulfate was added to the supernatant to 50% (wt/v) ammonium sulfate saturation. The solution was centrifuged again after stirring for 1 hr. The pellet from this step contains RuBisCO, and was kept at −20 C until used.

[0395] Obtaining Plant Proteins.

[0396] Moong bean seed 8S protein was purified by ammonium sulfate fractionation as described. Pellet was resuspended in 20 mM potassium phosphate pH pH 7.4 and 0.5M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16000 g, 10 min and protein concentrated to desired concentration. Pea globulins purified by ammonium sulfate fractionation as described. Protein pellet was resuspended in 20 mM potassium phosphate pH pH 7.4 and 0.4M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16000 g, 10 min and protein concentrated to desired concentration. Pea albumin purified by ammonium sulfate fractionation as described. Protein pellet was resuspended in 20 mM potassium phosphate pH pH 7.4 and 0.1M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16000 g, 10 min and protein concentrated to desired concentration.

[0397] Constructing a Muscle Tissue Analog

[0398] Moong bean seed 8S protein was purified by ammonium sulfate fractionation as described above, For preparation of gels, 200 g of pellet was dissolved in 400 ml of dialysis buffer (20 mM potassium phosphate, 400 mM NaCl, pH 7.3) and the resulting solution dialyzed for 6 hours against 51 of dialysis buffer, replaced twice with fresh buffer. Protein solution was centrifuged at 12,000 g for 15 min to remove debris. Protein was concentrated by dialyzing for 36 hours against 51 of 30% w/w solution of PEG 8000 (polyethylene glycol, molecular weight 8000) in dialysis buffer. Final protein concentration was 150 mg/ml.

[0399] Leghemoglobin was purified from soybean root nodules. Legume root nodules were cleaned to remove soil and extraneous root tissues prior to root nodule lysis in 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mm EDTA and 1 mM ascorbic acid. Root nodules were lysed by grinding root-nodules using juicer blender. Unsoluble material was separated by centrifugation. Root nodule lysate was fractionated using ammonium sulfate in two steps, first ammonium sulfate was added to 60% wt/v and solution incubated for 1 hr, 4° C. Pellet was discarded and supernatant brought to 90% wt/v ammonium sulfate and incubated for 12 hr, 4° C. Leghemoglobin was collected as a precipitated pellet in 90% ammonium sulfate fraction and resuspended in 20 mM potassium phosphate, 1 mM EDTA, 100 mM sodium chloride. SDS-PAGE gel analysis determined that protein solution contains 70% leghemoglobin and 30% other root nodule proteins. Ammonium sulfate was removed using size-exclusion chromatography in the same buffer. Leghemoglobin was concentrated by dialyzing for 48 hr against 30% PEG 8000 (polyethylene glycol, molecular weight 8000) in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride. Total protein concentration was 57 mg/ml. UV-VIS spectra suggested that leghemoglobin was in heme-iron oxidized state. Thus, leghemoglobin was incubated with 5 mM sodium hydrosulfite for 5 min and sodium hydrosulfite was removed using size-exclusion chromatography in 20 mM potassium phosphate, 100 mM sodium chloride buffer. Leghemoglobin was further concentrated to 35.4 mg/ml. UV-VIS spectra analysis confirmed that leghemoglobin is in heme-iron reduced state.

[0400] Transglutaminase was obtained commercially from (Activa TI, Ajimoto). Stock solution (20% wt/v) was made in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride buffer.

[0401] To prepare "dark" muscle tissue analog (FIG. **10**), 43 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 37 ml of leghemoglobin solution (46.5 mg/ml leghemoglobin and 20 mg/ml of other soybean root nodule protein) in 20 mM potassium phosphate, 100 mM NaCl, pH 7.3). 20 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly mixed, divided into two 50 ml Falcon tubes and incubated overnight at room temperature. Final protein concentrations were 65 mg/ml for moong bean protein, 18 mg/ml of leghemoglobin, 91 mg/ml total protein.

[0402] "White" muscle analog (FIG. **11**) was prepared by mixing 43 ml moong bean protein solution (150 mg/ml) with 45 ml of 11.7 mg/ml solution of leghemoglobin and 0.8% (wt/v) of transglutaminase solution. Final protein concentrations were 63 mg/ml for moong bean protein, 5.2 mg/ml of leghemoglobin, 68 mg/ml total protein.

[0403] The "dark" muscle tissue analog formed an opaque gel of dark chocolate color, smooth uniform texture, with glistening surface, and a small amount (<1 ml) of dark red, venous blood colored liquid on top. The gel was freely standing, elastic but fragile, similar in appearance to thin Jell-O. The gel has a medium aroma with notes of beans and blood clearly discernible. The flavor is dominated by notes of beans and iron/blood, with weaker grassy and medicinal/chemical flavors. The taste is salty, with a long aftertaste of blood.

[0404] The "white" muscle tissue analog was very similar, but with much lighter, cappuccino-like, color. It was also more fragile, 2-3-fold less strong against compression.

[0405] Fat Tissue Analog

[0406] Fat tissue analog using moong bean 8S globulin fraction was prepared as follows: 15 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 15 ml of rice bran oil. 6 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly emulsified using a homogenizer (VWR) at speed #2. Emulsion was aliquoted into 1.6 eppendorf tubes and incubated overnight at room temperature. After that, tubes were heated at 95° C. for 5 min in a heat block, and allowed to cool down to room temperature on a bench. Final concentrations were 75 mg/ml for moong bean protein, 50% w/w oil.

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[0407] Fat tissue analog using pea globulin (100 mg/ml) was prepared by the same method. Additionally, fat tissue analog was prepared from pea globulin, and either rice bran or canola oil, in bulk by the same method, but without aliquoting emulsions into eppendorf tubes. Instead, emulsions in 50 ml Falcon tubes were rotated overnight on a nutator, and were subsequently incubated at 90° C. for 30 min.

[0408] Fat tissue analog based on moong beans (FIG. **12**) and prepared in eppendorf tubes formed an opaque gel of off-white color, smooth uniform texture, with no visible discernible liquid that was not incorporated into the gel. The gel was freely standing, elastic and springy. The gel has a slight, pleasant aroma and a mild and pleasant flavor. The taste is mildly salty.

[0409] Fat tissue analog based on pea globulin (FIG. **13**) and prepared in eppendorf tubes was very similar to moong bean-based fat analog, except that it gave up a little of oil upon compression. Fat tissue analog prepared in 50 ml Falcon tubes were similar in appearance, texture and aromas, but substantially softer (2-fold softer for canola oil, and 3-fold softer for rice bran oil, according to compressibility measurements).

[0410] Connective Tissue Analog

[0411] Connective tissue analog prototypes were developed using zein protein sourced from 100% yellow corn gluten meal, or from commercial sources, such as Amazein (Prairie Gold, Bloomington, Ill.). Zein proteins were solubilized in 70-90% ethanol with desired ratios at 1:3 to 1:5 (solids:solution). By precipitating zein proteins, for example by a change in pH, in a controlled manner, large zein structures result with physicochemical properties that can be manipulated as desired. For example, FIG. **14** shows connective-tissue analog strands that were created using a 1:3 ratio in 70% ethanol, loaded into a syringe with a 23 gauge needle (ID 0.337 mm). The solution was slowly extruded from the bottom of a 5 inch-high vessel into an excess of 5 M NaCl solution. The ethanol-zein solution being less dense than the NaCl solution, floated upward, drawing out a fibrous stand of solidifying zein. The NaCl was constantly stirred as the strands began to develop to assist in the strand lengthening. The strands bunch together and become a hard, dense mass.

[0412] Ground Beef Replica Prototypes Made from Gels of Plant Proteins and Plant Oils.

[0413] A ground beef prototype patty was made by combining 62% (wt/wt) muscle analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "white muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (FIG. **15** panel A). A ground beef prototype patty was made by combining 62% muscle analog (62% "dark muscle analog" and 38% "white muscle analog), 29% fat tissue analog (from moong bean seed 8S protein and rice bran oil), 5% connective tissue analog (FIG. **15** panel B). A ground beef prototype patty was made by combining 71% (wt/wt) muscle tissue analog (composed of 60% "white" muscle analog, 40% "dark" muscle analog), 23% fat tissue (from pea seed globulin proteins and canola oil) (FIG. **15** panel C). A ground beef prototype patty was made by combining 67% "White" muscle analog, with 28% fat tissue analog (from pea globulins and rice bran oil), (FIG. **15**, panel D)

[0414] In a further test, the effect of cooking the ground beef replica patties was evaluated by grilling on a 350° F. pan. A ground beef patty analog was made by combining 62% (wt/wt) muscle tissue analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (FIG. **16**). The panel on the left shows the patty before cooking and the panel on the right shows the same patty after cooking for about 2 minutes. Observers described the aroma of the cooking ground beef replica as distinctly "beefy".

SEQUENCE LISTING

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<400> SEQUENCE: 1

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Ser Ile Leu Glu Lys Ala Pro Ala Ala Lys Asp Leu Phe Ser Phe Leu
        35                  40                  45

Ala Asn Gly Val Asp Pro Thr Asn Pro Lys Leu Thr Gly His Ala Glu
    50                  55                  60

Lys Leu Phe Ala Leu Val Arg Asp Ser Ala Gly Gln Leu Lys Ala Ser
65                  70                  75                  80

Gly Thr Val Val Ala Asp Ala Ala Leu Gly Ser Val His Ala Gln Lys
                85                  90                  95

Ala Val Thr Asp Pro Gln Phe Val Val Val Lys Glu Ala Leu Leu Lys
            100                 105                 110
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-continued

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<400> SEQUENCE: 2

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            20                  25                  30

Phe Lys Glu Glu Pro Thr Val Ser Val Leu Phe Gln Asn Pro Ile Ser
        35                  40                  45

Ser Gln Ser Arg Lys Leu Met Gln Val Leu Gly Ile Leu Val Gln Gly
    50                  55                  60

Ile Asp Asn Leu Glu Gly Leu Ile Pro Thr Leu Gln Asp Leu Gly Arg
65                  70                  75                  80

Arg His Lys Gln Tyr Gly Val Val Asp Ser His Tyr Pro Leu Val Gly
                85                  90                  95

Asp Cys Leu Leu Lys Ser Ile Gln Glu Tyr Leu Gly Gln Gly Phe Thr
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            20                  25                  30

Arg Val Lys His Tyr Phe Lys Asn Thr Asn Met Glu His Gln Ala Lys
        35                  40                  45

Gln Gln Glu Asp Phe Leu Thr Met Leu Leu Gly Gly Pro Asn His Tyr
    50                  55                  60

Lys Gly Lys Asn Met Ala Glu Ala His Lys Gly Met Asn Leu Gln Asn
65                  70                  75                  80

Ser His Phe Asp Ala Ile Ile Glu Asn Leu Ala Ala Thr Leu Lys Glu
                85                  90                  95

Leu Gly Val Ser Asp Gln Ile Ile Gly Glu Ala Ala Lys Val Ile Glu
            100                 105                 110

His Thr Arg Lys Asp Cys Leu Gly Lys
        115                 120
```

1.-162. (canceled)

163. A meat substitute product, comprising:

a) a muscle replica;

b) a fat tissue replica; and

c) a connective tissue replica;

wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat.

164. The meat substitute product of claim **163**, comprising two or more of said muscle replica, fat tissue replica, and connective tissue replica.

165. The meat substitute product of claim **163**, wherein (i) said muscle replica accounts for 40-90% of said product by weight, (ii) said fat tissue replica accounts for 1-60% of said product by weight, and (iii) said connective tissue replica accounts for 1-30% of said product by weight.

166. The meat substitute product of claim **163**, comprising:

a) 60-90% water;

b) 5-30% protein content;

c) 1-20% of a fat or fat replica;

wherein said protein content comprises one or more isolated, purified plant proteins.

167. The meat substitute product of claim **166**, wherein said protein content is derived from a non-animal source.

168. The meat substitute product of claim **167**, wherein said non-animal source is a plant source, or a genetically modified yeast or bacteria.

169. (canceled)

170. The meat substitute product of claim **163**, wherein 50% or more of said protein content by weight are one or more isolated purified proteins.

171. The meat substitute product of claim **170**, wherein each of said one or more isolated proteins is isolated and purified separately from different plant species.

172. The meat substitute product of claim **170**, wherein one or more of said isolated proteins is selected from the group consisting of: hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

173. The meat substitute product of claim **163**, further comprising one or more isolated and purified iron-containing proteins.

174. The meat substitute product of claim **173**, wherein said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, and flavohemoglobins.

175. The meat substitute composition of claim **174** wherein said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1.

176. The meat substitute product of claim **163**, wherein said meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

177. The meat substitute product of claim **174**, wherein said meat substitute product contains less than 1% wheat gluten.

178. The meat substitute product of claim **175**, wherein said meat substitute product contains no wheat gluten.

179. The meat substitute product of claim **176**, wherein said meat substitute product is characterized by one or more of the following contains no soy protein isolate, no soy protein concentrate, or no tofu.

180. (canceled)

181. (canceled)

182. The meat substitute product of claim **179**, wherein said meat substitute product contains less than 5% carbohydrates.

183. (canceled)

184. The meat substitute product of claim **163**, wherein said meat substitute product is characterized by one or more of the following: contains no tofu, no soy protein, contains less than 1% cellulose, contains less than 5% insoluble carbohydrates, or no wheat gluten.

185. (canceled)

186. The meat substitute product of claim **163**, wherein said meat substitute product contains no animal products and less than 5% carbohydrates.

187. (canceled)

188. (canceled)

189. (canceled)

190. (canceled)

191. (canceled)

192. The meat substitute product of claim **163**, wherein said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.

193-222. (canceled)

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EXHIBIT 1B

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(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, FT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, Cl, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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(54) **Title:** METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES

(57) Abstract: This document relates to food products containing highly conjugated heterocyclic rings complexed to an iron ion and one or more flavor precursors, and using such food products to modulate the flavor and/or aroma profile of other foods. The food products described herein can be prepared in various ways and can be formulated to be free of animal products.

EXHIBIT 1C

(54) METHOD METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES

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(22) Filed: **Jul. 12, 2013**

Related U.S. Application Data

(63) Continuation-in-part of application No. PCT/US2012/ 046560, filed on Jul. 12, 2012.

EXHIBIT 1C

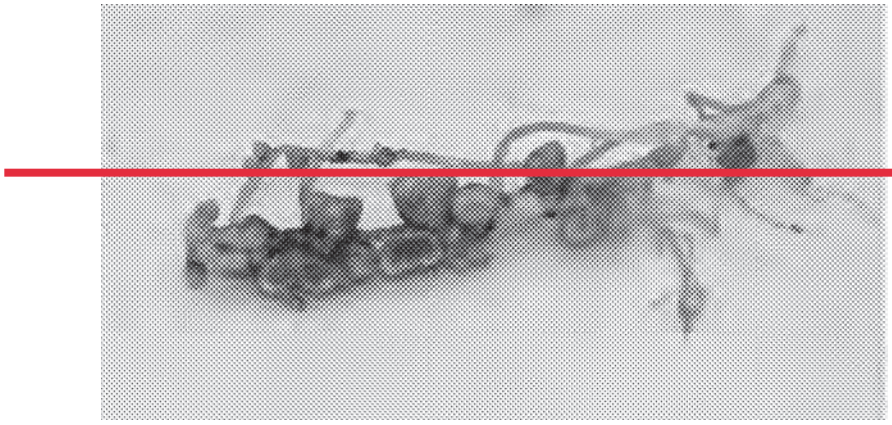


Figure 1

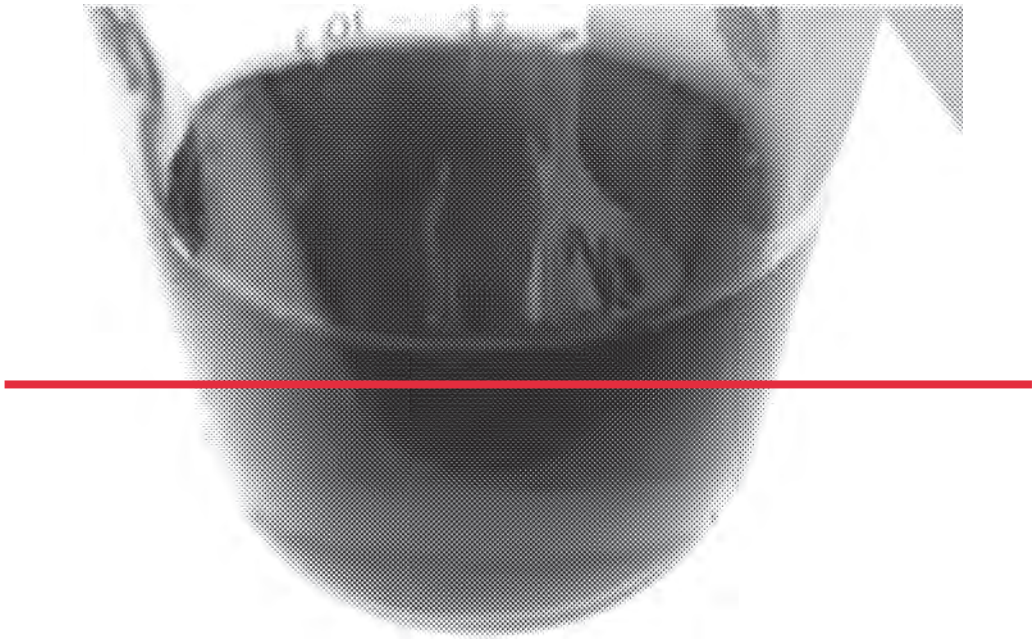


Figure 2

EXHIBIT 1C

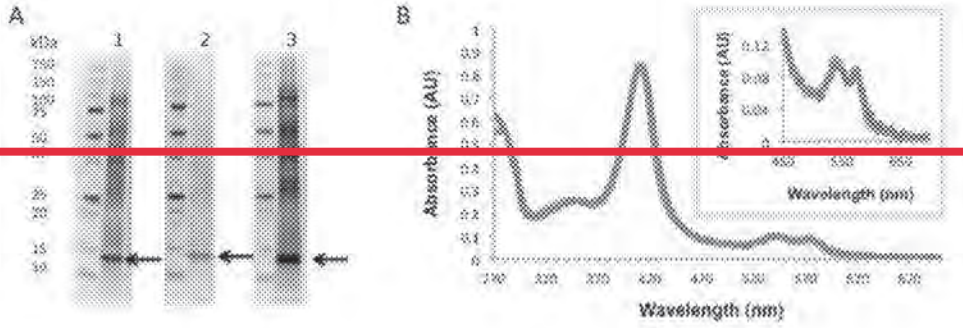


Figure 3

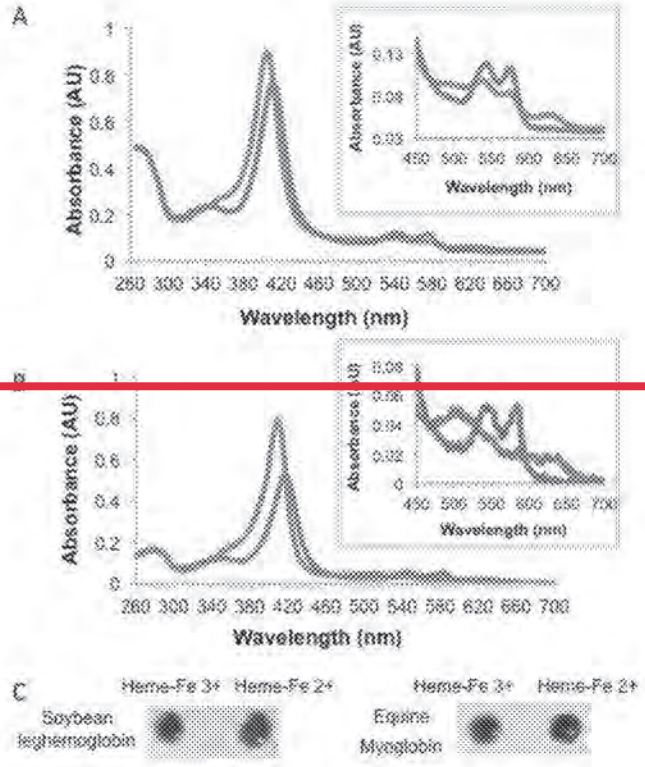


Figure 4

EXHIBIT 1C

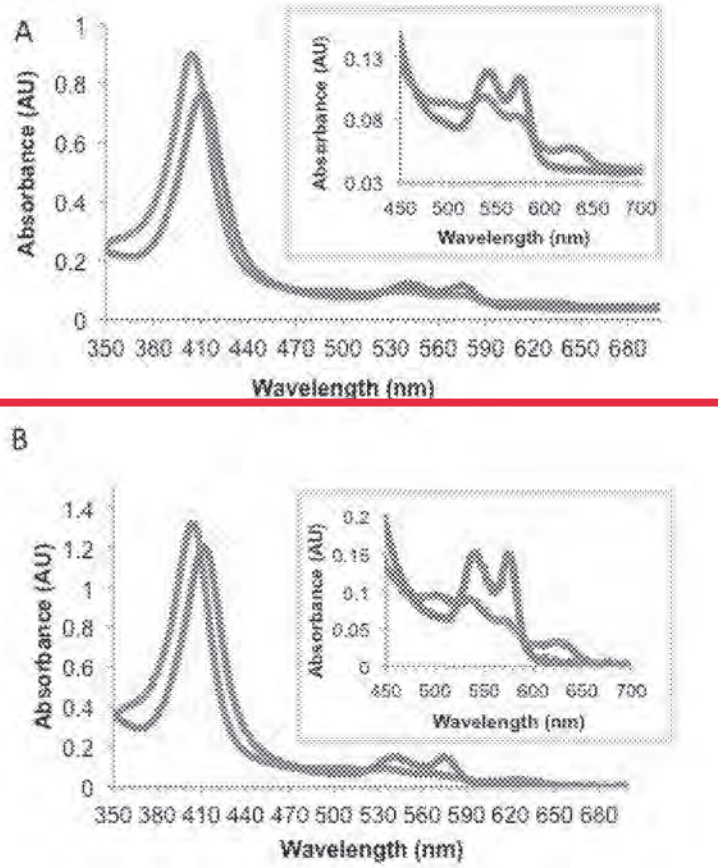


Figure 5

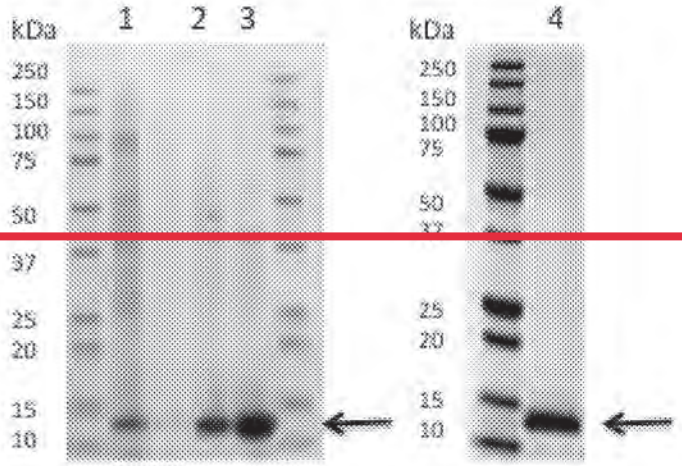


Figure 6

EXHIBIT 1C

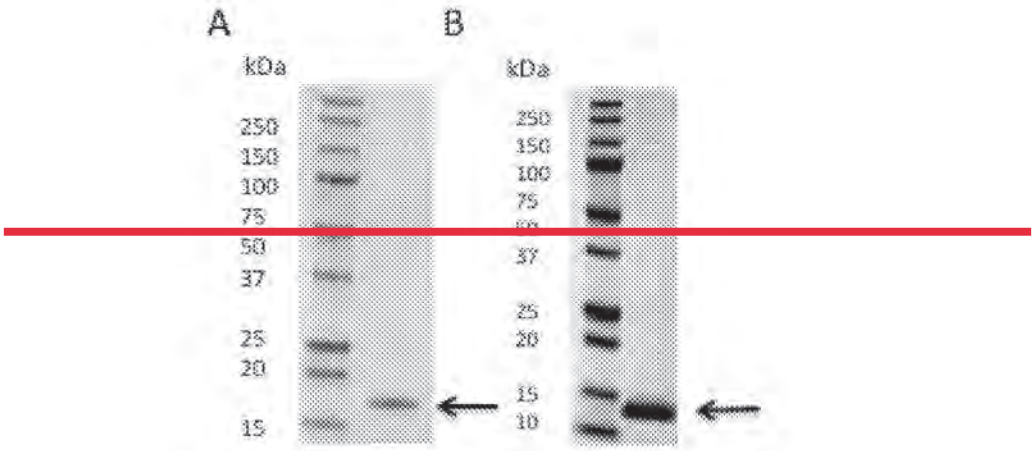


Figure 7

EXHIBIT 1C



Figure 8

EXHIBIT 1C

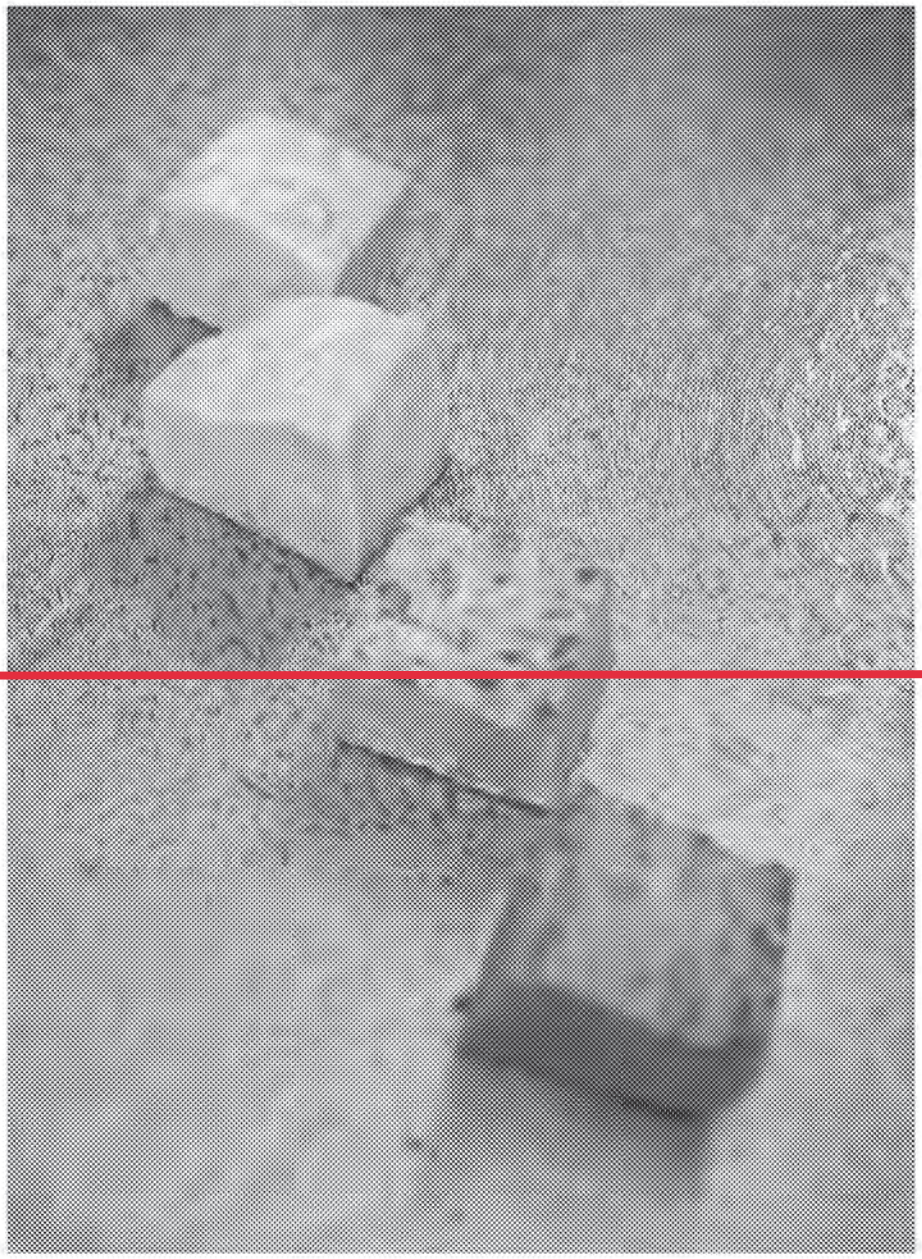


Figure 9

EXHIBIT 1C

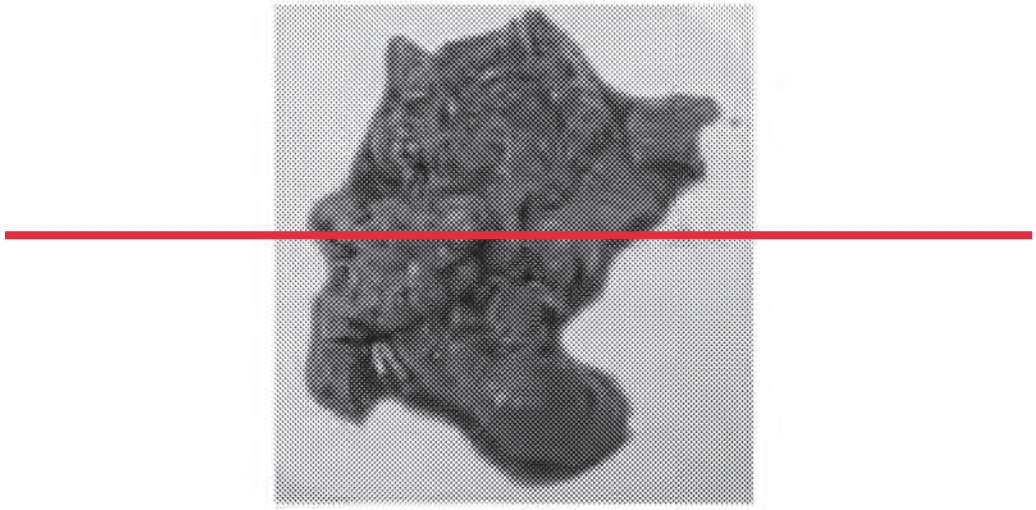


Figure 10

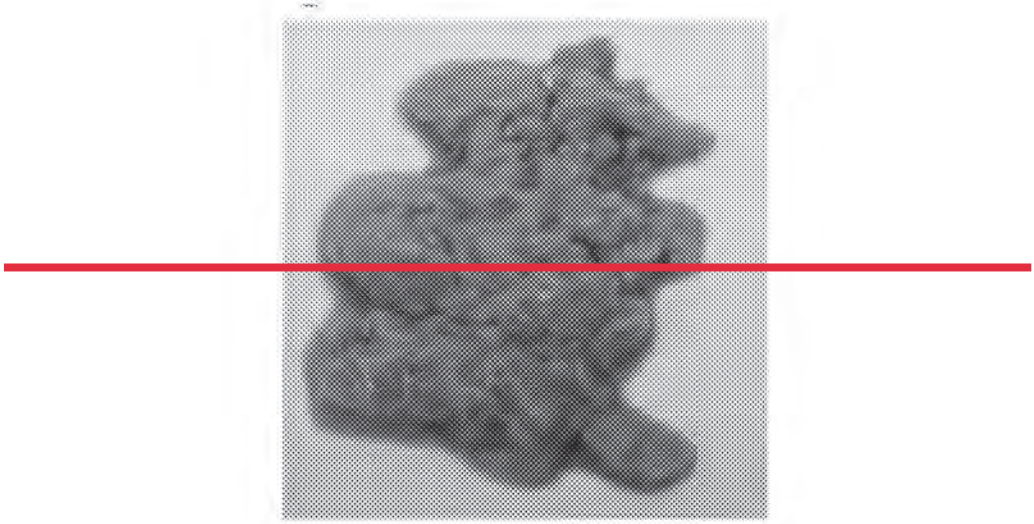


Figure 11

EXHIBIT 1C

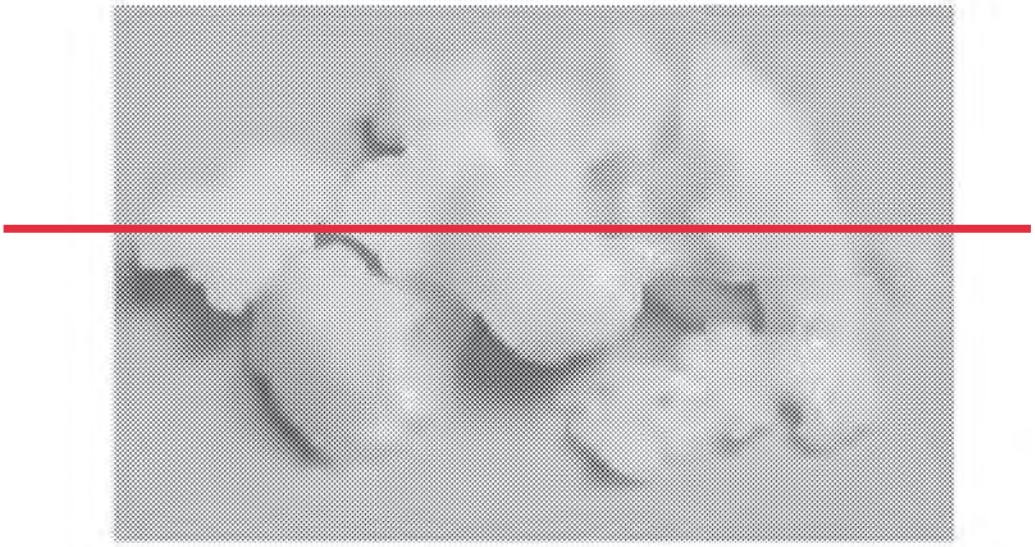


Figure 12

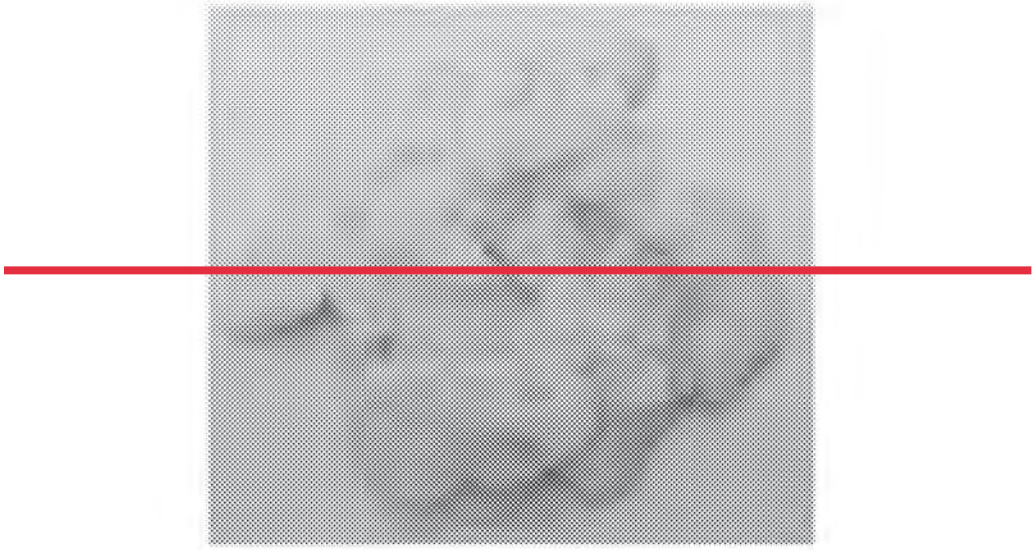


Figure 13

EXHIBIT 1C

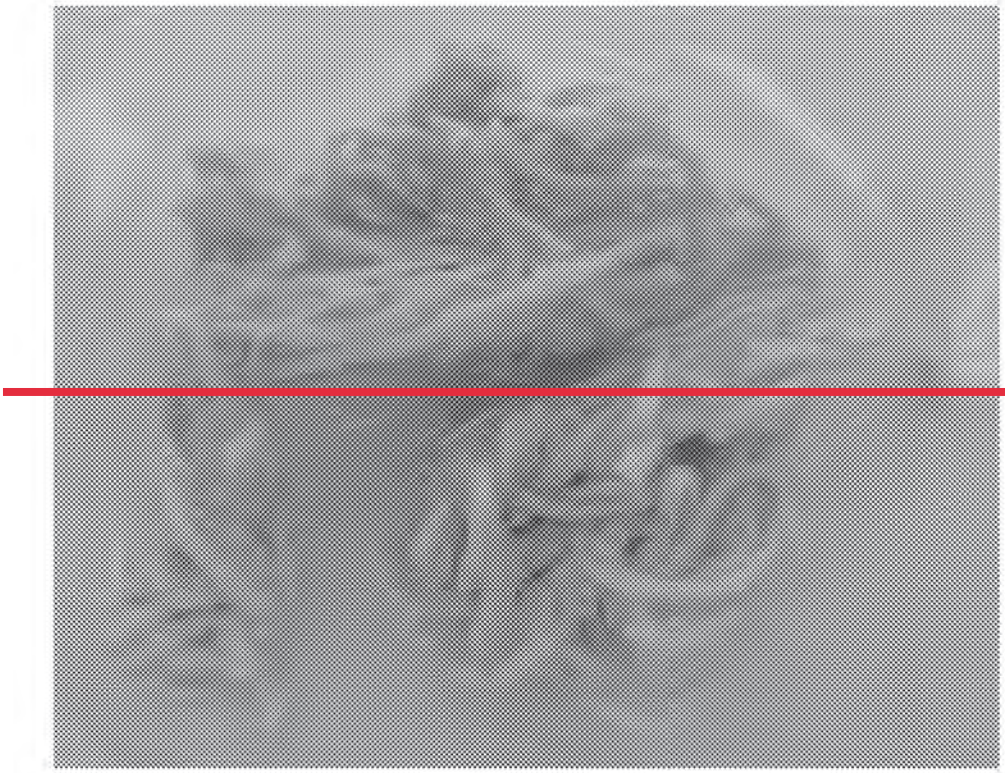


Figure 14

EXHIBIT 1C

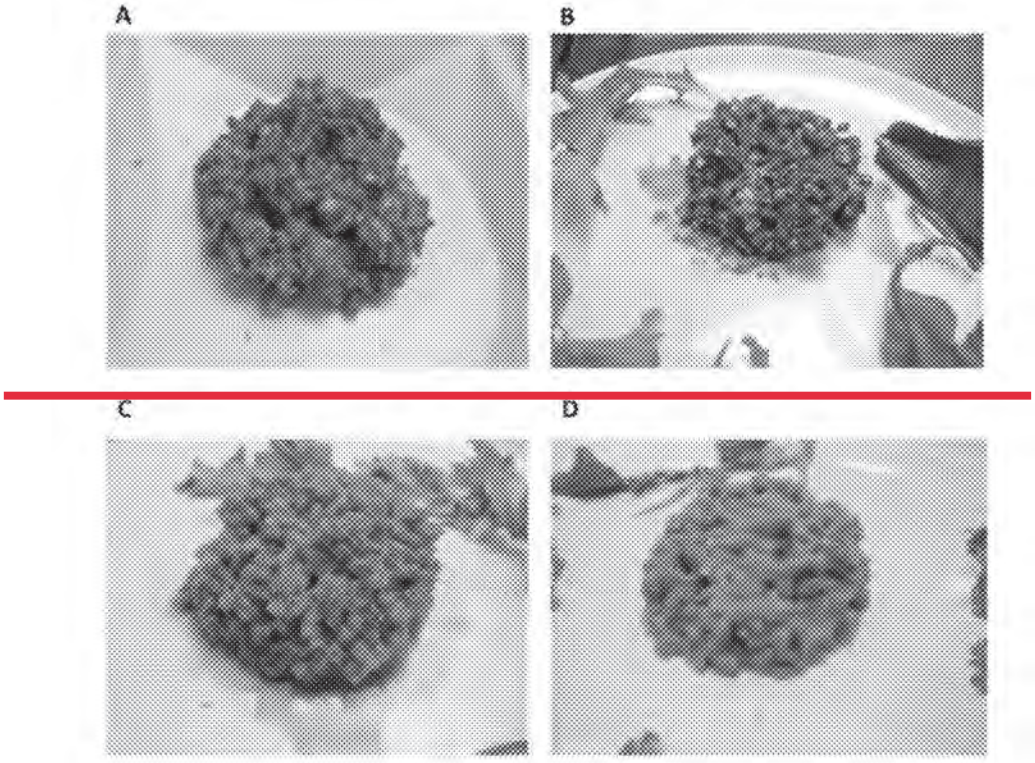


Figure 15

EXHIBIT 1C

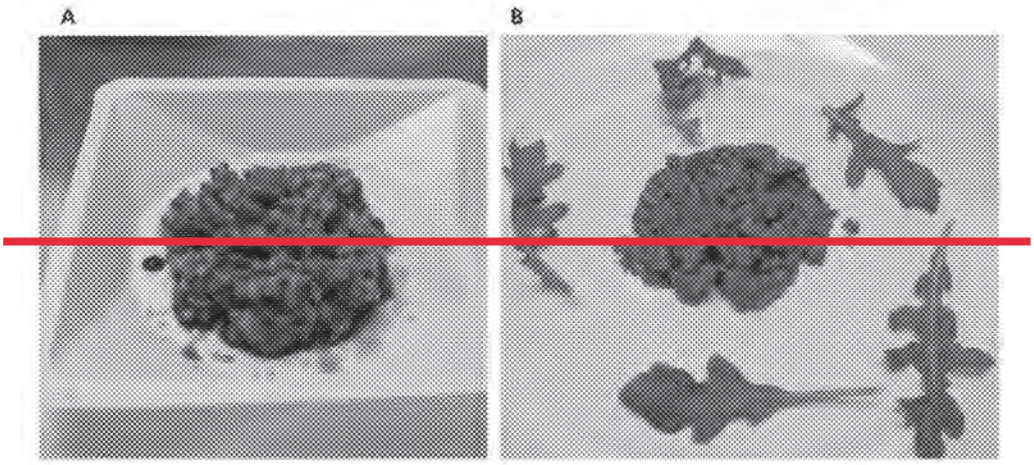


Figure 16

EXHIBIT 1C

FIGURE 1

SEQ ID NO:1 *Vigna radiata*

MTTTLERGFTEEQEALVVKSWNVMKKNSGELGLKFFLKIFEIAPSAQKLFSFLRDSTVP
LEQNPKLKPHAVSVFVMTCDSAVQLRKAGKVTVRESNLKKLGATHFRTGVANEHFEVTK
FALLETIKEAVPEMWSPAMKNAWGEAYDQLVDAIKYEMKPPSS

SEQ ID NO:2 *Methylacidiphilum infernorum*

MIDQKEKELIKESWKRIEPNKNEIGLLFYANLFKEEPTVSVLFQNPISSQSRKLMQVLG
ILVQGIDNLEGLIPTLQDLGRRHKQYGVVDSHYPLVGDCLLKSIQEYLGQGFTEEAKAA
WTKVYGIAAQVMTAE

SEQ ID NO:3 *Aquifex aeolicus*

MLSEETIRVIKSTVPLLKEHGTEITARMYELLFSKYPKTKELFAGASEEQPKKLANAII
AYATYIDRLEELDNAISTIARSHVRRNVKPEHYPLVKECLLQAIEEVLNPGEEVLKAWE
EAYDFLAKTLITLEKKLYSQP

SEQ ID NO:4 *Glycine max*

MGAFTEKQEALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLSNGVDPSNPKL
TGHAEKLFGLVRDSAGQLKANGTVVADAALGSIHAQKAITDPQFVVVKEALLKTIKEAV
GDKWSDELSSAWEVAYDELAAAIKKAF

SEQ ID NO:5 *Hordeum vulgare*

MSAAEGAVVFSEEKEALVLKSWAIMKKDSANLGLRFFLKIFEIAPSARQMFPFLRDSDV
PLETNPKLKTHAVSVFVMTCEAAAQLRKAGKITVRETTLKRLGGTHLKYGVADGHFEVT
RFALLETIKEALPADMWGPEMRNAWGEAYDQLVAAIKQEMKPAE

Magnaporthe oryzae, (SEQ ID NO:6)

```
   1 mdgavrldwt gldltgheih dgvpiasrvq vmvsfplfkd qhiimsskes psrksstigq
  61 strngscqad tqkgqlppvg ekpkpvkenp mkklkemsqr plptqhgdgt yptekkltgi
 121 gedlkhirgy dvktllamvk sklkgeklkd dktmlmervm qlvarlptes kkraeltdsl
 181 inelwesldh pplnylgpeh syrtpdgsyn hpfnpqlgaa gsryarsvip tvtppgalpd
 241 pglifdsimg rtpnsyrkhp nnvssilwyw atiiihdifw tdprdintnk sssyldlapl
 301 ygnsqemqds irtfkdgrmk pdcyadkrla gmppgvsvll imfnrfhnhv aenlalineg
 361 grfnkpsdll egeareaawk kydndlfqva rlvtsglyin itlvdyvrni vnlnrvdttw
 421 tldprqdaga hvgtadgaer gtgnavsaef nlcyrwhsci sekdskfvea qfqnifgkpa
 481 sevrpdemwk gfakmeqntp adpgqrtfgg fkrgpdgkfd dddlvrcise avedvagafg
 541 arnvpqamkv vetmgiiqgr kwnvaglnef rkhfhlkpys tfedinsdpg vaealrrlyd
```

```
 601 hpdnvelypg lvaeedkqpm vpgvgiapty tisrvvlsda vclvrgdrfy ttdftprnlt
 661 nwgykevdyd lsvnhgcvfy klfirafpnh fkqnsvyahy pmvvpsenkr ilealgradl
 721 fdfeapkyip prvnitsygg aeyiletqek ykvtwheglg flmgegglkf mlsgddplha
 781 qqrkcmaaql ykdgwteavk afyagmmeel lvsksyflgn nkhrhvdiir dvgnmvhvhf
 841 asqvfglplk taknptgvft eqemygilaa ifttiffdld psksfplrtk trevcqklak
 901 lveanvklin kipwsrgmfv gkpakdepls iygktmikgl kahglsdydi awshvvptsg
 961 amvpnqaqvf aqavdyylsp agmhyipeih mvalqpstpe tdalllgyam egirlagtfg
1021 syreaavddv vkedngrqvp vkagdrvfvs fvdaardpkh fpdpevvnpr rpakkyihyg
1081 vgphaclgrd asqiaitemf rclfrrrnvr rvpgpqgelk kvprpggfyv ymredwgglf
1141 pfpvtmrvmw dde
```

Fusarium oxysporum (SEQ ID NO:7)

```
   1 mkgsatlafa lvqfsaasql vwpskwdeve dllymqggfn krgfadalrt cefgsnvpgt
  61 qntaewlrta fhdaithdak agtggldasi ywessrpenp gkafnntfgf fsgfhnprat
 121 asdltalgtv lavgacngpr ipfragrida ykagpagvpe pstnlkdtfa aftkagftke
 181 emtamvacgh aiggvhsvdf peivgikadp nndtnvpfqk dvssfhngiv teylagtskn
 241 plvasknatf hsdkrifdnd katmkklstk agfnsmcadi ltrmidtvpk svqltpvlea
 301 ydvrpyitel slnnknkihf tgsvrvritn nirdnndlai nliyvgrdgk kvtvptqqvt
 361 fqggtsfgag evfanfefdt tmdakngitk ffiqevkpst katvthdnqk tggykvddtv
 421 lyqlqqscav leklpnaplv vtamvrdara kdaltlrvah kkpvkgsivp rfqtaitnfk
 481 atgkkssgyt gfqaktmfee qstyfdivlg gspasgvqfl tsqampsqcs
```

Fusarium graminearum (SEQ ID NO:8)

```
   1 masatrqfar aatratrngf aiaprqvirq qgrryyssep aqksssawiw ltgaavagga
  61 gyyfygnsas satakvfnps kedyqkvyne iaarleekdd yddgsygpvl vrlawhasgt
 121 ydketgtggs ngatmrfape sdhganagla aardflqpvk ekfpwitysd lwilagvcai
 181 qemlgpaipy rpgrsdrdvs gctpdgrlpd askrqdhlrg ifgrmgfndq eivalsgaha
 241 lgrchtdrsg ysgpwtfspt vltndyfrll veekwqwkkw ngpaqyedks tkslmmlpsd
 301 ialiedkkfk pwvekyakdn daffkdfsnv vlrlfelgvp faqgtenqrw tfkpthqe
```

SEQ ID NO: 9 *Chlamydomonas eugametos*

MSLFAKLGGREAVEAAVDKFYNKIVADPTVSTYFSNTDMKVQRSKQFAFLAYALGGASE
WKGKDMRTAHKDLVPHLSDVHFQAVARHLSDTLTELGVPPEDITDAMAVVASTRTEVLN
MPQQ

SEQ ID NO:10 *Tetrahymena pyriformis*

MNKPQTIYEKLGGENAMKAAVPLFYKKVLADERVKHFFKNTDMDHQTKQQTDFLTMLLG
GPNHYKGKNMTEAHKGMNLQNLHFDAIIENLAATLKELGVTDAVINEAAKVIEHTRKDM
LGK

SEQ ID NO:11 *Paramecium caudatum*

MSLFEQLGGQAAVQAVTAQFYANIQADATVATFFNGIDMPNQTNKTAAFLCAALGGPNA
WTGRNLKEVHANMGVSNAQFTTVIGHLRSALTGAGVAAALVEQTVAVAETVRGDVVTV

SEQ ID NO:12 *Aspergillus niger*

MPLTPEQIKIIKATVPVLQEYGTKITTAFYMNMSTVHPELNAVFNTANQVKGHQARALA
GALFAYASHIDDLGALGPAVELICNKHASLYIQADEYKIVGKYLLEAMKEVLGDACTDD
ILDAWGAAYWALADIMINREAALYKQSQG

SEQ ID NO:13 *Zea mays*

MALAEADDGAVVFGEEQEALVLKSWAVMKKDAANLGLRFFLKVFEIAPSAEQMFSFLRD
SDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRETTLKRLGATHLRYGVADGHF
EVTGFALLETIKEALPADMWSLEMKKAWAEAYSQLVAAIKREMKPDA

SEQ ID NO:14 *Oryza sativa subsp. japonica*

MALVEGNNGVSGGAVSFSEEQEALVLKSWAIMKKDSANIGLRFFLKIFEVAPSASQMFS
FLRNSDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRDTTLKRLGATHFKYGVG
DAHFEVTRFALLETIKEAVPVDMWSPAMKSAWSEAYNQLVAAIKQEMKPAE

SEQ ID NO:15 *Arabidopsis thaliana*

MESEGKIVFTEEQEALVVKSWSVMKKNSAELGLKLFIKIFEIAPTTKKMFSFLRDSPIP
AEQNPKLKPHAMSVFVMCCESAVQLRKTGKVTVRETTLKRLGASHSKYGVVDEHFEVAK
YALLETIKEAVPEMWSPEMKVAWGQAYDHLVAAIKAEMNLSN

SEQ ID NO:16 *Pisum sativum*

MGFTDKQEALVNSSWESFKQNLSGNSILFYTIILEKAPAAKGLFSFLKDTAGVEDSPKL
QAHAEQVFGLVRDSAAQLRTKGEVVLGNATLGAIHVQRGVTDPHFVVVKEALLQTIKKA
SGNNWSEELNTAWEVAYDGLATAIKKAMT

SEQ ID NO:17 *Vigna unguiculata*

MVAFSDKQEALVNGAYEAFKANIPKYSVVFYTTILEKAPAAKNLFSFLANGVDATNPKL
TGHAEKLFGLVRDSAAQLRASGGVVADAALGAVHSQKAVNDAQFVVVKEALVKTLKEAV
GDKWSDELGTAVELAYDELAAAIKKAY

SEQ ID NO:18 Bos taurus

MGLSDGEWQLVLNAWGKVEADVAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGNTVLTALGGILKKKGHHEAEVKHLAESHANKHKIPVKYLEFISDAIIHVLHA
KHPSDFGADAQAAMSKALELFRNDMAAQYKVLGFHG

SEQ ID NO:19 *Sus scrofa*

MGLSDGEWQLVLNVWGKVEADVAGHGQEVLIRLFKGHPETLEKFDKFKHLKSEDEMKAS
EDLKKHGNTVLTALGGILKKKGHHEAELTPLAQSHATKHKIPVKYLEFISEAIIQVLQS
KH PGDFGADAQGAMSKALELFRNDMAAKYKELGFQG

SEQ ID NO: 20 *Equus caballus*

MGLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHVLHS
KH PGDFGADAQGAMTKALELFRNDIAAKYKELGFQG

SEQ ID NO: 21 Nicotiana benthamiana

MSSFTEEQEALVVKSWDSMKKNAGEWGLKLFLKIFEIAPSAKKLFSFLKDSNVPL
EQNAKLKPHSKSVFVMTCEAAVQLRKAGKVVVRDSTLKKLGATHFKYGVADE
HFEVTKFALLETIKEAVPEMWSVDMKNAWGEAFDQLVNAIKTEMK

SEQ ID NO: 22 Bacillus subtilis

MGQSFNAPYEAIGEELLSQLVDTFYERVASHPLLKPIFPSDLTETARKQKQFLTQY
LGGPPLYTEEHGHPMLRARHLPFPITNERADAWLSCMKDAMDHVGLEGEIREFL
FGRLELTARHMVNQTEAEDRSS

SEQ ID NO: 23 Corynebacterium glutamicum

MTTSENFYDSVGGEETFSLIVHRFYEQVPNDDILGPMYPPDDFEGAEQRLKMFLS
QYWGGPKDYQEQRGHPRLRMRHVNYPIGVTAAERWLQLMSNALDGVDLTAEQ
REAIWEHMVRAADMLINSNPDPHA

SEQ ID NO: 24 Synechocystis PCC6803

MSTLYEKLGGTTAVDLAVDKFYERVLQDDRIKHFFADVDMAKQRAHQKAFLTY
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SEQ ID NO: 25 Synechococcus sp. PCC 7335

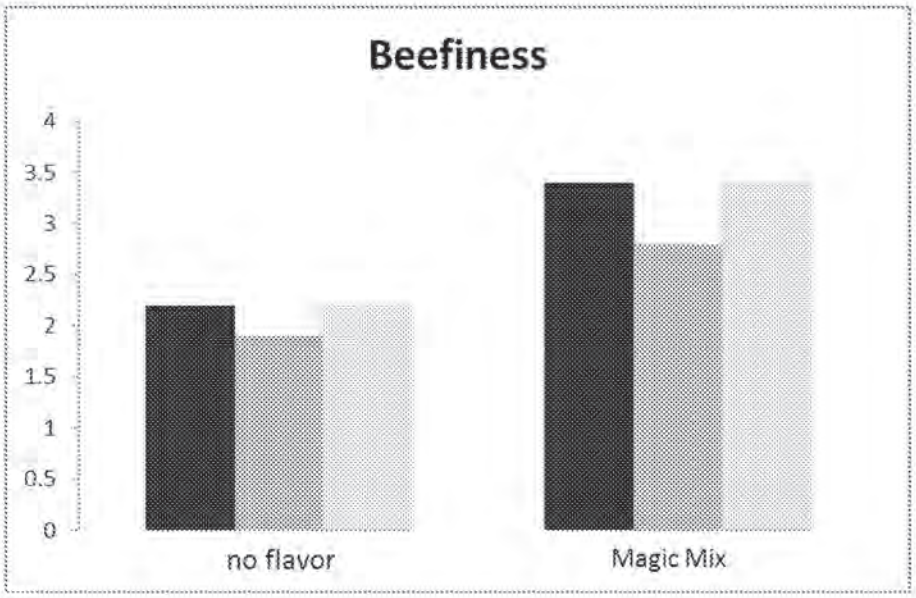
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TAWVEAYDVIASTMIEGADNPAAYLEPELTFYEWLDLYGEESPKVRNAIATLTH
FHYGEDPQDVQRDSRG

SEQ ID NO: 26 Nostoc commune

MSTLYDNIGGQPAIEQVVDELHKRIATDSLLAPVFAGTDMVKQRNHLVAFLAQIF
EGPKQYGGRPMDKTHAGLNLQQPHFDAIAKHLGERMAVRGVSAENTKAALDR
VTNMKGAILNK

SEQ ID NO: 27 Bacillus megaterium
MREKIHSPYELLGGEHTISKLVDAFYTRVGQHPELAPIFPDNLTETARKQKQFLTQ
YLGGPSLYTEEHGHPMLRARHLPFEITPSRAKAWLTCMHEAMDEINLEGPERDE
LYHRLILTAQHMINSPEQTDEKGFSH

FIGURE 2



CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 13/941,211, filed July 12, 2013, U.S. Application Serial No. 61/908,634, filed November 25, 2013, and to U.S. Application Serial No. 61/751,816, filed January 11, 2013, and is related to the following co-pending patent applications: Application Serial No. PCT/US12/46560; Application Serial No PCT/US 12/46552; Application Serial No. 61,876,676, filed September 11, 2013; and Application Serial No. 61/751,818, filed January 11, 2013, all of which are incorporated herein by reference.

Figure 12

Wavelength (nm)

TECHNICAL FIELD

This invention relates to food products and more particularly, to food products that include a highly conjugated heterocyclic ring complexed to iron such as a heme-cofactor and one or more flavor precursor molecules.

BACKGROUND OF THE INVENTION

[0002] Animal farming has a profound negative environmental impact. Currently it is estimated that 30% of Earth's land surface is dedicated to animal farming and that livestock account for 20% of total terrestrial animal biomass. Due to this massive scale animal farming accounts for more than 18% of net greenhouse gas emissions. Animal farming may be the largest human source of water pollution, and animal farming is by far the world's largest threat to biodiversity. It has been estimated that if the worlds human population could shift from a meat containing diet to a diet free of animal products, 26% of Earth's land surface would be freed for other uses. Furthermore the shift to a vegetarian diet would massively reduce water and energy consumption.

[0003] The consumption of meat has a profound negative impact on human health. The heath benefits of a vegetarian diet are well established. If the human population would shift to a vegetarian diet the cost savings in health care would be significant.

[0004] Hunger is a worldwide problem, yet the world's 4 major commodity crops (soybeans, maize, wheat, and rice) already supply more than 100% of the human population's requirements for calories and protein, including every essential amino acid.

Figure 5

H,,!

Food is any substance that is either eaten or drunk by any animal, including humans, for nutrition or pleasure. It is usually of plant or animal origin, and can contain essential nutrients, such as carbohydrates, fats, proteins, vitamins, or minerals. The substance is ingested by an organism and assimilated by the organism's cells in an effort to produce energy, maintain life, or stimulate growth.

Food typically has its origin in a photosynthetic organism, such as a plant. Some food is obtained directly from plants, but even animals that are used as food sources are raised by feeding them food which is typically derived from plants.

In most cases, the plant or animal food source is fractionated into a variety of different portions, depending upon the purpose of the food. Often, certain portions of the plant,

such as the seeds or fruits, are more highly prized by humans than others and these are selected for human consumption, while other less desirable portions, such as the stalks of grasses, are typically used for feeding animals.

[0005] Plant Current plant-based meat substitutes have largely failed to cause a shift to a vegetarian diet. The current state of the art for meat Meat substitute compositions involves the extrusion of soy/grain mixture, resulting in products are typically extruded soy/grain mixtures which largely fail to replicate the experience of cooking and eating meat. Common limitations of these plant-based meat substitute products are a texture and mouthfeel mouth-feel that are more homogenous than that of equivalent meat products. Furthermore, as the these products must largely be sold precooked pre-cooked, with artificial flavors and aromas built in pre-incorporated, they fail to replicate the aromas, flavors, and other key features, such as texture and mouth- feel, associated with cooking or cooked meat. As a result, these products appeal mainly largely to a limited consumer base that is already committed to vegetarianism/veganism, but have failed to appeal to the larger consumer segment accustomed to eating meat. It would be useful to have improved plant-based meat substitutes which better replicate the aromas and flavors of meat, particularly during and/or after cooking.

SUMMARY

Provided herein are methods and compositions for modulating the flavor and/or aroma profile of consumable food products, including animal- or non-animal (e.g., plant) based food products, or mixtures of animal- and non-animal-based food products. In some embodiments, the methods and compositions are useful for modulating the flavor and/or aroma profile of a consumable food product during and/or after the cooking process. In some embodiments, the methods and compositions are used to generate one or more chemical compounds that modulate the flavor and/or aroma profile of the consumable food product during and/or after the cooking process.

As provided herein, and without being bound by theory, certain characteristic meaty flavors and/or aromas (e.g., beefy, bacony, umami, savory, bloody, brothy, gravy, metallic, bouillon-like; see Tables 2, 7, and 11), including one or more specific chemical compounds

associated with the same (see Tables 3, 8, 9, 12, 14, 16, or 17), are believed to be produced during the cooking process of a consumable food product by chemical reaction of one or more flavor precursor molecules or compositions catalyzed by the

presence of a highly conjugated heterocyclic ring complexed to an iron ion (e.g., a heme moiety; or a porphyrin; a porphyrinogen; a corrin; a corrinoid; a chlorin; a bacteriochorophyll; a corphin; a chlorophyllin; a bacteriochlorin; or an isobacteriochlorin moiety complexed to an iron ion). Such highly conjugated heterocycylic moieties 5 include heterocyclic aromatic rings composed of one or more (2, 3, or 4 more) pyrrole, pyrrole-like, and/or pyrroline subunits. The highly conjugated heterocyclic ring complexed to an iron ion is referred to herein as an iron complex. In some embodiments, the heme moiety can be a heme cofactor such as a heme moiety bound to a protein; a heme moiety bound to a non-proteinaceous polymer; a heme moiety bound to a solid 10 support; or a heme moiety encapsulated in a liposome. In some embodiments, the flavors and/or aromas are not generated in the absence of the iron complex (e.g., in the absence of a ferrous chlorin) or are not generated in the absence of a heme-cofactor (e.g., in the absence of a heme-containing protein). Accordingly, as described herein, the iron complexes such as isolated chlorin-iron complexes or heme-cofactors (e.g., heme- 15 containing proteins) can be used to generate meaty flavors and/or aromas in a variety of food products, such as during the cooking process.

Combining one or more iron complexes such as a heme-cofactor (e.g., a heme-containing protein, including, for example a plant-derived heme protein such as a plant leghemoglobin (legH)), with one or more flavor precursor molecules or compositions 20 (see, e.g., Table 1 or Table 13) can generate or provide a range of savory and meaty

aromas and tastes (see, e.g., Tables 2, 7, and/or 11) in a cooked consumable food product. Flavor precursor molecules or compositions can be added to the uncooked food product in purified form and/or can be derived from ingredients in the uncooked consumable food product that contain and/or are enriched with one or more of the particular flavor 25 precursors or compositions, including, for example, yeast extract, vegetable oil, com oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, canola oil, sunflower oil, coconut oil, mango oil, or an algal oil. The resultant flavor and/or aroma profile

can be modulated by the type and concentration of the flavor precursors, the pH of the reaction, the length of cooking, the type and amount of iron complex (e.g., a heme cofactor such as a heme-containing protein), the temperature of the reaction, and the amount of water activity in the product, among other factors.

One or more flavor precursor molecules or compositions can be added along with a iron complex (e.g., ferrous chlorophyllin or a heme cofactor such as a heme-containing protein), to an uncooked food product, before and/or during the cooking process, to give the cooked consumable food product a particular meaty taste and smell, for example, the taste and smell of beef, bacon, pork, lamb, or chicken. Consumable food products can be animal or non-animal based (e.g., plant) food products, or combinations of an animal and non-animal based food product. For example, a plant based veggie burger or an animal - 10 based burger, such as a chicken burger, can be modified with the compositions and methods of the present disclosure to result in a burger having a cooked flavor and/or aroma profile that is more meat like, e.g., beef-like, lamb-like, pork-like, turkey-like, duck-like, deer-like, yak-like, bison-like or other desirable meat flavor.

Food products for use in the present disclosure include those that have an iron-complex (e.g., a heme cofactor such as a heme-containing protein), and one or more flavor precursor molecules included therein. The iron-complex such as a heme cofactor (e.g., a heme-containing protein) and the one or more flavor precursor molecules can be homogenously or heterogeneously included in the food products. A heme protein can be isolated and purified prior to inclusion in the food product. Non-limiting examples of consumable food products which can include an iron complex such as a heme-cofactor (e.g., a heme-containing protein) and one or more flavor precursor molecules include animal-based or non-animal (e.g., plant-based), or combinations of animal-based and non-animal-based, food products in the form of hot dogs, burgers, ground meat, sausages, steaks, filets, roasts, breasts, thighs, wings, meatballs, meatloaf, bacon, strips, fingers, nuggets, cutlets, or cubes.

Consumable food products for use in the present disclosure can be flavor additive

compositions, e.g., for addition to another consumable food product before, during, or after its cooking process. A flavor additive composition can include an iron complex such as a heme-cofactor (e.g., a heme-containing protein), and one or more flavor precursors.

A flavor additive composition can include a heme protein, e.g., an isolated and purified heme protein; such a flavor additive composition can be used to modulate the flavor and/or aroma profile of a consumable food product that comprises one or more flavor precursor molecules or compositions. A flavor additive composition can include one or more flavor precursor molecules or compositions; such a flavor additive composition can be used to modulate the flavor and/or aroma profile of a consumable food product that comprises the heme protein, e.g., an isolated and purified heme protein.

A flavor additive composition can be in the form, of but not limited to, soup or stew bases, bouillon, e.g., powder or cubes, flavor packets, or seasoning packets or shakers. Such flavor additive compositions can be used to modulate the flavor and/or aroma profile for a variety of consumable food products, and can be added to a consumable food product before, during, or after cooking of the consumable food product.

In some embodiments, a flavor additive composition such as one including an iron complex (e.g., ferrous chlorin or a heme protein) and one or more flavor precursors can be reacted (e.g., *in vitro)* with heating to generate a particular flavor and/or aroma profile of interest and the resultant product mixture can be added to the consumable food product of interest, which can then be eaten as-is or can be additionally modified, e.g., by additional cooking. In some embodiments, the iron complex can be removed from the resultant product mixture before adding the product mixture to the consumable food product of interest. For example, the iron complex can be removed from the product mixture using chromatographic techniques such as column chromatography, e.g., a column containing heme or iron-chlorin.

In some embodiments, the iron complex such as a heme-cofactor, e.g., a heme- protein, and the one or more flavor precursor flavor additive compositions can be soy- free, wheat-free, yeast-free, MSG-free, and free of protein hydrolysis products, and can taste meaty, highly savory, and without off odors or flavors.

In one aspect, this document features a food product that includes an iron complex such as a heme moiety, or a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochorophyll, a corphin, a chlorophyllin, a bacteriochlorin, or an isobacteriochlorin moiety complexed to an iron ion and one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine,

inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, com oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, sunflower oil, canola oil, olive oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone. The heme moiety can be a heme-containing protein, a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support. The heme-containing protein can be a plant, mammalian, a yeast or filamentous fungi, or bacterial heme-containing protein. The food product can include two to one hundred, two to fifty flavor precursors, two to forty flavor precursors, two to thirty-five flavor precursors, two to ten flavor precursors, or two to six flavor precursors. In some embodiments, the one or more flavor precursor molecules are selected from the group consisting of glucose, ribose, cysteine, a cysteine derivative, thiamine, alanine, methionine, lysine, a lysine derivative, glutamic acid, a glutamic acid derivative, IMP, GMP, lactic acid, maltodextrin, creatine, alanine, arginine, asparagine, aspartate, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, linoleic acid, and mixtures thereof. The heme-containing protein can be a non-symbiotic hemoglobin or a leghemoglobin (e.g., a plant leghemoglobin such as one from soybean, alfalfa, lupin, pea, cow pea, or lupin). The heme-containing protein can include an amino acid sequence having at least 80% sequence identity to a polypeptide set forth in SEQ ID NOs:l-26. The heme-containing protein can be isolated and purified. The food product further can include a food-grade oil, a seasoning agent, a flavoring agent, a protein, a protein concentrate, an emulsifier, a gelling agent, or a fiber. The food product can be a meat substitute, a soup base, stew base, snack food, bouillon powder, bouillon cube, a flavor packet, or a frozen food product. Any of the food products can be free of animal products. The food product can be sealed within a packet or shaker.

This document also features a method for producing a flavor compound. The method can include combining an iron complex (e.g., a heme moiety, a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochorophyll, a corphin, a chlorophyllin, a bacteriochlorin, or an isobacteriochlorin complexed to an iron) and one or more flavor precursor molecules to form a mixture, the one or more flavor precursor molecules selected from the group consisting of glucose, fructose, arabinose, ribose glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose,

sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, com oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, canola oil, olive oil, sunflower oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture to form one or more flavor compounds selected from the group consisting of phenylacetaldehyde, l-octen-3- one, 2-n-heptylfuran, 2-thiophenecarboxaldehyde, 3-thiophenecarboxaldehyde, butyrolactone, 2-undecenal, pyrazine, methyl-, furfural, 2-decanone, pyrrole, l-octen-3- ol, 2-acetylthiazole, (E)-2-octenal, decanal, benzaldehyde, (E)-2-nonenal, pyrazine, 1- hexanol, 1-heptanol, dimethyl trisulfide, 2-nonanone, 2-pentanone, 2-heptanone, 2,3- butanedione, heptanal, nonanal, 2-octanone, 1-octanol, 3-ethylcyclopentanone, 3-octen-2- one, (E,E)-2,4-heptadienal, (Z)-2-heptenal, 2-heptanone, 6-methyl-, (Z)-4-heptenal, (E,Z)-2,6-nonadienal, 3-methyl-2-butenal, 2-pentyl-furan, thiazole, (E, E)-2,4- decadienal,, hexanoic acid, 1-ethyl-5-methylcyclopentene, (E,E)-2,4-nonadienal, (Z)-2- decenal, dihydro-5-pentyl-2(3H)-furanone, trans-3-nonen-2-one, (E,E)-3,5-octadien-2- one, (Z)-2-octen-l-ol, 5-ethyldihydro-2(3H)-furanone, 2-butenal, l-penten-3-ol, (E)-2- hexenal, formic acid, heptyl ester, 2-pentyl-thiophene, (Z)-2-nonenal, 2-hexyl-thiophene, (E)-2-decenal, 2-ethyl-5-methyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine, 2-ethyl-1- hexanol, thiophene, 2-methyl-furan, pyridine, butanal, 2-ethyl-furan, 3-methyl-butanal, trichloromethane, 2-methyl-butanal, methacrolein, 2-methyl-propanal, propanal, acetaldehyde, 2-propyl-furan, dihydro-5-propyl-2(3H)- furanone, 1,3-hexadiene, 4-decyne, pentanal, 1-propanol, heptanoic acid, trimethyl-ethanethiol,, 1-butanol, 1- penten-3-one, dimethyl sulfide, 2-ethyl furan, 2-pentyl-thiophene, 2-propenal, 2-tridecen- l-ol, 4-octene, 2-methyl thiazole,, methyl-pyrazine, 2-butanone, 2-pentyl-furan, 2- 10 methyl-propanal,

butyrolactone,, 3-methyl-butanal, methyl-thiirane, 2-hexyl-furan,

butanal,, 2-methyl-butanal, 2-methyl-furan, furan, octanal, 2-heptenal, 1-octene, formic acid heptyl ester, 3-pentyl-furan, and 4-penten-2-one. The heme moiety can be a heme-containing protein, a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support. The method can include combining cysteine, ribose, lactic acid, 15 lysine, and/or thiamine with the heme-containing protein.

In another aspect, this document features a method for producing a flavor compound. The method includes combining an iron complex, such as a heme-containing protein, and one or more flavor precursor molecules to form a mixture, the one or more flavor precursor molecules selected from the group consisting of glucose, fructose, 20 ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, com oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, 25 olive oil, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, methionine, cysteine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture to form one or more

flavor compounds set forth in Tables 3, 8, or 9. For example, the flavor precursors can include cysteine, a sugar, and one or more other precursors.

This document also features a method for imparting a meat like flavor (e.g., beeflike, chicken like, pork-like, lamb-like, turkey-like, duck-like, deer-like, or bison-like) to 5 a food product. The method includes contacting the food product with a flavoring

composition, the flavoring composition comprising i) an iron complex, such as a heme moiety (e.g., a heme-containing protein); and ii) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6- phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, 10 maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP,

lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, com oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; wherein after heating the food product and the flavoring composition together, a meat like flavor (e.g., beef-like, chicken like, pork-like, lamb- like, turkey-like, duck-like, deer-like, or bison-like) is imparted to the food product. In some embodiments, the iron complex is removed from the food product. The flavoring composition further can include a seasoning agent, a flavoring agent, a protein, a protein concentrate, or an emulsifier. The flavoring composition can be sealed within a packet or shaker.

In another aspect, this document features a method of making a food product. The method includes combining an isolated heme-containing protein and one or more flavor precursor molecules to form a mixture, the one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6- phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin,

inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, sunflower oil, coconut oil, canola oil, flaxseed oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims. The word "comprising" in the claims may be replaced by "consisting essentially of" or with "consisting of," according to standard practice in patent law.

DESCRIPTION OF THE DRAWINGS

FIG. 1 contains amino acid sequences of exemplary heme-containing proteins.

FIG. 2 is a bar graph of the beefiness rating of the meat replica with or without the Magic Mix, both samples in triplicate with 1% w/v LegH protein. Tasters rated beefiness on a scale from 1-7, with 1 being not beefy at all and 7 being exactly like ground beef.

DETAILED DESCRIPTION

This document is based on methods and materials for modulating the taste and/or aroma profile of food products. As described herein, compositions containing one or

more flavor precursors and one or more highly conjugated heterocyclic rings complexed to an iron (referred to herein as an iron complex) can be used to modulate the taste and/or aroma profile of food products. Such iron complexes include heme moieties or other highly conjugated heterocylic rings complexed to an iron ion (referred to as an iron 5 complex). "Heme" refers to a prosthetic group bound to iron (Fe^{2+} or Fe^{3}) in the center of a porphyrin ring. Thus, an iron complex can be a heme moiety, or a porphyrin, porphyrinogen, corrin, corrinoid, chlorin, bacteriochorophyll, corphin, chlorophyllin, bacteriochlorin, or isobacteriochlorin moiety complexed to iron ion. The heme moiety that can be used to modulate the taste and/or aroma profile of food products can be a 10 heme cofactor such as a heme-containing protein; a heme moiety bound to a non-peptidic

polymer or other macromolecule such as a liposome, a polyethylene glycol, a carbohydrate, a polysaccharide, a cyclodextrin, a polyethylenimine, a polyacrylate, or derivatives thereof; a siderophore (i.e., an iron chelating compound); or a heme moiety bound to a solid support (e.g., beads) composed of a chromatography resin, cellulose, 15 graphite, charcoal, or diatomaceous earth.

In some embodiments, the iron complexes catalyze some reactions and produce flavor precursors without heating or cooking. In some embodiments, the iron complex destabilizes upon heating or cooking and releases the iron, e.g., the protein is denatured, so flavor precursors can be generated.

20 Suitable flavor precursors include sugars, sugar alcohols, sugar derivatives, oils (e.g., vegetable oils), free fatty acids, alpha-hydroxy acids, dicarboxylic acids, amino acids and derivatives thereof, nucleosides, nucleotides, vitamins, peptides, protein hydrolysates, extracts, phospholipids, lecithin, and organic molecules. Non-limiting examples of such flavor precursors are provided in Table 1.

25 **TABLE 1**

Flavor Precursor Molecules

Sugars, sugar alcohols, sugar acids, and sugar derivatives: glucose, fructose, ribose, sucrose, arabinose, glucose-6-phosphate,fructose-6-phosphate, fructose 1,6-diphosphate, inositol, maltose, molasses, maltodextrin, glycogen, galactose,

lactose, ribitol, gluconic acid and glucuronic acid, amylose, amylopectin, or xylose

Oils: coconut oil, mango oil, sunflower oil, cottonseed oil, safflower oil, rice bran oil, cocoa butter, palm fruit oil, palm oil, soybean oil, canola oil, com oil, sesame oil, walnut oil, flaxseed, jojoba oil, castor, grapeseed oil, peanut oil, olive oil, algal oil, oil from bacteria or fungi

Free fatty acids: caprylic acid, capric acid, lauric acid, myristic acid, palmititic acid, palmitoleic acid, stearic, oleic acid, linoleic acid, alpha linolenic acid, gamma linolenic acid, arachidic acid, arachidonic acid, behenic acid, or emcic acid

Amino acids and derivatives thereof: cysteine, cystine, a cysteine sulfoxide, allicin, selenocysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, 5-hydroxytryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, or tyrosine

Nucleosides and Nucleotides: inosine , inosine monophosphate (IMP), guanosine, guanoside monophosphate (GMP), adenosine, adenosine monophosophate (AMP)

Vitamins: thiamine, vitamin C, Vitamin D, Vitamin B6, or Vitamin E Misc: phospholipid, lecithin, pyrazine, creatine, pyrophosphate Acids: acetic acid, alpha hydroxy acids such as lactic acid or glycolic acid, tricarboxylic acids such as citric acid, dicarboxylic acids such as succinic acid or tartaric acid

Peptides and protein hydrolysates: glutathione, vegetable protein hydrolysates, soy protein hydrolysates, yeast protein hydrolysates, algal protein hydrolysatess, meat protein hydrolysates

Extracts: a malt extract, a yeast extract, and a peptone

In some embodiments, one flavor precursor or combinations of two to one hundred flavor precursors, two to ninety, two to eighty, two to seventy, two to sixty, or two to fifty flavor precursors are used. For example, combinations of two to forty flavor

Figure 6

precursors, two to thirty-five flavor precursors, two to ten flavor precursors, or two to six flavor precursors can be used with the one or more iron complexes (e.g., heme co-factors such as a heme-containing proteins). For example, the one or more flavor precursors can be glucose, ribose, cysteine, a cysteine derivative, thiamine, lysine, a lysine derivative, glutamic acid, a glutamic acid derivative, alanine, methionine, IMP, GMP, lactic acid, and mixtures thereof (e.g., glucose and cysteine; cysteine and ribose; cysteine, glucose or ribose, and thiamine; cysteine, glucose or ribose, IMP, and GMP; cysteine, glucose or ribose, and lactic acid). For example, the one or more flavor precursors can be alanine, arginine, asparagine, aspartate, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, pro line, threonine, tryptophan, tyrosine, valine, glucose, ribose, maltodextrin, thiamine, IMP, GMP, lactic acid, and creatine.

As used herein, the term "heme containing protein" can be used interchangeably with "heme containing polypeptide" or "heme protein" or "heme polypeptide" and includes any polypeptide that can covalently or noncovalently bind a heme moiety. In some embodiments, the heme-containing polypeptide is a globin and can include a globin fold, which comprises a series of seven to nine alpha helices. Globin type proteins can be of any class (e.g., class I, class II, or class III), and in some embodiments, can transport or store oxygen. For example, a heme-containing protein can be a non-symbiotic type of hemoglobin or a leghemoglobin. A heme-containing polypeptide can be a monomer, i.e., a single polypeptide chain, or can be a dimer, a trimer, tetramer, and/or higher order oligomers. The life-time of the oxygenated $Fe2^{+}$ state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption. The life-time of the unoxygenated Fe^{2+} state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or more under conditions in which the heme-protein- containing consumable is manufactured, stored, handled or prepared for consumption Non-limiting examples of heme-containing polypeptides can include an androglobin, a cytoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a

Figure 6

myoglobin, an crythrocruorin, a beta hemoglobin, an alpha hemoglobin, a protoglobin, a cyanoglobin, a cytoglobin, a histoglobin, a neuroglobins, a chlorocruorin, a truncated hemoglobin (e.g., HbN or HbO), a truncated 2/2 globin, a hemoglobin 3 (e.g., Glb3), a cytochrome, or a peroxidase.

Heme-containing proteins that can be used in the compositions and food products described herein can be from mammals (e.g., farms animals such as cows, goats, sheep, pigs, ox, or rabbits), birds, plants, algae, fungi (e.g., yeast or filamentous fungi), ciliates, or bacteria. For example, a heme-containing protein can be from a mammal such as a farm animal (e.g., a cow, goat, sheep, pig, ox, or rabbit) or a bird such as a turkey or 10 chicken. Heme-containing proteins can be from a plant such as *Nicotiana tabacum* or *Nicotiana sylvestris* (tobacco); *Zea mays* (com), *Arabidopsis thaliana,* a legume such as *Glycine max* (soybean), *Cicer arietinum* (garbanzo or chick pea), *Pisum sativum* (pea) varieties such as garden peas or sugar snap peas, *Phaseolus vulgaris* varieties of common beans such as green beans, black beans, navy beans, northern beans, or pinto beans, *Vigna unguiculata* varieties (cow peas), *Vigna radiata* (Mung beans), *Lupinus albus* (lupin), or *Medicago sativa* (alfalfa); *Brassica napus* (canola); *Triticum sps.* (wheat, including wheat berries, and spelt); *Gossypium hirsutum* (cotton); *Oryza sativa* (rice); *Zizania sps.* (wild rice); *Helianthus annuus* (sunflower); *Beta vulgaris* (sugarbeet); *Pennisetum glaucum* (pearl millet); *Chenopodium sp.* (quinoa); *Sesamum sp.* (sesame); *Linum usitatissimum* (flax); or *Hordeum vulgare* (barley). Heme-containing proteins can be isolated from fungi such as *Saccharomyces cerevisiae, Pichia pastoris, Magnaporthe oryzae, Fusarium graminearum, Aspergillus oryzae, Trichoderma reesei, Myceliopthera thermophile, Kluyvera lactis,* or *Fusarium oxysporum.* Heme-containing proteins can be isolated from bacteria such as *Escherichia coli, Bacillus subtilis. Bacillus licheniformis, Bacillus megaterium, Synechocistis sp., Aquifex aeolicus, Methylacidiphilum infernorum,* or thermophilic bacteria such as *Thermophilus.* The sequences and structure of numerous heme-containing proteins are known. See for example, Reedy, *et al.. Nucleic Acids Research,* 2008, Vol. 36, Database issue D307-D313 and the Heme Protein Database available on the world wide web at http ://hemeprotein.info/heme .php.

For example, a non-symbiotic hemoglobin can be from a plant selected from the group consisting of soybean, sprouted soybean, alfalfa, golden flax, black bean, black

Figure 6

eyed pea, northern, garbanzo, moong bean, cowpeas, pinto beans, pod peas, quinoa, sesame, sunflower, wheat berries, spelt, barley, wild rice, or rice.

Any of the heme-containing proteins described herein that can be used for producing food products can have at least 70% (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%) sequence identity to the amino acid sequence of the corresponding wild-type heme-containing protein or fragments thereof that contain a heme-binding motif For example, a heme-containing protein can have at least 70% sequence identity to an amino acid sequence set forth in FIG. 1, including a non-symbiotic hemoglobin such as that from *Vigna radiata* (SEQ ID NO:l), *Hordeum vulgare* (SEQ ID NO:5), *Zea mays* (SEQ ID NO: 13), *Oryza sativa subsp. japonica (rice)* (SEQ ID NO: 14), *or Arabidopsis thaliana* (SEQ ID NO: 15), a Hell's gate globin I such as that from *Methylacidiphiluni infernorum* (SEQ ID NO:2), a flavohemoprotein such as that 15 from *Aquifex aeolicus* (SEQ ID NOG), a leghemoglobin such as that from *Glycine max* (SEQ ID NO:4), *Pisum sativum* (SEQ ID NO: 16), or *Vigna unguiculata* (SEQ ID NO: 17), a heme-dependent peroxidase such as *from Magnaporthe oryzae,* (SEQ ID NO:6) or *Fusarium oxysporum* (SEQ ID NOG), a cytochrome c peroxidase from *Fusarium graminearum* (SEQ ID NO:8), a truncated hemoglobin from *Chlamydomonas* 20 *moewusii* (SEQ ID NO:9), *Tetrahymenapyriformis* (SEQ ID NO: 10, group I truncated), *Paramecium caudatum* (SEQ ID NO:l 1, group I truncated), a hemoglobin from *Aspergillus niger* (SEQ ID NO: 12), or a mammalian myoglobin protein such as the *Bos taurus* (SEQ ID NO: 18) myoglobin, *Sus scrofa* (SEQ ID NO: 19) myoglobin, *Equus caballus* (SEQ ID NO:20) myoglobin, a heme-protein from *Nicotiana benthamiana* (SEQ 25 ID NO:21), *Bacillus subtilis* (SEQ ID NO:22), *Corynebacterium glutamicum* (SEQ ID NOGS), Synechocystis PCC6803 (SEQ ID NO:24), Synechococcus sp. PCC 7335 (SEQ ID NO:25), or *Nostoc commune* (SEQ ID NO:26).

The percent identity between two amino acid sequences can be determined as follows. First, the amino acid sequences are aligned using the BLAST 2 Sequences 30 (B12seq) program from the stand-alone version of BLASTZ containing BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from Fish & Richardson's web site (e.g., www.fr.com/blast/) or the U.S. government's National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov). Instructions explaining

Figure 6

how to use the B12seq program can be found in the readme file accompanying BLASTZ. 5 B12seq performs a comparison between two amino acid sequences using the BLAST? algorithm. To compare two amino acid sequences, the options of B12seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seql.txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); 10 and all other options are left at their default setting. For example, the following

command can be used to generate an output file containing a comparison between two amino acid sequences: C:\B12seq-i c:\seql.txt-j c:\seq2.txt-p blastp -o c:\output.txt. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not 15 share homology, then the designated output file will not present aligned sequences.

Similar procedures can be following for nucleic acid sequences except that blastn is used.

Once aligned, the number of matches is determined by counting the number of positions where an identical amino acid residue is presented in both sequences. The percent identity is determined by dividing the number of matches by the length of the 20 full-length polypeptide amino acid sequence followed by multiplying the resulting value by 100. It is noted that the percent identity value is rounded to the nearest tenth. For example, 78.11, 78.12, 78.13, and 78.14 is rounded down to 78.1, while 78.15, 78.16, 78.17, 78.18, and 78.19 is rounded up to 78.2. It also is noted that the length value will always be an integer.

25 It will be appreciated that a number of nucleic acids can encode a polypeptide having a particular amino acid sequence. The degeneracy of the genetic code is well known to the art; i.e., for many amino acids, there is more than one nucleotide triplet that serves as the codon for the amino acid. For example, codons in the coding sequence for a given enzyme can be modified such that optimal expression in a particular species (e.g., 30 bacteria or fungus) is obtained, using appropriate codon bias tables for that species.

Heme-containing proteins can be extracted from the source material (e.g., extracted from animal tissue, or plant, fungal, algal, or bacterial biomass, or from the culture supernatant for secreted proteins) or from a combination of source materials (e.g., multiple plant species). Leghemoglobin is readily available as an unused by-product of 5 commodity legume

Figure 6

crops (e.g., soybean, alfalfa, or pea). The amount of leghemoglobin in the roots of these crops in the United States exceeds the myoglobin content of all the red meat consumed in the United States.

In some embodiments, extracts of heme-containing proteins include one or more non-heme-containing proteins from the source material (e.g., other animal, plant, fungal, 10 algal, or bacterial proteins) or from a combination of source materials (e.g., different animal, plant, fungi, algae, or bacteria).

In some embodiments, heme-containing proteins are isolated and purified from other components of the source material (e.g., other animal, plant, fungal, algal, or bacterial proteins). As used herein, the term "isolated and purified" indicates that the 15 preparation of heme-containing protein is at least 60% pure, e.g., greater than 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% pure. Without being bound by theory, isolating and purifying proteins can allow the food products to be made with greater consistency and greater control over the properties of the food product as unwanted material is eliminated. Proteins can be separated on the basis of their molecular weight, for example, by size 20 exclusion chromatography, ultrafiltration through membranes, or density centrifugation. In some embodiments, the proteins can be separated based on their surface charge, for example, by isoelectric precipitation, anion exchange chromatography, or cation exchange chromatography. Proteins also can be separated on the basis of their solubility, for example, by ammonium sulfate precipitation, isoelectric precipitation, surfactants, 25 detergents or solvent extraction. Proteins also can be separated by their affinity to another molecule, using, for example, hydrophobic interaction chromatography, reactive dyes, or hydroxyapatite. Affinity chromatography also can include using antibodies having specific binding affinity for the heme-containing protein, nickel NTA for His- tagged recombinant proteins, lectins to bind to sugar moieties on a glycoprotein, or other 30 molecules which specifically binds the protein.

Heme-containing proteins also can be recombinantly produced using polypeptide expression techniques (e.g., heterologous expression techniques using bacterial cells, insect cells, fungal cells such as yeast, plant cells such as tobacco, soybean, or *Arabidopsis,* or mammalian cells). In some cases, standard polypeptide synthesis 5 techniques (e.g., liquid-phase polypeptide synthesis techniques or solid-phase

Figure 6

polypeptide synthesis techniques) can be used to produce heme-containing proteins synthetically. In some cases, *in vitro* transcription-translation techniques can be used to produce heme-containing proteins.

The protein used in the consumable may be soluble in a solution. In some 10 embodiments, the isolated and purified proteins are soluble in solution at greater than 5, 10, 15, 20, 25, 50, 100, 150, 200, or 250g/L.

In some embodiments, the isolated and purified protein is substantially in its native fold and water soluble. In some embodiments, the isolated and purified protein is more than 50, 60, 70, 80, or 90% in its native fold. In some embodiments, the isolated 15 and purified protein is more than 50, 60, 70, 80, or 90% water soluble.

Modulating Flavor and/or Aroma Profiles

As described herein, different combinations of flavor precursors can be used with one or more iron complexes (e.g., a ferrous chlorin, a chlorin-iron complex, or a heme- 20 cofactor such as a heme-containing protein or heme bound to a non-peptidic polymer such as polyethylene glycol or to a solid support) to produce different flavor and aroma profiles when the flavor precursors and iron complexes are heated together (e.g., during cooking). The resultant flavor and/or aroma profile can be modulated by the type and concentration of the flavor precursors, the pH of the reaction, the length of cooking, the 25 type and amount of iron complex (e.g., a heme-cofactor such as heme-containing protein, heme bound to non-peptidic polymer or macromolecule, or heme bound to a solid support), the temperature of the reaction, and the amount of water activity in the product, among other factors. In embodiments in which a heme moiety is bound to a solid support such as cellulose or a chromatography resin, graphite, charcoal, or diatomaceous earth,
30 the solid support (e.g., beads) can be incubated with sugars and/or one or more other flavor precursors to generate flavors, and then the solid support with attached heme moiety can be re-used, i.e., incubated again with sugars and/or one or more other flavor precursors to generate flavors.

Table 2 provides non-limiting examples of flavor types that can be generated by 5 combining one or more flavor precursors and one or more heme co-factors (e.g., heme- containing

Figure 6

proteins). See also Tables 7 and/or 11.

TABLE 2

Flavor Types	
beef	beef broth
beef dripping	cheesy
cold-cut deli meat	squash
bacon	sharp
meaty	fruity
brothy	floral
ramen	musty
egg	fried food
malty	caramel
bready	barbeque
sulfur	chocolate
fried chicken	sweet
browned	potato
pretzel	french toast
grassy	breadcrust
bloody	mushroom
broccoli	chicken
brothy	cumin
buttery	umami
metallic	raisin

Figure 6

yeasty	goaty
vegetable broth	

Flavor and aroma profiles are created by different chemical compounds formed by chemical reactions between the heme co-factor (e.g., heme-containing protein) and flavor precursors. Gas chromatography-mass spectrometry (GCMS) can be used to separate and identify the different chemical compounds within a test sample. For example, volatile chemicals can be isolated from the head space after heating a heme-containing protein and one or more flavor precursors.

Table 3 provides non-limiting examples of compounds that can be produced. See also Tables 8, 9, 12, and/or 14.

TABLE 3

Compounds Produced

phenylacetaldehyde	2-butenal, 2-ethyl-	1,3-hexadiene
l-octen-3-one	acetonitrile	4-decyne
2-n-heptylfuran		pentanal
2-thiophenecarboxaldehyde	(E)-2-Hexenal	1-propanol
3 -thiophenecarboxaldehy de	4-ethyl-phenol,	heptanoic acid
1-octene	3-octanone	ethanethiol
butyrolactone	styrene	2-methyl-1 -heptene
2-undecenal	furan, 3-pentyl-	(E)-4-octene
propyl-cyclopropane	formic acid, heptyl ester	2-methyl-2-heptene
methyl-pyrazine	(E)-2-Heptenal	pentanoic acid
1 -hydroxy-propanone	6-methyl-5-hepten-2-one	nonanoic acid
acetic acid	n-caproic acid vinyl ester	1,3-dimethyl-benzene
furfural	2-ethyl-2-hexenal	
2-decanone	l-hepten-3-ol	toluene
pyrrole	1 -ethyl-1 -methyl-cyclopentane	1-butanol
l-octen-3-ol	3-ethyl-2-methyl-1,3-hexadiene	2,3,3-trimethyl-pentane
2-acetylthiazole	2-pentyl-thiophene	isopropyl alcohol
(E)-2-octenal	(Z)-2-nonenal	2,2,4,6,6-pentamethyl-heptane
decanal	2-n-octylfuran	phenol

Figure 6

benzaldehyde	2-hexyl-thiophene	l-penten-3-one
(E)-2-Nonenal	4-cyclopentene-1,3-dione	dimethyl sulfide
pyrazine	1-nonanol	thiirane
1-pentanol	(E)-2-decenal	(E)-2-octen-l-ol
trans-2-(2-pentenyl)furan	4-ethyl-benzaldehyde	2,4-dimethyl-1 -heptene
1-hexanol	l,7-octadien-3-ol	l,3-bis(l,l-dimethylethyl)-benzene
1-heptanol	octanoic acid	heptane
dimethyl trisulfide	2-ethyl-5 -methyl-pyrazine	4,7-dimethyl-undecane
2-nonanone	3 -ethyl-2,5 -dimethy 1-pyrazine	acetophenone
2-pentanone	1,3,5-cycloheptatriene	tridecane
2-heptanone	2-ethyl-1 -hexanol	thiophosphoramide, s-methyl ester
2,3-butanedione	4-methyl-octanoic acid	2-methyl-thiazole
heptanal	m-aminophenylacetylene	3 -(1 -methy lethoxy)-propanenitrile,
nonanal	benzene	2,4-bis(l,l-dimethylethyl)-phenol
2-octanone	thiophene	3-ethyl-2,2-dimethyl-pentane
2-butanone	2-methyl-furan	3-ethyl-pentane
octanal	pyridine	2,3,4-trimethyl-pentane
1-octanol	furan	2,4,6-trimethy l-o ctane
3 -ethylcyclopentanone	butanal	2,6-dimethyl-nonane
8-methyl-1 -undecene	2-ethyl-furan	2-hexyl-furan
3-octen-2-one	carbon disulfide	4-methyl-5-thiazoleethanol
2,4-Heptadienal, (E,E)-	Furan, 2-hexyl-:2	4-penten-2-one
(Z)-2-heptenal	3 -methy 1-butanal	4-methylthiazole
6-methyl-2-heptanone	2-methyl-butanal	2-methyl-3 -pentanone
(Z)-4-heptenal	methacrolein	2,3-pentanedione
(E,Z)-2,6-nonadienal	octane	(E)-2-tridecen-1 -ol
3 -methyl-2-butenal	ethanol	2-thiophenemethanamine
2-pentyl-furan	2-methyl-propanal	(Z)-2-nonenal,
thiazole	acetone	methyl thiolacetate
(E,E)-2,4-decadienal	propanal	methyl ethanoate
hexanoic acid	methyl-thiirane	isothiazole
1 -ethyl-5 -methylcyclopentene	acetaldehyde	3,3 -dimethyl-hexane

~~Figure 6~~

(E,E)-2,4-nonadienal	2-propenal	4-methyl-heptane
(Z)-2-decenal	2-propyl-furan	2,4-dimethyl-heptane
dihydro-5-pentyl-2(3h)-furanone	dihydro-5-propyl-2(3H)-furanone	2,3,4-trimethyl-heptane
trans-3 -nonen-2-one	dihydro-3 -(2H)-thiophenone	2-methyl-heptane
(E,E)-3,5-octadien-2-one	2,2,6-trimethyl-decane	2-methyl-3 -furanthiol
(Z)-2-octen-l-ol	3,3'-dithiobis[2-methyl-furan	4-amino-1,2,5-oxadiazole-3-carbonitrile
5-ethyldihydro-2(3h)-furanone	1-heptene	1,2-benzisothiazol-3(2H)-one
2-butenal	1,3-octadiene	2-acetyl-propen-2-ol,
l-penten-3-ol	1-nonene	l-decen-3-one
1 -(ethylthio)-2-(methylthio)-buta-1,3-diene		

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein is heated in the presence of ground chicken, to increase specific volatile flavor and odorant components typically elevated in beef. For example, propanal, butanal, 2-ethyl-furan, heptanal, octanal, trans-2-(2- 5 pentenyl)furan, (Z)-2-heptenal, (E)-2-octenal, pyrrole, 2,4-dodecadienal, 1-octanal, (Z)-2- decenal, or 2-undecenal can be increased in the presence of the heme-containing protein, which can impart a more beefy flavor to the chicken.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein is heated in the presence of cysteine 10 and glucose or other combinations of flavor precursors to provide a different profile of volatile odorants than when any subset of the three components are used individually. Volatile flavor components that are increased under these conditions include but are not limited to furan, acetone, thiazole, furfural, benzaldehyde, 2-pyridinecarboxaldehyde, 5- methyl-2-thiophenecarboxaldehyde, 3 -methyl-2-thiophenecarboxaldehyde, 3 - 15 thiophenemethanol and decanol. See, e.g., Tables 8 and 9. Under these conditions, cysteine and glucose alone or in the presence of iron salts such as ferrous glucanate produced a sulfurous, odor, but addition of heme-containing proteins reduced the

sulfurous odor and replaced it with flavors including but not limited to chicken broth,

Figure 6

burnt mushroom, molasses, and bread.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein is heated in the presence of cysteine 5 and ribose to provide a different profile of volatile odorants. Heating in the presence of ribose created some additional compounds as compared to when a heme-containing protein and glucose were heated together. See Tables 8 and 9.

In some embodiments, an iron complex (e.g., a ferrous chlorophillin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the 10 presence of thiamine and a sugar to affect the formation of 5-Thiazoleethanol, 4-methyl- furan, 3,3'-dithiobis[2-methyl-furan, and/or 4-Methylthiazole. These compounds are known to be present in meat and have beefy, meaty taste notes.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of a 15 nucleotide such as inosine monophosphate and/or guanosine monophosphate to control the formation of flavor compounds such as (E)-4-octene, 2-ethyl-furan, 2-pentanone, 2,3-butanedione, 2-methyl-thiazole, methyl-pyrazine, tridecane, (E)-2-octenal, 2-thiopenecarboxaldehyde, and/or 3-thiopenecarboxaldehyde. These compounds are known to be present in meat and have a beefy, meaty, buttery, and or savory flavor notes.

20 In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of lysine, a sugar such as ribose, and cysteine to control the formation of flavor compounds such as dimethyl trisulfide, nonanal, 2-pentyl thiophene, 2-nonenal furfural, 1-octanol, 2- nonenal, thiazole, 2-acetylthiazole, phenylacetaldehyde, and/or 2-acetylthiazole. These 25 compounds are known to be present in meat and some have a beefy, meaty, and or savory flavor.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of lactic acid, a sugar such as ribose, and cysteine to control the formation of the flavor 30 compounds nonanal, thiazole, 2-acetylthiazole, and/or 8-methyl 1-undecene. These compounds are known to be present in meat and have beefy, savory, browned, bready, and malty notes.

Figure 6

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of 5 amino acids, sugars such as glucose, ribose, and maltodextrin, lactic acid, thiamine, IMP, GMP, creatine, and salts such as potassium chloride and sodium chloride, to control the formation of flavor compounds such as l,3-bis(l,l-dimethylethyl)-benzene, 2-methyl 3- furanthiol, and/or bis(2-methyl-4,5-dihydro-3-furyl) disulfide. These compounds are known to be present in meat and have beefy notes. See also Table 14.

In some embodiments, a particular type of heme-containing protein is chosen to control the formation of flavor compounds. See, for example, the results of Table 9, which shows that the addition of different types of heme-proteins (LegH, Barley, *B. myoglobin, or A. aeolicus)* in flavor reaction mixtures containing one or more flavor precursor compounds results in many of the same key meat flavors, including but not limited to pentanone, 3-methyl butanal, 2-methyl butanal, 2-heptenal, 1-octene, nonanal, 2-propenal, 2-decenal, 2-nonanone, 2-octanone, 2-tridecen-l-ol, 2-octanone, 2-octenal, 4- methyl-2-heptanone, octanal, 2-undecenal, butyrolactone, l-octen-3-one, 3-methylheptyl acetate, and 2-pentyl-thiophene. These differences in flavor compounds can change the overall taste profile.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein and one or more flavor precursors can be reacted (e.g., *in vitro)* with heating to generate a particular flavor and/or aroma profile of interest and the resultant flavor additive composition can be added to the consumable food product of interest, which can then be eaten as-is or can be additionally modified, e.g., by additional cooking.

In some embodiments, any undesirable flavors can be minimized by deodorizing with activated charcoal or by removing enzymes such as lipoxygenases (LOX), which can be present in trace amounts when using preparations of plant proteins, and which can convert unsaturated triacylglycerides (such as linoleic acid or linolenic acid) into smaller and more volatile molecules. LOX are naturally present in legumes such as peas, soybeans, and peanuts, as well as rice, potatoes, and olives. When legume flours are fractionated into separate protein fractions, LOX can act as undesirable “time-bombs”

Figure 6

that can cause undesirable flavors on aging or storage. Compositions containing plant proteins (e.g., from ground plant seeds) can be subjected to purification to remove LOX using, for example, an affinity resin that binds to LOX and removes it from the protein sample. The affinity resin can be linoleic acid, linolenic acid, stearic acid, oleic acid, propyl gallate, or epigalloccatechin gallate attached to a solid support such as a bead or resin. See, e.g., WO2013138793. In addition, depending on the protein component of the food product, certain combinations of antioxidants and/or LOX inhibitors can be used as effective agents to minimize off-flavor or off-odor generation especially in the presence of fats and oils. Such compounds can include, for example, one or more of P- carotene, a-tocopherol, caffeic acid, propyl gallate, or epigallocatechin gallate.

In some embodiments, specific flavor compounds, such as those described in Tables 3, 8, 9, 12, 14, 16, or 17 can be isolated and purified from the flavor additive composition. These isolated and purified compounds can be used as an ingredient to create flavors useful to the food and fragrance industry.

A flavor additive composition can be in the form, of but not limited to, soup or stew bases, bouillon, e.g., powder or cubes, flavor packets, or seasoning packets or shakers. Such flavor additive compositions can be used to modulate the flavor and/or aroma profile for a variety of food products, and can be added to a consumable food product before, during, or after cooking of the food product.

Food Products

Food products containing one or more flavor precursors and one or more heme-containing proteins can be used as a base for formulating a variety of additional food products, including meat substitutes, soup bases, stew bases, snack foods, bouillon powders, bouillon cubes, flavor packets, or frozen food products. Meat substitutes can be formulated, for example, as hot dogs, burgers, ground meat, sausages, steaks, filets, roasts, breasts, thighs, wings, meatballs, meatloaf, bacon, strips, fingers, nuggets, cutlets, or cubes.

~~Figure 6~~

In addition, food products described herein can be used to modulate the taste and/or aroma profile of other food products (e.g., meat replicas, meat substitutes, tofu, mock duck or other gluten based vegetable product, textured vegetable protein such as textured soy protein, pork, fish, lamb, or poultry products such as chicken or turkey 5 products) and can be applied to the other food product before or during cooking. Using the food products described herein can provide a particular meaty taste and smell, for example, the taste and smell of beef or bacon, to a non-meat product or to a poultry product.

Food products described herein can be packaged in various ways, including being 10 sealed within individual packets or shakers, such that the composition can be sprinkled or spread on top of a food product before or during cooking.

Food products described herein can include additional ingredients including food- grade oils such as canola, com, sunflower, soybean, olive or coconut oil, seasoning agents such as edible salts (e.g., sodium or potassium chloride) or herbs (e.g., rosemary, 15 thyme, basil, sage, or mint), flavoring agents, proteins (e.g., soy protein isolate, wheat glutin, pea vicilin, and/or pea legumin), protein concentrates (e.g., soy protein concentrate), emulsifiers (e.g., lecithin), gelling agents (e.g., k-carrageenan or gelatin), fibers (e.g., bamboo filer or inulin), or minerals (e.g., iodine, zinc, and/or calcium).

Food products described herein also can include a natural coloring agent such as 20 turmeric or beet juice, or an artificial coloring agent such as azo dyes, triphenylmethanes, xanthenes, quinines, indigoids, titanium dioxide, red #3, red #40, blue #1, or yellow #5.

Food products described herein also can include meat shelf life extenders such as carbon monoxide, nitrites, sodium metabisulfite, Bombal, vitamin E, rosemary extract, green tea extract, catechins and other anti-oxidants.

25 Food products described herein can be free of animal products (e.g., animal heme-containing proteins or other animal products).

In some embodiments, the food products can be soy-free, wheat-free, yeast-free, MSG-free, and/or free of protein hydrolysis products, and can taste meaty, highly savory, and without off odors or flavors.

Assessment of Food Products

Food products described herein can be assessed using trained human panelists.

EXHIBIT 1C

The evaluations can involve eyeing, feeling, chewing, and tasting of the product to judge product appearance, color, integrity, texture, flavor, and mouth feel, etc. Panelists can be 5 served samples under red or under white light. Samples can be assigned random three- digit numbers and rotated in ballot position to prevent bias. Sensory judgments can be scaled for "acceptance" or "likeability" or use special terminology. For example, letter scales (A for excellent, B for good, C for poor) or number scales may be used (1 = dislike, 2 = fair, 3 = good; 4 = very good; 5=excellent). A scale can be used to rate the 10 overall acceptability or quality of the food product or specific quality attributes such beefiness, texture, and flavor. Panelists can be encouraged to rinse their mouths with water between samples, and given opportunity to comment on each sample.

In some embodiments, a food product described herein can be compared to another food product (e.g., meat or meat substitute) based upon olfactometer readings. In 15 various embodiments, the olfactometer can be used to assess odor concentration and odor thresholds, odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of appreciation, or relative intensity of odors.

In some embodiments, an olfactometer allows the training and automatic evaluation of expert panels. In some embodiments, a food product described herein 20 causes similar or identical olfactometer readings. In some embodiments, the differences between flavors generated using the methods of the invention and meat are sufficiently small to be below the detection threshold of human perception.

In some embodiments, volatile chemicals identified using GCMS can be evaluated. For example, a human can rate the experience of smelling the chemical 25 responsible for a certain peak. This information could be used to further refine the profile of flavor and aroma compounds produced using a heme-containing protein and one or more flavor precursors.

Characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions molecules including amino acids, fats and sugars 30 which are found in plants as well as meat. Therefore, in some embodiments, a food

product is tested for similarity to meat during or after cooking. In some embodiments human ratings, human evaluation, olfactometer readings, or GCMS measurements, or combinations thereof, are used to create an olfactory map of the food product. Similarly, an

Figure 6

olfactory map of the food product, for example, a meat replica, can be created. These 5 maps can be compared to assess how similar the cooked food product is to meat.

In some embodiments, the olfactory map of the food product during or after cooking is similar to or indistinguishable from that of cooked or cooking meat. In some embodiments the similarity is sufficient to be beyond the detection threshold of human perception. The food product can be created so its characteristics are similar to a food 10 product after cooking, but the uncooked food product may have properties that are different from the predicate food product prior to cooking.

These results will demonstrate that the compositions of the invention are judged as acceptably equivalent to real meat products. Additionally, these results can demonstrate that compositions of the invention are preferred by panelist over other 15 commercially available meat substitutes. So, in some embodiments the present invention provides for consumables that are significantly similar to traditional meats and are more meat like than previously known meat alternatives.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

20

Figure 6

EXAMPLES

Example 1: Addition of heme-protein increases beefy qualities of replica burgers

Replica burgers containing the ingredients in Table 4 and the flavor precursors 5 cysteine (10 mM), glutamic acid (10 mM), glucose (10 mM), and thiamine (1 mM) were prepared. Water was added to make up the balance. See, for example, U.S. Provisional Application No. 61/751,816, filed January 11, 2013. Control burgers were prepared as in Table 4 with precursors cysteine (10 mM), glutamic acid (10 mM), glucose (10 mM), and thiamine (1 mM) except LegH was omitted.

After cooking for 5 minutes at 150 C, the replica burgers were evaluated by a trained sensory panel. Panelists were served samples under red lights and each panelist individually evaluated the samples. Samples were assigned a random three-digit number and rotated in ballot position to prevent bias. Panelists were asked to evaluate cooked replica burger samples on multiple flavor, aroma, taste, texture and appearance attributes 15 including but not limited to: beefiness, bloody quality, savory quality, and overall acceptability using a 7-point scale from l=dislike extremely, to 7=like extremely. Panelists were encouraged to rinse their mouths with water between samples, and to fill out a survey to record their evaluation of each sample.

When replica burgers containing the LegH were compared to the control replica 20 burgers without LegH, the samples containing LegH were rated significantly beefier, bloodier, more savory, and overall preferred compared to those that did not include LegH. See Table 5.

TABLE 4

25 **Replica Burger Ingredients**

Replica burger	"<> precooked u w
Pea vicilin	3.86
Soy protein concentrate (SPC)	2.52
Bamboo fiber	0.34

Figure 6

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NaCl	0.54
Pea legumin	2
Soy Protein Isolate (SPI) (Solae, St. Louis, MO)	4.68
Wheat gluten	4.68
Coconut oil	15
Soy lecithin	0.1
k-carrageenan	1
LegH	1

TABLE 5

Sensory evaluation of replica burger with Heme

Allribule	Beef 20 SO	No 1 leme	r„ 1 leme
lleel\iiess			
mean	5.33	1.30	3.20
STDEV	1.58	0.67	0.79
Blood}			
mean	4.00	1.10	2.78
STDEV	1.32	0.32	1.64
Sa\or\			
mean	4.67	3.00	5.10
STDEV	1.22	1.63	0.57

5 Example 2: Replica burgers with a flavor precursor mixture taste beefy and bloody

Replica burgers containing a flavor precursor mixture of glucose, cysteine, thiamine, and glutamic acid and 1% LegH pre-cooked w/w (see Table 4) were prepared as described in Example 1, and evaluated by a trained sensory panel after the burgers 10 were cooked for 5 minutes at 150 C. Control burgers included LegH and all other ingredients except for the flavor precursor mixture.

Panelists were asked to evaluate the samples overall improvement in taste and

Figure 6

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descriptively analyze each sample using a 5-point scale from l=dislike extremely, to 5=like extremely. Panelists were encouraged to rinse their mouths with water between samples, and to fill out a survey to record their evaluation of each sample. The replicate 5 burgers which included LegH and the flavor precursor mixture were described as having bouillon, gravy, meaty, bloody, savory, and beefy notes on taste, and were preferred to the same replica burger with LegH but no added flavor precursor mixture. See, Table 6

TABLE 6

Improvement of overall taste with precursors added to LegH burgers

	with precursors	without precursors
Average	3.5	1.8
STDV	0.6	0.5

Example 3: Replica burgers with flavor precursor mixture resulting in a bacon taste

Replica burgers (see Table 4) were cooked with different precursor mixes (see Table 7) and 1% LegH and evaluated by a trained sensory panel after the burgers were 15 cooked for 5 minutes at 150 C. Control burgers contained LegH and all of the other ingredients except for the flavor precursors. Panelists were asked to evaluate each sample and descriptively analyze of each sample. 5-point scale from l=dislike extremely, to 5=like extremely. Panelists were encouraged to rinse their mouths with water between samples, and to fill out a survey to record their evaluation of each sample. A replica 20 burger with a precursor mixture of 10 mM glucose, 10 mM ribose, 10 mM cysteine, 1 mM thiamine, 1 mM glutamic acid, 1 mM GMP, and LegH was described as having a bacon aroma and taste, and overall meatiness, savory quality, a very umami quality, a brothy quality, and slight beefy notes. See Table 7 for a summary of the flavor description for the various combinations of flavor precursors and heme-containing 25 protein.

Figure 6

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TABLE 7

Flavors generated by addition of precursors to LegH (1 %)

Precursor (concentration)						Flavor Description
	ribose (10 mM)	cysteine (10 mM)				some kind of cold-cut/sliced deli meat
	ribose (10 mM)	cysteine (10 mM)			IMP (2 mM)	bread crust with beef drippings, sweet, grassy, umami
	ribose (10 mM)	cysteine (10 mM)			lactic acid (1 mM)	bready, malty, browned, breadcrust
	ribose (10 mM)	cysteine (10 mM)			lysine (5 mM)	savory, beefy, little grassy, brothy, bread
	ribose (10 mM)	cysteine (10 mM)			alanine (5 mM)	savory, weak beefy, brothy, little metallic
	ribose (10 mM)	cysteine (10 mM)			I+G (2 mM)	savory, weak beefy, brothy, sweet
	ribose (10 mM)	cysteine (10 mM)			methionine	cooked potato
	ribose (10 mM)	cysteine (10 mM)		glutamic acid (5 mM)		little meaty, pretzel, brothy, savory, sweet, chocolate
glucose (10 mM)	ribose (10 mM)	cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)		slight beefy, browned, grasssy,
glucose (10 mM)	ribose (10 mM)	cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	IMP (2 mM)	bacon, very umami, savory, brothy, slight beef
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)		beef jerky, bloody, meaty, brothy
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	lactic acid (1 mM)	savory, beefy, bloody, meaty, savory, gravy

Figure 8

glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	lysine (5 mM)	roast beef
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	alanine (5 mM)	boiled beef, sweet
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	I+G (2 mM)	beefy with a sulfury note
glucose (10 mM)		cysteine (10 mM)			I+G (2 mM)	sweet, malty, umami, meaty
glucose (10 mM)					I+G (2 mM)	savory, roast beef, grassy
glucose (10 mM)				glutamic acid (5 mM)		umami, savory, meaty, sweaty, fermented

Figure 8

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Example 4: Type of sugar modulates flavor compounds created in the presence of Hemeprotein

The addition of different sugars to flavor reaction mixtures containing a hemeprotein and one or more flavor precursor compounds resulted in distinct differences in the flavor compounds generated and the overall flavor profile. LegH heme protein at 1% pre-cooked w/w/ was mixed with cysteine (10 mM) and glucose (20 mM) at pH 6 in phosphate buffer to form a flavor reaction mixture and heated to 150 C for 3 minutes; this reaction created flavor compounds known to be present in meat; *see* Table 8. Similarly, a flavor reaction mixture made when LegH heme protein at 1% was mixed with cysteine 10 (10 mM) and ribose (20 mM) at pH 6 and heated to 150 C for 3 minutes created flavor compounds known to be in meat; *see* Table 8.

The characteristic flavor and fragrance components were mostly produced during the cooking process when the flavor precursor molecules reacted with the heme-protein. Gas chromatography-mass spectrometry (GCMS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to separate and identify different substances within a test sample. Samples were evaluated by GCMS to identify the flavor compounds generated after heating and also evaluated for their sensory profiles. Volatile chemicals were isolated from the head space around the flavor reactions. The profile of the volatile chemicals in the headspace around the flavor reaction mixtures is shown in Table 8. In particular, the use of ribose created some additional compounds as compared to glucose, as shown in Table 8.

Notably, the control mixtures of cysteine with ribose or glucose heated in the absence of the LegH heme-protein did not generate the same set of flavor compounds. The flavor reaction mixtures containing LegH also were evaluated by a blinded trained sensory panel, which described the samples with ribose as having beefy, savory, brothy, and gravy-like notes, and the samples with glucose as savory, bloody, metallic, raw meat, and bouillon-like.

~~Figure 8~~

TABLE 8

Flavor compounds generated with cysteine, LegH, and either glucose or ribose in the flavor reaction mixture.

LegH 1%		
Compounds civulcd	e\steine (10 m\l). glucose (20 m\l)	cysieine (10 m\1). ribose (20 m\l)
benzaldehyde	X	X
2-butanone	X	X
dimethyl trisulfide	X	X
2-pentyl-fiiran	X	X
2-methyl-propanal	X	X
thiazole	X	X
butyrolactone	X	X
2-acetylthiazole	X	X
pentanal	X	X
3 -methy 1-butanal	X	X
methyl-thiirane	X	X
nonanal	X	X
heptanal	X	X
2,3-butanedione	X	X
1,3,5 -cycloheptatriene	X	X
propyl-cyclopropane	X	X
2-hexyl-furan	X	X
butanal	X	X
2-methyl-butanal	X	X
2-ethyl-furan		X
2-octanone	X	X
propanal	X	X

Figure 8

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~~Figure 8~~

trichloromethane	X	
2-methyl-furan	X	X
furan	X	X
pyrazine	X	X
thiophene	X	X
1,3-dimethyl-benzene	X	X
octane		X
octanal	X	X
thiazole	X	X
2-pentanone		X
furfural	X	X
2-nonanone	X	X
(Z)-2-heptenal	X	X
(E)-2-heptenal	X	X
1-octene	X	X
formic acid, heptyl ester	X	X
2-pentyl-thiophene		X
l-octen-3-one	X	X
3-pentyl-furan	X	X
2-propenal		X
(E)-2-tridecen-1 -ol		X
benzene		X
(E)-4-octene		X
l-penten-3-one		X
4-penten-2-one	X	X
2-methyl-thiazole		X
methyl-pyrazine		X
trans-2-(2-pentenyl)furan		X
3-ethylcyclopentanone		X

Figure 8

pyrrole	X	X
2-thiophenecarboxaldehyde		X
3 -thiophenecarboxaldehy de		X

Example 5: Heme-protein in the presence of thiamine affects the production of certain flavor compounds

The addition of thiamine in a flavor reaction mixtures with a heme protein and 5 other flavor precursors affected the formation of 5-Thiazoleethanol, 4-methyl-furan, 3,3'- dithiobis[2-methyl-thiazole, and 4-methylthiazole. These compounds are known to be present in meat and have beefy, meaty taste notes.

Flavor reaction mixtures at pH 6 containing LegH (1%), cysteine (10 mM), thiamine (1 mM), either glucose or ribose (20 mM), and with or without glutamic acid 10 (10 mM) were prepared and subsequently heated to 150 C for 3 minutes. These flavor

reaction samples then were evaluated by GCMS for the flavor compounds generated and evaluated by a trained panel for their sensory profiles. Volatile chemicals were isolated from the head space around the flavor reactions. GCMS showed 4-methyl-5- thiazoleethanol, 3,3'-dithiobis[2-methyl]-furan, and 4-methylthiazole compounds were 15 created by a mixture of LegH with thiamine, a sugar (either glucose or ribose), and cysteine. The same flavor reaction mixtures without thiamine did not generate these compounds; additionally these compounds were not generated when heme-proteins were not present in the flavor reaction mixtures.

The flavor reaction samples also were evaluated by a blinded trained sensory 20 panel, which described the samples with the addition of thiamine as more complex in taste and more beefy, meaty, and savory.

Example 6: Heme-proteins with nucleotides controls particular flavor compound production.

25 The addition of inosine monophosphate and guanosine monophosphate in mixes with heme protein and other precursors controlled the formation of flavor compounds (E)-4-octene, 2-ethyl-furan, 2-pentanone, 2,3-butanedione, 2-methyl-thiazole,, methyl- pyrazine, tridecane, (E)-2-octenal, 2-thiophenecarboxaldehyde, and 3- thiophenecarboxaldehyde. These compounds are known to be present in meat and have a beefy, meaty, buttery, and or

Figure 8

savory flavor notes.

Reactions containing heme protein at 1% (LegH) with cysteine (10 mM), and 5 glucose (20 mM), 1 mM IMP and 1 mM GMP, at pH 6.0 were prepared and heated to 150C for 3 minutes. Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors reacting heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the 10 flavor reaction and identified using GCMS, creating a profile of the volatile chemicals in the headspace around the flavor reaction mixture. GCMS showed 4-octene, 2-ethyl furan, 2-pentanone, 2,3-butanedione, 2-methyl-thiazole, methyl-pyrazine, tridecane, 2-octenal, 2-thiophenecarboxaldehyde, 3-thiophenecarboxaldehyde compounds were created by a mixture of hemeprotein LegH with IMP, GMP, glucose, and cysteine. The same samples 15 without IMP and GMP did not generate these compounds, additionally these compounds were also not created when heme-proteins were not present, just precursor molecules. Sensory evaluation by blinded trained panelist found the samples with the addition of inosine and guanosine as described as having more complexity in taste and more beefy, meaty, brothy and savory. Figure 2 shows the abundance of the novel flavor compounds 20 created with heme protein at 1% was mixed in a reaction at pH 6, with cysteine (10 mM), and glucose (20 mM), IMP (1 mM) and GMP (1 mM), and detected by solid phase microextraction (SPME) and then detected by GCMS.

Example 7: Flavor generation with the addition of a particular organic acid

25 The addition of lactic acid in mixes with heme protein, ribose, and cysteine controlled the formation of the flavor compounds nonanal, thiazole, 2-acetylthiazole, and 8-methyl-l-undecene. These compounds are known to be present in meat.

Reactions containing heme protein at 1%, cysteine (10 mM), and ribose (20 mM), and lactic acid (ImM), pH 6.0, were prepared and heated to 150C for 3 minutes.

30 Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors reacting heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction and identified using GCMS, creating a profile of the generated compounds. Nonanal,

5 thiazole, 2-acetylthiazole, and 8-methyl-1-undecene compounds were created by a

Figure 8

mixture of LegH with lactic acid, ribose, and cysteine. The same samples without lactic acid did not generate these compounds, additionally these compounds were not created in the absence of heme-proteins.

Sensory evaluation by blinded trained panelist found the samples with the addition of lactic acid as described as beefy, savory, browned, bready, and having malty notes. The sample with everything but lactic acid rated lower in browned, bready and malty notes.

Example 8: Flavor generated with the addition of a particular amino acid.

The addition of lysine in mixes with heme protein ribose, and cysteine controlled the formation of flavor compounds dimethyl trisulfide, nonanal, 2-pentyl-thiophene, furfural, 2-nonenal, 1-octanol, 2-nonenal, thiazole, 2-acetylthiazole, phenylacetaldehyde, 2-acetylthiazole. These compounds are known to be present in meat and some have a beefy, meaty, and or savory flavor.

Reactions containing heme protein at 1%, cysteine (10 mM), and ribose (20 mM), and lysine (ImM), at pH 6.0, were prepared and heated to 150C for 3 minutes. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors could react with the heme-protein.

These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction. Dimethyl trisulfide, nonanal, 2-pentyl-thiophene, furfural, 2-nonenal, 1-octanol, 2-nonenal, thiazole, 2-acetylthiazole, phenylacetaldehyde, 2-acetylthiazole compounds were created by a mixture of LegH with lactic acid, ribose, and cysteine. The same samples without lactic acid did not generate these compounds, additionally these compounds were not created when heme-proteins were not present, just precursor molecules. Sensory evaluation by blinded trained panelist found the samples with the addition of lysine as described as roast beefy, savory, and browned. The addition of lysine increased the roasted browned notes.

Example 9 - Flavor compound production by different Heme-proteins

The addition of different types of heme-proteins (LegH, Barley, *B. myoglobin,* or *A.*

~~Figure 8~~

aeolicus) in flavor reaction mixtures containing one or more flavor precursor compounds results in many of the same key meat flavors, including but not limited to 2- 10 pentyl-furan, 2,3-Butanedione, Thiophene, 2-methyl-thiazole, Pyrazine, Furan, Pyrrole, 2-methyl-furan and distinct differences in the flavor compounds, including but not limited to 2-pentyl-thiophene, Nonanal, 2-Nonanone, and l-Octen-3-one. These differences in flavor compounds can change the overall taste profile. The different types of heme-protein were LegH, Barley, *B. myoglobin, or A. aeolicus* used at 1% w/w in a 15 reaction mixed with cysteine (10 mM) and ribose (10 mM) at pH 6. The pre- reaction mixture was heated to 150 C for 3 minutes; this reaction created flavor compounds known to be present in meat; see Table 9. The characteristic flavor and fragrance components are mostly produced during the cooking process where the flavor precursor molecules react with the heme-protein. Samples were evaluated by GCMS to identify the 20 flavor compounds generated after heating and also evaluated for their sensory profiles.

Volatile chemicals were isolated from the head space around the flavor reactions. Table 9 shows the similarity and differences in volatile flavor compounds created by the different types of heme-proteins.

TABLE 9

Flavor compounds created by different heme-protein when heated with ribose and cysteine.

Name	LegH	Barley	*B. myoglobin*	*A. aeolicus*
Furan	x	x	x	x
Thiazole	x	x	x	x
benzaldehyde	x	x	x	x
2-acetylthiazole	x	x	x	x

Figure 8

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2-methyl-propanal	x	x	x	x
furfural	x	x	x	x
2,3-butanedione	x	x	x	x
2-pentyl-furan	x	x	x	x
2-pentanone	x	x		
pyrazine	x	x	x	x
dimethyl trisulfide	x	x	x	x
3 -methy 1-butanal	x	x		x
2-methyl-thiazole	x	x	x	x
pentanal	x	x	x	x
1,3,5-cycloheptatriene	x	x	x	x
methacrolein	x	x	x	x
heptanal	x	x	x	x
2-methyl-butanal	x	x		x
isothiazole	x	x	x	x
thiophene	x	x	x	x
propanal	x	x	x	x
2-heptenal	x		x	x
methyl-pyrazine	x	x	x	x
1-octene	x		x	x
butanal	x	x	x	x
2-acetyl-propen-2-ol	x	x	x	x
pyrrole	x	x	x	x
2-methyl-furan	x	x	x	x
nonanal		x	x	x
2-propenal		x	x	x
2-decenal		x	x	x
2-nonanone		x		x
2-octanone		x	x	x
2-tridecen-l-ol,			x	x
2-octanone			x	
2-octenal			x	x
4-methyl-2-heptanone			x	x
octanal			x	x
2-undecenal				x
butyrolactone				x
l-octen-3-one				x
3-methylheptyl acetate				x

Figure 8

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| 2-pentyl-thiophene | | | | x |

Example 10 - Generation of Meat flavors from different lipids

Several different samples including oils (canola oil or coconut oil), free fatty acids (FFA) (linoleic acid (C18:2), oleic acid (08:1), stearic acid (08:0), or myristic acid 5 (04:0)) and phospholipids (PL) (beef heart polar lipids extract, Biolipon95 (from Perimond), or NatCholinePC40 (from Perimond)) were tested for their ability to produce beefy flavor in the absence and in the presents of other precursors. Oils, FFAs, and PLs were added to 50 mM potassium phosphate buffer (PPB) pH 6.0 or a Maillard reaction mix (MRM) containing 50 mM potassium phosphate pH 6.0, 5 mM Cysteine, 10 mM 10 Glucose, 0.1 mM Thiamine, and 0.1% (w/v) LegHemoglobin. Lipids in combination with MRM were designed to capture the cross reactions of lipid degradation and Maillard reaction productions while lipids in phosphate buffer functioned as a lipid control. The oils were added at 3% of the total 1 mL volume of solution while FFAs and PLs were added at 1% of the total 1 mL volumes. All samples were cooked at 150°C for 3 mins, 15 cooled to 50°C and then analyzed using GCMS (SPME fiber sampling of headspace). After all samples were analyzed by GCMS the caps were removed and samples were smelled by a trained flavor scientist and aromas recorded.

Table 10
Legend showing components of each sample

Sample Name	Solution	Additives
MRM None	Maillard Reaction Mix	None
MRM Linoelic Acid	Maillard Reaction Mix	1% linoleic acid
MRM Oleic Acid	Maillard Reaction Mix	1% oleic acid
MRMC14	Maillard Reaction Mix	1% C14:0 free fatty acid
MRMC18	Maillard Reaction Mix	1% Cl8:0 free fatty acid
MRM Canola	Maillard Reaction Mix	3% Canola Oil
MRM Coconut	Maillard Reaction Mix	3% Coconut Oil
MRM BeefHeart	Maillard Reaction Mix	1% BeefHeart Polar Lipids Extract
MRM Biolipon95	Maillard Reaction Mix	1% Biolipon95 (emulsifier)

Figure 8

MRM_NatCholinePC40	Maillard Reaction Mix	1% NatCholinePC40 (emulsifier)
KPhosb Linoelic Acid	PPB, pH 6	1% linoelic acid
KPhosb Oleic Acid	PPB, pH 6	1% oleic acid
KPhos6_C14	PPB, pH 6	1% C14:0 free fatty acid
KPhos6_C18	PPB, pH 6	1% Cl8:0 free fatty acid
KPhosb Canola	PPB pH 6	3% Canola Oil
KPhosbCoconut	PPB, pH 6	3% Coconut Oil
KPhosb BeefHeart	PPB, pH 6	1% BeefHeart Polar Lipids Extract
KPhosb Biolipon95	PPB, pH 6	1% Biolipon95 (emulsifier)
KPhosb NatCholinePC40	PPB, pH 6	1% NatCholinePC40 (emulsifier)

Table 11 contains the aroma descriptions and Table 12 contains the GCMS data from the most interesting samples analyzed. Many of the lipids introduced a "fatty" aroma to MRM that was otherwise absent. The combinations of Linoleic Acid or 5 NatCholinePC40 in MRM produced the greatest abundance of fatty compounds suggesting that these lipids may improve the flavor perception of beef tallow. Linoleic Acid and NatCholinePC40 also showed high abundance of earthy-mushroom aromas.

The addition of lipids to MRM significantly increased the abundance of "nutty & roasted" aromas. Less desirable "green" aroma compounds were most prominent in 10 samples with unsaturated free fatty acids (linoleic acid or oleic acid) or phospholipids. In general, the addition of lipids significantly increased the number of target beef compounds made.

TABLE 11

15 **Aroma descriptions of each sample after it was cooked.**

Sample Names	Aroma Descriptions
MRMjDnly	brothy, malty, beef stew
KPhos6_BeefHeart	fatty, creamy, beef tallow, slight sweet, slight roasted nutty
MRM_BeefHeart	fatty, beef tallow, old meat, mushroom
KPhos6_Biolipon95	fatty, fresh
MRM_Biolipon95	fatty, brothy, hay, malty green

Figure 8

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KPhos6_NatCholinePC40	light fatty, fresh
MRM_NatCholinePC40	fatty, beef tallow, brothy
K-Phos6_C14	light/faint plastic/waxy
MRM_C14	brothy, beefy, minty, fresh
K-Phos6_C18	light/faint plastic/waxy
MRM_C18	beefy with cucumber &/or pepper aroma
K-Phos6_Canola	fresh, cucumber
MRM_Canola	fatty, brothy, oil, roasted nuts
K-Phos6_Coconut	nothing
MRM_Coconut	brothy, beefy, slight fatty, crackers
K-Phos6_Oleic Acid	fresh, cucumber, camphorous/minty-like
MRM_Oleic Acid	herbal, plastic, slight cheesy, brothy
K-Phos6_Linoelic Acid	light plastic
MRMJJnoelic Acid	fatty, light waxy, brothy, herbal

Table 12

List of aromatic compounds found in Beef by GCMS and a chart showing which were detected in each lipid plus MRM sample.

Compounds in Beef	MRM only	MRM BeefHeart	MRM_NatCholinePC40	MRM Linoleic acid
(s)-isopropyl lactate	N	N	N	N
1-ethyl-5-methylcyclopentene	Y	Y	Y	Y
1-heptanol	N	Y	N	N
l-hepten-3-ol	N	Y	Y	Y
1-heptene	N	Y	Y	Y
2-methyl-1 -heptene	N	N	N	N
1-hexanol	N	Y	Y	Y
2-ethyl-1 -hexanol	N	N	N	N
1-nonanol	N	N	Y	N
1-nonene	N	Y	Y	N
1-octanol	N	Y	Y	N
l-octen-3-ol	N	Y	Y	Y

Figure 8

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1-octen-3-one		Y	Y	Y
1-octene	N	N	N	N
1-pentanol	N	Y	Y	Y
1-penten-3-ol	N	Y	Y	N
1-propanol	N	N	N	N
8-methyl-1 -undecene	N	Y	Y	Y
1,3-hexadiene	N	N	N	Y
3-ethyl-2-methyl-1,3-hexadiene	N	Y	Y	Y
1,3-octadiene		N	N	Y
1,3,5-cycloheptatriene	N	N	N	N
2.3-dihydro-5,6-dimethyl-1.4- dioxin	N	N	N	N
1,7-octadien-3-ol	N	Y	N	N
lh-pyrrole-2-carboxaldehyde	N	N	N	N
2-methyl-1 H-pyrrole	N	N	N	N
2-acetyl-2-thiazoline		N	N	N
2-acetylthiazole	Y	Y	Y	Y
2-butanone	N	Y	Y	Y
2-butenal	N	Y	Y	Y
2-ethyl-2-butenal	N	N	N	Y
3 -methyl-2-butenal	N	N	Y	Y
3 -methyl-2-cyclohexen-1 -one	N	N	N	N
2-decanone	Y	Y	Y	N
(E)-2-decenal	N	N	N	N
(Z)-2-decenal	Y	Y	Y	Y
2-furanmethanol	N	N	N	N
2-heptanone	Y	Y	Y	Y
6-methyl-2-heptanone	N	N	Y	N
(E)-2-heptenal	N	Y	Y	Y
(Z)-2-heptenal	N	N	N	.....Y .....
(E)-2-hexenal	N	Y	Y	Y
2-ethyl-2-hexenal	N	N	N	N
2-methyl-2-heptene		N	N	N
2-n-heptylfuran		N	N	N
2-n-octylfuran	Y	Y	Y	N

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~~Figure 8~~

2-nonanone	N	Y		N
(E)-2-nonenal	Y	Y	Y	Y
(Z)-2-nonenal	N	N	N	Y
2-octanone	Y	Y	Y	Y
(Z)-2-octen-l-ol	Y	Y	Y	Y
(E)-2-octenal	N	Y	Y	Y
2-pentanone	N	Y	Y	N
1 -propoxy-2-propanol	N	N	N	N
1 -(acetyloxy)-2-propanone	Y	N	N	N
1 -hydroxy-2-propanone	Y	N	N	N
2-propenal	N	N	N	Y
2-thiophenecarboxaldehyde	Y	Y	Y	Y
2-undecenal	N	Y	Y	Y
2,3-butanedione	N	N	N	Y
2,3-pentanedione	N	N	N	N
(E,E)-2,4-decadienal	N	Y	Y	Y
2,4-decadienal	N	N	N	Y
(E,E)-2,4-heptadienal	N	Y	Y	Y
(E,E)-2,4-nonadienal	N	Y	Y	Y
2,6-dimethylpyrazine	N	N	N	N
(E,Z)-2,6-nonadienal	N	N	Y	N
5-ethyldihydro-2(3H)-furanone	N	Y	Y	Y
5-methyl-2(3H)-furanone	N	N	N	N
dihydro-5-pentyl-2(3H)-furanone	N	N	Y	Y
dihydro-5-propyl-2(3H)-furanone	N	N	N	N
2(5H)-furanone	N	N	N	N
tetrahydro-6-methyl-2H-pyran-2-one	N	N	N	N
3 -ethylcyclopentanone	N	Y	Y	Y
3-hexanone	N	N	N	N
3-methyl-2-thiophenecarboxaldehyde	N	N	N	N
3-octanone	Y	Y	N	Y
3-octen-2-one	N		Y	
3 -thiophenecarboxaldehyde	N	Y	Y	Y

Figure 8

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~~Figure 8~~

(E,E)-3,5-octadien-2-one	N	N	Y	Y
dihydro-2-methyl-3 (2H)-furanone	N	N	N	N
4-cyanocyclohexene	N	N	N	N
4-cyclopentene-1,3-dione	N	N		N
4-decyne	N		N	N
(Z)-4-heptenal	N	Y	Y	Y
4-methyloctanoic acid	N	N	N	N
(E)-4-octene	N	N	N	N
2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one	Y	N	N	N
6-methyl-5-hepten-2-one	Y	N	N	N
acetaldehyde	N	N	N	Y
acetic acid	N	N	N	N
acetic acid ethenyl ester	Y	N	N	N
acetoin	Y	N	N	N
acetone	1111 11	N	N	Y
acetonitrile	N	N	N	Y
benzaldehyde	Y	Y	Y	Y
4-ethyl-benzaldehyde	N	Y	Y	N
benzene	1111 1111	N	N	N
benzoic acid, hydrazide		N	N	N
butanal		N	N	Y
2-methyl-butanal	N	N	N	N
3 -methy 1-butanal		N	N	N
butanoic acid	N	N	N	N
butyrolactone	Y	Y	N	Y
caprolactam	N	N	N	N
carbon disulfide	N	N	N	Y
1 -ethyl-1 -methyl-cyclopentane	Y	Y	Y	Blli
propyl-cyclopropane	N	N		Y
decanal	N	Y	¥	N
dihydro-3 -(2H)-thiophenone	N	N	N	N
Dimethyl sulfide	Y	N	N	N
dimethyl sulfone	N	N	N	N
dimethyl trisulfide	Y	Y	N	N
ethanethiol	N	N	N	N

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~~Figure 8~~

ethanol	N	N	N	Y
1 -(1 (H)-pyrrol-2-yl)-ethanone	N	N	N	N
1 -(2-furanyl)-ethanone	N	N	N	N
ethosuximide	Y	N	N	N
formic acid, heptyl ester	Y	Y	N	N
furan	Y	N	N	Y
2-ethyl-furan	...Y....	N	N	N
2-hexyl-furan	Y	N	N	Y
2-methyl-fiiran	N	N	N	Y
2-pentyl-furan	N	Y	Y	Y
2-propyl-furan	N	N	Y	Y
3-methyl-furan	Y	N	N	N
3-pentyl-furan	Y	Y	Y	Y
furfural	N	Y	Y	Y
heptanal	N	Y	Y	Y
heptanoic acid	N	N	N	Y
2-methyl-hex-2-yn-4-one	N	N	N	N
hexanoic acid	N	N	N	Y
hydrogen sulfide	N	N	N	N
m-aminophenylacetylene	N	N	N	N
maleic anhydride	N	N	N	N
methacrolein	N	N	N	N
methanethiol	N	N	N	N
methyl ethanoate	N	N	N	N
methyl isobutyl ketone	Y	N	N	N
n-caproic acid vinyl ester	N	Y	■■■1	N
nonanal	N	Y	Y	Y
3 -methyl-nonane	Y	N	N	N
nonanoic acid	Y	N	N	N
octanal	N	Y	Y	Y
octane	N	N	N	Y
octanoic acid	N	N	N	Y
oxalic acid, isobutyl pentyl ester	Y	N	N	N
p-cresol	N	N	N	N
pentanal	N	N	N	Y
pentanoic acid	Y	N	N	Y

Figure 8

Figure 8

4-ethyl-phenol	N	Y	Y	N
phenylacetaldehyde	Y	Y	Y	Y
(p-hydroxyphenyl)-phosphonic acid	Y	N	N	N
propanal	N	N	N	Y
2-methyl-propanal	N	N	N	N
propanoic acid	N	N	N	N
2-methyl-propanoic acid	Y	N	N	N
propanoic acid, ethenyl ester	N	N	N	N
pyrazine	N	Y	N	Y
2-ethyl-5 -methyl-pyrazine	N	N	N	N
2-ethyl-6-methyl-pyrazine	N	N	N	N
2,3-dimethyl-pyrazine	N	N	N	N
2,5-dimethyl-pyrazine	N	N	N	N
3 -ethyl-2,5 -dimethy 1-pyrazine	Y	N	N	N
ethyl-pyrazine	N	N	N	N
methyl-pyrazine	N	N	N	N
trimethyl-pyrazine	Y	N	N	N
pyridine	Y	N	Y	N
pyrrole	IIIIII	Y	HH	Y
styrene	Y	N	Y	N
thiazole	Y	Y	Y	Y
methyl-thiirane	N	N	N	N
thiophene	N	N	N	Y
2-hexyl-thiophene	Y	N	Y	N
2-pentyl-thiophene	N	Y	N	N
trans-2-(2-pentenyl)furan	N	Y	Y	N
trans-3 -nonen-2-one	N	Y	Y	Y
undecanoic acid	N	N	N	N
Total # of Compounds Detected:	54	63	66	76

In samples having fatty or creamy aromas, 2,4-decadienal, (E,E)-2,4-nonadienal, (E,E)-2,4-heptadienal, and/or (E,E)-2,4-decadienal were detected in the KPhos6_BeefHeart, MRM BeefHeart, MRM_BioLipon95, MRM_NatCholinePC40,

Figure 8

Kphos6_Canola, MRM Canola, KPhos6_01eic Acid, KPhos6_Linoleic acid and MRM Linoleic acid samples. For (E,E)-2,4-decadienal, the strongest signal intensity was in the MRM_NatCholinePC40 sample, followed by the MRM Linoleic acid, KPhos6_Linoleic acid, MRM BeefHeart, MRM_BioLipon95, KPhos6_BeefHeart,

5 MRM Oleic Acid, and KPhos6_01eic Acid samples. For (E,E)-2,4-heptadienal, the strongest signal intensity was in the MRM_NatCholinePC40 sample followed by the MRM Canola sample. (E,E)-2,4-heptadienal also was detected in the MRM_BioLipon95, MRM BeefHeart, and MRM Linoleic acid samples. For (E,E)-2,4- nonadienal, the strongest signal intensity was in the MRM Canola and MRM Linoleic 10 acid samples. (E,E)-2,4-nonadienal also was detected in the Kphos6_Canola,

MRM_NatCholinePC40, MRM_BioLipon95, MRM BeefHeart, and KPhos6_Linoleic acid samples. For 2,4-decadienal, the strongest signal intensity was in the MRM Linoleic acid sample. 2,4-decadienal also was detected in KPhos6_Linoleic acid, MRM Canola, and KPhos6_01eic Acid samples.

15 In samples having earthy or mushroom aromas, 3-octen-2-one, l-octen-3-one, 3-octanone, and/or l-octen-3-ol were detected in the KPhos6_BeefHeart, MRM BeefHeart, Kphos_BioLipon95, MRM_BioLipon95, Kphos_NatCholinePC40, MRM_NatCholinePC40, MRM Canola, KPhos6_01eic Acid, MRM Oleic Acid, KPhos6_Linoleic acid, and MRM Linoleic acid samples. For l-octen-3-ol, the strongest 20 signal intensity was in the MRM Linoleic acid sample, followed by

MRM_NatCholinePC40, KPhos6_Linoleic acid, MRM BeefHeart, KPhos6_BeefHeart, MRM Canola, MRM_BioLipon95, KPhos6_01eic Acid, and MRM Oleic Acid samples. 3-octanone was detected in the MRM Oleic Acid, KPhos6_Linoleic acid, and MRM Linoleic acid samples. For l-octen-3-one, the strongest signal intensity was in the 25 MRM Linoleic acid and MRM BeefHeart samples, followed by KPhos6_Linoleic acid, MRM_NatCholinePC40, KPhos6_BeefHeart, MRM_BioLipon95, MRM Oleic Acid, and KPhos6_01eic Acid samples. For 3-octen-2-one, the strongest signal intensity was in the KPhos6_Linoleic acid sample, followed by MRM Linoleic acid, MRM_NatCholinePC40, KPhos6_BeefHeart, KPhos6_01eic Acid, MRM Oleic Acid,

30 MRM BeefHeart, MRM_BioLipon95, MRM Canola, Kphos_BioLipon95, and

Figure 8

Kphos_NatCholinePC40. Pyrazine was detected in the MRM Coconut, MRM C18, MRM C14, and MRM_BioLipon95 samples.

In samples having a nutty and roasted aroma, thiazole and 2-acetylthiazole were the most abundant compounds detected, along with pyrazine, methyl pyrazine, trimethyl 5 pyrazine, and 3-ethyl-2,5-dimethylpyrazine. 2-acetylthiazole was detected in all samples with MRM and most abundant in samples with MRM Beefheat, MRM_biolipon95,

MRM Canola, and MRM coconut. Thiazole was created in samples with MRMCoconut, MRM BeefHeat, MRM_Biolipon95, MRM C14, MRM C18, MRM Canola, MRM Oleic acid and MRM Linoleic acid and MRM_NatCholinePC40. Pyrazine was 10 present in the largest amount in samples with MRM-Coconut, followed by samples MRM BeefHeat, MRM_Biolipon95, MRM C14, MRM C18, MRM Canola having roughly equal amount, MRM Oleic acid and MRM Linoleic acid sample had even less. Methyl-pyrazine was present in MRM_Biolipon95 and MRM Coconut. 3-ethyl-2,5- dimethyl- pyrazine and trimethyl- pyrazine, were present only without phospholipids in 15 the MRM.

In samples having green, vegetable, or grass aromas, 1-heptanol, l-hepten-3-ol, 1-hexanol, (E)-2-heptenal, (Z)-2-heptenal, (E)-2-hexenal, 2-pentyl-furan, and/or heptanal were detected in the KPhos6_BeefHeart, MRM BeefHeart, Kphos_BioLipon95, MRM_BioLipon95, Kphos_NatCholinePC40, MRM_NatCholinePC40, Kphos_C14, 20 MRM C14, Kphos_C18, MRM C18, MRM Canola, MRM Coconut, KPhos6_01eic Acid, MRM Oleic Acid, KPhos6_Linoleic acid, and MRM Linoleic acid samples. For 2-pentyl-furan, the strongest signal intensity was in the KPhos6_BeefHeart sample, followed by the KPhos6_Linoleic acid, MRM_BioLipon95, MRM Linoleic acid, MRM BeefHeart, MRM Oleic Acid, MRM_NatCholinePC40, MRM Canola, 25 KPhos6_01eic Acid, and Kphos_NatCholinePC40 samples. For (E)-2-heptenal, the strongest signal intensity was in the MRM BeefHeart, MRM Canola, MRM Oleic Acid, and KPhos6_Linoleic acid samples, followed by the KPhos6_01eic Acid, MRM_BioLipon95, KPhos6_BeefHeart, MRM Linoleic acid, MRM_NatCholinePC40, Kphos_BioLipon95, and Kphos_NatCholinePC40 samples. For (Z)-2-heptenal, the 30 strongest signal intensity was in the MRM Linoleic acid sample. MRM Linoleic acid

also was detected in the KPhos6_Linoleic acid sample. For heptanal, the strongest signal

Figure 8

intensity was in the MRM Oleic Acid sample, followed by the KPhos6_01eic Acid, MRM C14, MRM C18, MRM Canola, MRM BeefHeart, MRM_NatCholinePC40, MRM Linoleic acid, and KPhos6_BeefHeart samples. For , (E)-2-hexenal, the strongest 5 signal intensity was in the MRM Linoleic acid sample, followed by the

MRM_NatCholinePC40, KPhos6_Linoleic acid, and MRM Oleic Acid samples.

Example 11 - Creation of beefy flavors using complex precursor mixtures

A formulation was prepared (the "magic mix," see Table 13 containing the 10 estimated concentrations of amino acids, sugars, and other small molecules in beef based on their values reported in literature. The magic mix was tested for its ability to produce beefy flavors in the presence of LegHemoglobin (LegH). The magic mix and 1% w/v LegH were added to the meat replica, pH 6.0 (see Table 4) and baked in a convection oven for 7 minutes at 160°C. A control sample was prepared by adding 1% w/v LegH to 15 the meat replica, pH 6.0 and baking in a convection oven for 7 minutes at 160°C.

The meat replica sample containing only LegH, was compared to the meat replica sample containing the magic mix and LegH by a sensory panel and GCMS analysis. Live tasters rated the flavored meat replicas for beefiness, bitterness, and levels of savory flavors, and off flavors. Each property was rated on a 7 point scale in which 7 was the 20 highest amount of the specified property (e.g., a standard 80:20 ground beef would be rated 7 on the beefy scale). The Magic Mix flavor was rated one point higher in beefy character than the LegH only sample (LIG. 1).

To determine which chemical products were produced upon heating, a solution of Magic Mix was prepared with 1% w/v LegH at pH 6.0. The samples were cooked with 25 shaking at 150°C for three minutes, then Solid Phase Micro Extraction (SPME) was performed for twelve minutes at 50°C to extract the volatile compounds above the headspace of the reaction. A search algorithm was used to analyze the retention time and mass fingerprint information of the volatile compounds and assign chemical names to peaks. Table 14 shows the compounds identified in both the Magic Mix + LegH (MM,

30 average of two samples) and in the LegH alone in buffer (LegH, average of five samples)

samples. The compounds in Table 14 are listed in order of the retention time (R.T., in

Figure 8

seconds), and are designated as having a zero peak area (0), or a small (S), medium (M), or large (L) average peak area. Hundreds of compounds were identified between the samples, many of which are characteristic of beefy aroma, including but not limited to 5 l,3-bis(l,l-dimethylethyl)-benzene, 2-methyl 3-furanthiol, and Bis(2-methyl-4,5-dihydro-3-furyl) disulfide, which increased in the samples containing the Magic Mix and LegH.

Table 13

Chemical entities added to the Magic Mix

Chemical entity	m M
Alanine	5.6
Arginine	0.6
Asparagine	0.8
Aspartate	0.8
Cysteine	0.8
Glutamic acid	3.4
Glutamine	0.7
Glycine	1.3
Histidine	0.6
Isoleucine	0.8
Leucine	0.8
Lysine	0.7
Methionine	0.7
Phenylalanine	0.6
Proline	0.9
Threonine	0.8
Tryptophan	0.5
Tyrosine	0.6

Figure 8

Valine	0.9
glucose	5.6
Ribose	6.7
Maltodextrin	5.0
Thiamine	0.5
GMP	0.24
IMP	0.6
Lactic acid	1.0
creatine	1.0
NaCl	10
KC1	10
Kphos pH 6.0	10

Figure 8

TABLE 14

Compounds identified with GC-MS analysis in samples with MM and LegH, or LegH alone (average of five samples)

R.T.(s)	Name	MM with LegH	LegH alone
248	acetaldehyde	L	S
256.3	carbon disulfide	L	S
264.3	dimethyl sulfide	S	0
265	oxalic acid, isobutyl pentyl ester	M	0
268.1	2,3,4-trimethyl-pentane	M	0
269.2	methanethiol	S	0
283.4	propanal	M	0
285.4	octane	M	0
287.1	furan	M	0
295.3	2-methyl-propanal	L	S
297.6	acetone	L	S
319.3	2-propenal	M	S
338.1	2-methyl-furan	M	S
342.1	butanal	L	S

Figure 8

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344.2	2,4-dimethyl-1 -heptene	M	0
346.3	methacrolein	M	0
357.4	methyl-thiirane	L	0
360.2	3-methyl-furan	S	0
363.7	butanone	L	S
368.9	2,3 -dihydro-5 -methyl-furan	M	S
376.4	2-methyl-butanal	L	M
381.1	3 -methy 1-butanal	L	M
390.6	isopropyl alcohol	0	S
399.6	ethanol	L	M
406.2	2-propenoic acid, methyl ester	M	0
408.2	benzene	S	0
414.4	methyl vinyl ketone	M	0
416.4	2,2,4,6,6-pentamethyl-heptane	M	0
422.6	2-ethyl-furan	S	0
438.4	2-ethylacrolein	M	0
449.9	2-pentanone	S	0
453.2	pentanal/2,3-butanedione	L	0
453.8	2,3-butanedione	L	M
472.8	4,7-dimethyl-undecane	M	S
485.9	2-methyl-pentanal	M	0
492.6	2-methyl-1 -penten-1 -one	S	0
496.6	(E)-3-penten-2-one	M	0
508.6	l-penten-3-one	M	0
510.6	trichloromethane	M	M
520.4	p-dithiane-2,5-diol	M	0
525.5	3 -methyl-pentanal	M	0
535.1	(E)-5-decene	M	0
536.5	toluene	M	S
537.9	2-butenal	M	S
543.8	4-penten-2-one	M	0
550.8	methyl thiolacetate	M	0
683.7	p-xylene	S	0
727.4	dimethyl selenone	M	0
738.3	methyl isopropyl disulphide	M	0
755	2-heptanone	M	0
758.7	heptanal	L	0
781.9	1,3-diisopropoxy-1,3-dimethyl-1,3-	S	M

Figure 8

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~~Figure 8~~

	disilacyclobutane		
789.4	3 -methyl-2 -butenal	M	0
793.4	4-methyl-2-heptanone	M	0
810.4	pyrazine	M	0
818.8	isothiazole	S	0
827.1	acetyl valeryl	M	0
831.8	2-pentyl-furan	L	0
851	2-methyl-thiazole	S	0
853.3	isothiocyanato-methane	S	0
870.9	thiazole	L	0
879.2	styrene	M	0
890.7	1 -(methylthio)-propane	M	0
895.6	methyl-pyrazine	M	0
910.5	thiocyanic acid, methyl ester	S	0
918.6	4-methylthiazole	M	0
921.4	2-octanone	M	0
923.9	2-methyl-cyclopentanone	M	0
927.9	octanal	L	S
934.3	tridecane	M	0
948.8	trans-2-(2-pentenyl)furan	S	0
961.9	1 -hydroxy-2-propanone	M	0
974.5	(E)-2-heptenal	M	0
987.4	5 -methyl-1 -undecene	M	0
993.8	2-hexyl-furan	M	0
1007.8	7 -methyl-(E)-5 -undecene	M	0
1024.1	2-methyl-5 -(methylthio)-furan,	S	0
1058.6	2-butyl-1-decene	M	0
1079.3	dimethyl trisulfide	L	S
1085.3	2-nonanone	M	0
1093.2	nonanal	L	M
1142.3	1,3 -bis(1,1 -dimethylethyl)-benzene	M	0
1149.6	(E)-2-octenal	M	0
1164.5	1-heptanol	M	0
1193.5	methional	L	0
1198.8	acetic acid	M	S
1207.2	furfural	M	0
1242.1	2-decanone	M	0
1250.8	decanal	M	0

Figure 8

EXHIBIT 1C

Figure 8

1265.2	l-decen-3-one	M	0
1283.3	pyrrole	M	0
1292.6	5 -ethenyl-4-methyl-thiazole	M	0
1294.3	benzaldehyde	L	M
1303.7	2-n-octylfuran	M	0
1305.6	(E)-2-nonenal	M	0
1341.4	1-octanol	M	0
1361.1	2-methyl-1 (H)-pyrrole	S	0
1391.7	2-undecanone	M	0
1401.2	(E)-2-octen-l-ol	M	0
1448	butyrolactone	S	S
1456.3	(E)-2-decenal	M	0
1462.4	phenylacetaldehyde	L	S
1466.3	2-acetylthiazole	L	0
1471.3	acetophenone	M	S
1475.4	1-nonanol	M	0
1487	methyl (methylthio)methyl disulfide	M	0
1497.1	5-(2-chloroethyl)-4-methylthiazole	L	0
1497.5	1 -(ethylthio)-2-(methylthio)-buta-1,3-diene	L	S
1512	3 -thiophenecarboxaldehyde	M	0
1518.8	2-nonen-4-one	M	0
1531.7	2-thiophenecarboxaldehyde	S	0
1543.9	dodecanal	M	0
1551.6	4-ethyl-2-methyl-pyrrole	S	0
1558.2	3 -(methy lthio)-propanenitrile	S	0
1561.2	3-decen-2-one	M	0
1613.1	bis(2-methyl-4,5-dihydro-3-furyl) disulfide	M	0
1615.6	1,10-undecadiene	M	0
1619.5	2-undecenal	S	0
1668.9	2-phenylpropenal	M	0
1692.3	(Z)-3-decen-l-ol, acetate	M	0
1733.1	3-phenyl-furan	S	0
1739.7	4-nitrophenyl 2-thiophenecarboxylic acid ester	S	0
1741.2	5 -formyl-4-methylthiazole	M	0
1749.7	pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester	M	0
1765.5	benzyl alcohol	S	0

Figure 8

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~~Figure 8~~

1774.2	pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester	S	0
1796.9	dodecanal	M	0
1806.1	(1 -ethyl-1 -propenyl)-benzene	S	0
1825.6	1-undecanol	M	S
1827.9	2-methyl-3 -furanthiol	M	0
1828.3	2-methyl-3-(methylthio) furan	M	0
1836.1	4-chloro-2,6-bis(1,1 -dimethylethyl)-phenol	S	0
1844.1	phenol	S	S
1845.3	[(methylsulfonyl)methyl]-benzene	S	0
1850.3	(e)-2-tridecen-1 -ol	M	0
1859.9	1 -heptyl-1,2,3,4-tetrahydro-4-methyl-naphthalene	S	0
1863.2	2,4-decadienal	S	0
1905.1	3,3 '-dithiobis [2-methyl]-furan	M	0
1906.9	3,5-di-tert-butylbenzoic acid	S	0
1909.6	4-ethoxy-benzoic acid, ethyl ester	S	0
1921.5	3 -(pheny hnethy l)-2,5 -piperazinedione	S	0
1944.5	9-octadecenal	M	0
1959.7	3,5 -bis(1,1 -dimethylethyl)-phenol	M	S
1968.4	4-methyl-5 -thiazoleethanol	M	S
2007.8	1, F-(1,2-cyclobutanediyl)bis-cis-benzene	S	0
2019.8	benzoic acid	S	S
2026.4	4-quinolinecarboxaldehyde	S	0
2027.8	m-aminophenylacetylene	M	0

Example 12 - Ferrous Chlorin Catalyzes Production of Meat-like flavor compounds

Fresh green spinach (10 lb) was added to 500 mL water and finely ground in a Vitamix blender to yield 2 L of green suspension. Acetone (8L) was added with mixing and the material was allowed to extract for 1 hour. The material was filtered through Whatman filter paper and the acetone was removed on a rotary evaporator (Buchi). To the residual green suspension (500 mL) was added 2 mL of 10 M HC1, causing the 10 suspension to turn brown. To this was added Ig of FeCh.dFLO in 10 mL FLO. The

Figure 8

solution was shaken then left at 4°C for 16 hours. This suspension was extracted with diethyl ether (3x50mL) to give a bright green organic phase, the combined organics were washed with saturated sodium chloride solution, dried over sodium sulfate, filtered and evaporated to leave a black paste (l.lg). The pellet was dissolved in chloroform for 5 fractionation.

Chlorophyll and Ferrous chlorin crude fractions were stored at -20°C. Crude extracts were fractionated by reverse-phase high-pressure liquid chromatography (RP-HPLC). HPLC conditions are outlined in Table 15. Both chlorophyll and ferrous chlorophyll were eluted from the column with a peak retention time of 7.6 minutes. Eluted material was collected from 7.3 - 8.0 minutes. Collected fractions were pooled and stored on ice. Collected fractions were re-chromatographed and showed a single peak with retention time 7.6 minutes. The desired fractions were pooled, then 10% sunflower oil was added, methanol was removed on a rotary evaporator (Buchi).

TABLE 15

HPLC conditions for purification of chlorophyll and ferrous chlorin from crude extract.

Sample:	Chlorophyll or Fe-chlorin (~2mg/mL in CHCfr)
System:	Agilent 1100 with Chemstation
Column:	Zorbax Bonus-RP (4.6 x 250 mm, 5uM)
Mobile phase:	acetonitrile, methanol, ethyl acetate (60:20:20) isocratic flow
Temperature:	30°C
Flow Rate:	1.0 mL per minute
Injection volume:	0.05 mL

Preparation of Flavor Reaction Containing Ferrous Chlorin or Leghemoglobin

A solution of ferrous chlorophyll was mixed with the Magic Mix (Table 13) to a final concentration of 0.35% ferrous chlorin, 1% glycerol, 0.005% tween-20, 5% sunflower oil, 100 mM NaCl, 20 mM phosphate at pH 6. Leghemoglobin (0.35%) at pH 6 in phosphate buffer (20 mM), 100 mM NaCl, was mixed with the Magic Mix (Table 13), 1% glycerol, and 0.005% tween-20. The flavor reaction mixtures were heated to

Figure 8

150°C for 3 minutes; this reaction created flavor compounds known to be present in meat, created by hemoglobin and also created by ferrous chlorin; *see* Table 16.

The characteristic flavor and fragrance components were mostly produced during 5 the cooking process when the flavor precursor molecules reacted with the heme-protein or the ferrous chlorophyll. Samples were evaluated by GCMS to identify the flavor compounds generated after heating. Volatile chemicals were isolated from the headspace around the flavor reactions. The profile of the volatile chemicals in the headspace around the flavor reaction mixtures that were similar between heme-protein and ferrous chlorin 10 are shown in Table 16. Notabily, many of the compounds created by the ferrous chlorin are important in the flavor of meat.

TABLE 16

Flavor Compounds created by both Ferrous Chlorin and LegH with Magic Mix.

1-heptanol	acetone
1-hexanol	acetonitrile
1-octanol	benzaldehyde
l-octen-3-ol	butanal
l-octen-3-one	2-methyl-butanal
1-pentanol	dimethyl trisulfide
2-acetylthiazole	ethyl acetate
2-butenal	furan
3 -methyl-2-butenal,	2-ethyl-furan
(Z)-2-decenal	2-hexyl furan
6-methyl-2-heptanone	2-pentyl-furan
(E)-2-heptenal	furfural
(E)-2-hexenal	heptanal
2-methyl-3 -furanthiol	aminophenylacetylene
(E)-2-nonenal	methacrolein
(E)-2-octenal	methional
2-pentanone	octanal
1 -hydroxy-2-propanone	octane
2-thiophenecarboxaldehyde	oxalic acid, diallyl ester
2-undecenal	2,3-butanedione

~~Figure 8~~

3 -methyl-3 -buten-2-one	2-methyl-propanal
3 -thiophenecarboxaldehyde	pyrazine
(E)-4-octene,	2,3- dimethy 1-pyr azine
methyl-pyrazine	2,5-dimethyl-pyrazine
thiazole	

Example 13 - Flavor creation by immobilized Hemin

Preparation of hemin linked CM Sepharose.

200 mg of bovine hemin (Sigma Aldrich) was loaded into a scintillation vial. A 5 small magnetic stir bar, 800 pL acetonitrile, 64 pL 4-methylmorpholine, and 71 mg of N-hydroxysuccinimide were added in that order. The vial was placed in an ice bath and chilled then 118 mg of N-(3-dimethylaminopropyl)-N·-ethyl-carbodiimide hydrochloride was added with stirring, followed by 845 pL of Jeffamine ED900. This was stirred while allowing the black mixture to warm to ambient temperature. Chloroform (10 mL) was 10 added to the mixture followed by water (4 mL). A flashlight was used to distinguish between organic and aqueous layers since both were black and the organic layer was pipetted off and concentrated to a dark black oil. The oil was dissolved in a 4:1 mixture of acetonitrile and ethanol to make an approximately 10% strength solution that was inky black in color.

15 2 mL of water swelled and equilibrated CM Sepharose was equilibrated in a BioRad minicolumn with 3 volumes of acetonitrile. The resin was resuspended in 1 mL acetonitrile and pipetted into a scintillation vial. This was followed with 44 microliters 4-methylmorpholine, 23 mg N-hydroxysuccinimide, and 39 mg of solid N-(3-dimcthylaminopropyl)-N'-cthyl-carbodiimidc hydrochloride. The mixture was vortexed 20 vigorously and then shaken for three hours. To this white solid was added *570* microliters of inky black 20% strength hemin coupled diamine. The black solid was vortexed and shaken for an hour. The slurry strongly resembled Turkish coffee. The mixture was poured into a BioRad minicolumn and filtered, washed with acetonitrile until what came out no longer resembled espresso, then switched to deionized water, and 25 finally 20 mM pH 9 sodium carbonate buffer. The black solid was washed until the effluent ran clear and then resuspended in 2 mL of buffer for storage until use.

Figure 8

Flavor reaction

The flavor reaction was created with heme protein (equine myoglobin -Sigma) at 0.35% in a phosphate buffer (20 mM) at pH 6.0 with 100 mM NaCl, this was mixed with 5 Magic Mix (Table 13). Another flavor reaction was created with Immobilized Hemin at 0.35% in a phosphate buffer (20 mM) at pH 6.0 with 100 mM NaCl, this was mixed with Magic Mix (Table 13). The flavor reaction mixtures were heated to 150°C for 3 minutes; this reaction created flavor compounds known to be present in meat.

The characteristic flavor and fragrance components were mostly produced during the cooking process when the flavor precursor molecules reacted with the Heme-protein or the immobilized Hemin. Samples were evaluated by GCMS to identify the flavor compounds generated after heating. Volatile chemicals were isolated from the headspace around the flavor reactions. As can be seen in Table 17, immobilized hemin catalyzed production of compounds similar to those whose production was catalyzed by myoglobin 15 free in solution. Notably, the profiles of flavor compounds, measured by GCMS,

produced by cooking mixtures containing the immobilized hemin and the heme-protein, respectively, were very similar.

TABLE 17

Flavor compounds produced by cooking mixtures containing either myoglobin free in solution or hemin coupled to a solid support

Flavor compound	myoglobin	hemin-linker-resin
2-methyl-5-(methylthio)-thiophene	Low	
dihydro-5-propyl-2(3H)-furanone	Low	
octane	Low	
pyrrole	Low	Low
methanethiol	Low	Low
2-thiophenecarboxaldehyde	Low	Low
methyl-pyrazine	Low	Low
1 -hydroxy-2-propanone	Low	Low
propanal	Low	Low

Figure 8

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thiophene	Low	medium
pyridine	Low	Low
2-methyl-furan	Low	medium
oxalic acid, butyl propyl ester	Low	Low
pyrazine	medium	Low
oxalic acid, diallyl ester	medium	medium
2-butenal	medium	large
furfural	medium	medium
nonanal	medium	medium
2-ethyl-furan	medium	Low
ethanol	medium	very large
tert-butanol	medium	
3,3 '-dithiobis [2-methyl] -furan	medium	medium
m-aminophenylacetylene	medium	medium
2,5 -dihydro-3,4-dimethyl-furan	medium	medium
2-acetylthiazole	medium	medium
cyclohexane	medium	
ethyl tert-butyl ether	medium	
carbon disulfide	medium	medium
thiazole	medium	medium
acetonitrile	medium	large
2-pentyl-furan	medium	Low
3 -thiophenecarboxaldehyde	medium	medium
2-methyl-butanal	medium	medium
thiazole	medium	large
2-methyl-3 -furanthiol	larege	large
2-propenal	large	large
3 -methyl-2 -butenal	large	medium
2-methyl-3-(methylthio) furan	large	large
ethyl acetate	large	medium
methacrolein	large	medium
methyl-thiirane	large	large
methional	large	large
methyl alcohol	large	medium
2-butanone	large	Low
2,3-butanedione	large	medium
acetone	large	large
furan	large	medium

Figure 8

benzaldehyde	large	medium
methyl thiolacetate	large	medium
acetaldehyde	very large	very large
2-methyl-propanal	very large	very large
dimethyl trisulfide	very large	very large
3 -methy 1-butanal	very large	very large
propyl-cyclopropane		medium
(E)-2-octenal		medium
2-n-propylaziridine		medium
thiirane		medium
ethyl formate		medium
methyl vinyl ketone		medium
2-propenoic acid, ethyl ester		medium
1-nonanol		large
1-octene		large
1-heptanol		large
1-dodecene		large
phorone		very large

Example 14. The combination of precursors with Heme protein drives flavor reactions.

Three samples were compared: precursor mix alone, 1% heme protein alone, and 5 precursor mix with 1% heme. The precursor mix was made of glucose (20 mM), ribose (20 mM), cysteine (10 mM), thiamine (1 mM), and glutamic acid (1 mM). Reactions were all at pH 6.0, prepared and heated to 150°C for 3 minutes. These three samples were run in duplicate. These samples were evaluated by GCMS for the flavor compounds generated. Characteristic flavor and fragrance components were mostly produced during 10 the cooking process where precursors could react with the heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction. The flavor compounds created in each sample is indicated in Table 18. As shown most of the flavor molecules were created on when the precursors are combined 15 with the heme protein.

Figure 8

Table 18
Flavor molecules created by the combination of LegH and precursor mix.

Compound	Precursor mix	LegH	Precursor mix + Leg H
carbon disulfide	medium	medium	high
isopropyl alcohol	medium	medium	low
2-methyl-furan	low		low
butanal	low		medium
thiophene	low		low
2,3-butanedione	low	low	high
furan	low		medium
2,4-dimethyl-1 -heptene		high	high
acetone		high	high
dimethyl trisulfide		medium	medium
2-methyl-heptane		medium	medium
2-pentanone		medium	
pentanal		medium	medium
2-pentyl-furan		medium	medium
2-methyl-propanal		low	high
2-acetatyl-1 -propene		low	low
2-methyl-butanal		low	medium
1,3-dimethyl-benzene		low	low
octane		low	low
benzene		low	low
benzaldehyde			very high
2-butanone			very high
furfural			very high
thiazole			high
nonanal			high
thiazole			high
2-acetylthiazole			medium
3 -methy 1-butanal			medium
(Z)-2-heptenal			medium
heptanal			medium
methyl-thiirane			medium
3-ethyl-pentane			medium
phenylacetaldehyde			medium
2-hexyl-furan			medium

Figure 8

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2-nonanone			medium
propanal			medium
pyrazine			medium
(Z)-2-heptenal			medium
2-methyl-1 -heptene			medium
2-ethyl-furan			medium
octanal			medium
(E)-4-octene			low
(E)-2-octenal			low
2-methyl-thiazole			low
2-propenal			low
l-octen-3-one			low
1-octene			low
2-octanone			low
dimethyl sulfide			low
3-pentyl-furan			low
2-n-octylfuran			low
2-pentyl-thiophene			low

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction 5 with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

~~Figure 8~~

WHAT IS CLAIMED IS:

[0006] Disclosed herein are improved methods and compositions which more accurately replicate the characteristics that consumers value in the preparation and consumption of meat and which overcome the shortcomings and drawbacks of current meat substitutes.

SUMMARY OF THE INVENTION

[0007] In some aspects, the invention provides a meat substitute composition comprising a protein content, wherein one or more isolated and purified proteins accounts for 10% or more of said protein content by weight, wherein said meat substitute composition accurately mimics the taste, texture, or color of a meat product derived from animal sources.
[0008] In one embodiment, the meat substitute composition accurately mimics the color of said meat product in its raw state and in a cooked state after cooking.
[0009] In another embodiment, the one or more isolated and purified proteins accounts for 25% or more of said protein content by weight.
[0010] In another embodiment, the one or more isolated and purified proteins accounts for 50% or more of said protein content by weight.
[0011] In another embodiment, the one or more isolated and purified proteins accounts for 75% or more of said protein content by weight.
[0012] In another embodiment, the one or more isolated and purified proteins accounts for 90% or more of said protein content by weight.
[0013] In another embodiment, gluten does not account for 10% or more of said protein content by weight.
[0014] In another embodiment, each of said isolated, purified proteins is separately isolated and purified.
[0015] In another embodiment, the meat substitute composition comprises 1-7 isolated and purified proteins.
[0016] In another embodiment, said 1-7 isolated and purified proteins are each isolated from different plant species. [0017] In some embodiments the meat substitute comprises less than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 individual proteins isolated from one or more plant species.
[0018] In another embodiment, said protein content comprises no more than trace amounts of any other proteins derived from the one or more plant species.
[0019] In another embodiment, said one or more isolated and purified proteins are selected from the group consisting of leghemoglobin, non-symbiotic hemoglobin, hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyano- globin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglo- bins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructoki- nases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-l,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-l,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.
[0020] In another embodiment, said one or more isolated and purified proteins are not isolated from an animal.
[0021] In another embodiment, said one or more isolated and purified proteins are isolated from a single plant source. [0022] In another embodiment, said one or more isolated and purified proteins are isolated from multiple plant sources. [0023] In another embodiment, wherein said one or more isolated, purified proteins are isolated from a genetically modified organism.
[0024] In some embodiments, said genetically modified oiganism is a genetically modified bacteria or yeast organism.
[0025] In some embodiments, said isolated, purified protein has been formed into fibers.
[0026] In particular embodiments, said fibers resemble skeletal muscle fibers.
[0027] In yet more particular embodiments, said fibers are asymmetric fibers.
[0028] In some embodiments, the meat substitute composition further comprises one or more isolated and purified iron-containing proteins.
[0029] In some embodiments, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non- symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuro- globin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.
[0030] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSV-
VFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAE KLFALVRDSAGQLKASGTWADAALGS-
VHAQKAVTDPQFVVKEALLKTIKAAVGDKWSDE LSRAWEVAYDELAAAIKKA.
[0031] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 2. SEQ ID NO 2: MIDQKEKELI KESWKRIEPN KNEIGLLFYA NLFKEEPTVS VLFQN- PISSQ SRKLMQVLGI

Figure 8

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LVQGIDNLEG LIPTLQDLGR RHKQYGWDS HYPLVGDCLL KSIQEYLGQG FTEEA- KAAWT KVYGIAAQVM TAE. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 80% homology to SEQ ID NO 2. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 90% homology to SEQ ID NO 2. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 98% homology to SEQ ID NO 2.
[0032] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 3. SEQ ID NO 3: MRKQPTVFEK LGGQAAMHAA VPLFYKKVLA DDRVKHYFKN TNMEHQAKQQ EDFLTMLLGG PNHYKGKNMA EAH- KGMNLQN SHFDAIIENL AATLKELGVS DQI- IGEAAKV IEHTRKDCLG K. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 80% homology to SEQ ID NO 3. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 90% homology to SEQ ID NO 3. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 98% homology to SEQ ID NO 3.
[0033] In some embodiments, the isolated and purified proteins are assembled into one or more gels.
[0034] In some embodiments, the meat substitute composition further comprises one or more fats.
[0035] In particular embodiments, said one or more fats are derived from a plant source.
[0036] In another aspect, the invention provides a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from nonanimal sources.
[0037] In some embodiments, said indicator is a visual indicator that accurately mimics the color transition of a meat product during said cooking progression.
[0038] In one embodiment, said color transition is from red to brown.
[0039] In one embodiment, said color transition is from pink to white or tan.
[0040] In one embodiment, said visual indicator transitions from a translucent to opaque color during said cooking progression.
[0041] In some embodiments, the indicator is an olfactory indicator that indicates cooking progression.
[0042] In one embodiment, said olfactory indicator is one or more volatile odorants released during cooking.
[0043] In some embodiments, said indicator comprises one or more isolated, purified iron-containing proteins.
[0044] In particular embodiments, said one or more isolated, purified iron-containing proteins is in a reduced state before cooking.
[0045] In one embodiment, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non- symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuro- globin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.
[0046] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSV- VFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAE KLFALVRDSAGQLKASGTWADAALGS- VH AQKAVTDPQF VWKEALLKTIKAAV GDKWSDE LSRAWEVAYDELAAAIKKA.
[0047] In some embodiments, said one or more isolated and purified iron-containing proteins are not isolated from an animal. In some embodiments compositions of the invention do not contain any proteins from an animal.
[0048] In particular embodiments, said one or more isolated and purified iron-containing proteins are isolated from one or more plant sources.
[0049] In yet more particular embodiments, said one or more isolated, purified proteins are isolated from the root nodules, roots, seeds, leaves, or stems of said one or more plant sources.
[0050] In other particular embodiments, said one or more plant sources are soy or pea plants.
[0051] In one embodiment, said one or more plant sources comprises one or more plants of the legume family.
[0052] In some embodiments, said one or more isolated and purified iron carrying proteins in a reduced or oxidized state has a similar UV-VIS profile to a myoglobin protein derived from an animal source when in an equivalent reduced or oxidized state.
[0053] In a particular embodiment, the difference between the peak absorbance wavelength of said one or more isolated and purified iron-containing proteins and the peak absorbance wavelength of myoglobin derived from an animal source is less than 5%.
[0054] In some embodiments, said one or more isolated, purified proteins are isolated from a genetically modified oiganism.
[0055] In one embodiment, said genetically modified organism is a genetically modified bacteria or yeast organism. [0056] In some embodiments, the meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum. [0057] In particular embodiments, the meat substitute product additionally contains less than 1% wheat gluten. [0058] In a more particular embodiment, said meat substitute product contains no wheat gluten.
[0059] In other particular embodiments, said meat substitute product contains no soy protein isolate.
[0060] In other particular embodiments, said meat substitute product contains no soy protein concentrate.
[0061] In other particular embodiments, said meat substitute product contains no soy protein.
[0062] In a more particular embodiment, said meat substitute product contains less than 5% carbohydrates.
[0063] In other particular embodiments, said meat substitute product contains no tofu.
[0064] In some embodiments, said meat substitute product contains no tofu, and no wheat gluten.
[0065] In some embodiments, said meat substitute product contains no soy protein, and no wheat gluten.
[0066] In some embodiments, said meat substitute product contains no animal products and less than 5% carbohydrates. [0067] In some embodiments, said meat substitute product contains less than 1% cellulose.
[0068] In some embodiments, said meat substitute product contains less than 5% insoluble carbohydrates.

Figure 8

[0069] In some embodiments, said meat substitute product contains no soy protein, and less than 1% cellulose.
[0070] In some embodiments, said meat substitute product contains no soy protein, and less than 5% insoluble carbohydrates.
[0071] In some embodiments, said meat substitute product contains no wheat gluten, and less than 1% cellulose.
[0072] In some embodiments, said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.
[0073] In another aspect, the invention provides a muscle tissue replica comprising a protein content, wherein said protein content comprises one or more isolated and purified proteins, wherein said muscle tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source.
[0074] In some embodiments, said one or more isolated and purified proteins accounts for at least 50% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 40% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 30% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 20% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 10% of said protein content by weight.
[0075] In some embodiments, said one or more isolated and purified proteins accounts for at least 50% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 40% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 30% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 20% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 10% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 5% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 1 % of said composition content by weight.
[0076] In some embodiments, said protein content is derived from one or more non-animal sources.
[0077] In particular embodiments, said one or more nonanimal sources is a plant source.
[0078] In other particular embodiments, said one or more non-animal sources is a genetically modified yeast or bacteria.
[0079] In some embodiments, each of said one or more isolated proteins is isolated and purified separately.
[0080] In some embodiments, said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chloroeruorin, erythroeruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyano- globin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglo- bins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructoki- nases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-l,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-l,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.
[0081] In one embodiment, said seed storage protein is moong bean 8S protein.
[0082] In some embodiments, said protein content is suspended in a gel.
[0083] In some embodiments, said protein content is in the form of a gel.
[0084] In one embodiment, said gel comprises an isolated, purified cross-linking enzyme.
[0085] In some embodiments, said isolated, purified cross- linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.
[0086] In a particular embodiment, said isolated, purified cross-linking enzyme is transglutaminase.
[0087] In some embodiments, said protein content has been assembled into fibers.
[0088] In particular embodiments, said fibers are arranged isotropically.
[0089] In one embodiment, said fibers are asymmetric fibers.
[0090] In some embodiments, the muscle tissue replica further comprises one or more isolated and purified iron-containing proteins.
[0091] In some embodiments, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non- symbiotic hemoglobin, chlorocmorin, erythrocmorin, neuro- globin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohe- moglobins.
[0092] In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKA-NIPQYSWFYTSILEKAPAAKDLFS- FLANGVDPTNPKLTGHAE KLFALVRDSAGQLKAS-GTWADAALGSVHAQKAVTDPQFVWKEALLKTIKA AVGDKWSDE LSRAWEVAYDELAAAIKKA. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 80% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron- containing proteins comprises an amino acid sequence with at least 90% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 95% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 98%

Figure 8

homology to SEQ ID NO 1.
[0093] In particular embodiments, the muscle tissue replica comprises a protein content, wherein (i) one isolated and purified protein that is not an iron-containing protein accounts for 40-95% of said protein content, (ii) one or more isolated and purified iron-containing proteins accounts for 1-20% of said protein content, and (iii) one or more cross- linking reagents accounts for 0.1 -35% of said protein content. [0094] In one embodiment, said protein content accounts for 5-50% of said replica by weight or by weight/volume. [0095] In one embodiment, said one isolated and purified protein is moong bean 8S protein.
[0096] In one embodiment, said one or more isolated and purified iron-containing proteins is leghemoglobin.
[0097] In one embodiment, said one or more cross-linking reagents is transglutaminase.
[0098] In some embodiments, the muscle tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum. [0099] In particular embodiments, the muscle tissue replica additionally contains less than 1 % wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 5% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 10% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 0.1% wheat gluten. [0100] In a more particular embodiment, said muscle tissue replica contains no wheat gluten.
[0101] In other particular embodiments, said muscle tissue replica contains no soy protein isolate.
[0102] In other particular embodiments, said muscle tissue replica contains no soy protein concentrate.
[0103] In other particular embodiments, said muscle tissue replica contains no soy protein.
[0104] In a more particular embodiment, said muscle tissue replica contains less than 5% carbohydrates.
[0105] In other particular embodiments, said muscle tissue replica contains no tofu.
[0106] In some embodiments, said muscle tissue replica contains no tofu, and no wheat gluten.
[0107] In some embodiments, said muscle tissue replica contains no soy protein, and no wheat gluten.
[0108] In some embodiments, said muscle tissue replica contains no animal products and less than 5% carbohydrates. [0109] In some embodiments, said muscle tissue replica contains less than 1% cellulose.
[0110] In some embodiments, said muscle tissue replica contains less than 5% insoluble carbohydrates.
[0111] In some embodiments, said muscle tissue replica contains no soy protein, and less than 1% cellulose.
[0112] In some embodiments, said muscle tissue replica contains no soy protein, and less than 5% insoluble carbohydrates.
[0113] In some embodiments, said muscle tissue replica contains no wheat gluten, and less than 1% cellulose.
[0114] In some embodiments, said muscle tissue replica contains no wheat gluten, and less than 5% insoluble carbohydrates.
[0115] In some embodiments, the muscle tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum. [0116] In particular embodiments, the meat replica contains less than 1% wheat gluten. In particular embodiments, the meat replica contains less than 5% wheat gluten. In particular embodiments, the meat replica contains less than 10% wheat gluten. In particular embodiments, the meat replica contains less than 0.1% wheat gluten.
[0117] In a more particular embodiment, the meat replica contains no wheat gluten.
[0118] In other particular embodiments, the meat replica contains no soy protein isolate.
[0119] In other particular embodiments, the meat replica contains no soy protein concentrate.
[0120] In other particular embodiments, the meat replica contains no soy protein.
[0121] In a more particular embodiment, the meat replica contains less than 5% carbohydrates.
[0122] In other particular embodiments, the meat replica contains no tofu.
[0123] In some embodiments, the meat replica contains no tofu, and no wheat gluten.
[0124] In some embodiments, the meat replica contains no soy protein, and no wheat gluten.
[0125] In some embodiments, the meat replica contains no animal products and less than 5% carbohydrates.
[0126] In some embodiments, the meat replica contains less than 1% cellulose. In some embodiments, the meat replica contains less than 0.1% cellulose. In some embodiments, the meat replica contains less than 10% cellulose. In some embodiments, the meat replica contains less than 5% cellulose.
[0127] In some embodiments, the meat replica contains less than 5% insoluble carbohydrates.
[0128] In some embodiments, the meat replica contains no soy protein, and less than 1% cellulose.
[0129] In some embodiments, the meat replica contains no soy protein, and less than 5% insoluble carbohydrates. [0130] In some embodiments, the meat replica contains no wheat gluten, and less than 1% cellulose.
[0131] In some embodiments, the meat replica contains no wheat gluten, and less than 5% insoluble carbohydrates.
[0132] In another aspect, the invention provides a fat tissue replica comprising a gelled emulsion, said gelled emulsion comprising a protein solution with fat droplets suspended therein.
[0133] In some embodiments, said fat droplets are derived from a non-animal source.
[0134] In some embodiments, said fat droplets are comprised of one or more plant oils.
[0135] In some embodiments, said one or more plant oils is selected from the group consisting of com oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phos- phatidic acids, lysophosphatidic acids, oleic

Figure 8

acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachi- donic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid.

[0136] In one embodiment, said one or more plant oils is rice bran oil or canola oil.

[0137] In some embodiments, said protein solution comprises one or more isolated, purified proteins.

[0138] In some embodiments, said one or more isolated, purified proteins accounts for 75% or more of the protein in said protein solution.

[0139] In some embodiments, said one or more isolated, purified proteins are derived from a non-animal source. [0140] In some embodiments, said non-animal source is a plant source.

[0141] In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

[0142] In some embodiments, each of said one or more isolated proteins is isolated and purified separately.

[0143] In some embodiments, said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyano- globin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglo- bins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructoki- nases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-l,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-l,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

[0144] In some embodiments, said one or more isolated, purified proteins is an albumin protein, a seed storage protein, or pea globulin protein.

[0145] In particular embodiments, said albumin protein is isolated pea albumin protein.

[0146] In some embodiments, said seed storage protein is moong bean 8S protein.

[0147] In some embodiments, said gelled emulsion comprises a protein solution comprising 1 -3 isolated and purified proteins, wherein said solution accounts for 30-70% of the volume of said emulsion; a plant oil, wherein said plant oil accounts for 30-70% of the volume of said emulsion; and an isolated, purified cross-linking enzyme, wherein said cross- linking enzyme accounts for 0.5-5% of said emulsion by wt/volume; wherein said plant oil is emulsified in said protein solution, wherein said emulsion is formed into a gel by said cross-linking enzyme.

[0148] In other embodiments said gelled emulsion comprises a protein solution comprising 1 -3 isolated and purified proteins, wherein said solution accounts for 1-30% of the volume of said emulsion; a plant oil, wherein said plant oil accounts for 70-99% of the volume of said emulsion; and an isolated, purified cross-linking enzyme, wherein said cross- linking enzyme accounts for 0.5-5% of said emulsion by wt/volume; wherein said plant oil is emulsified in said protein solution, wherein said emulsion is formed into a gel by said cross-linking enzyme.

[0149] In some embodiments, the fat replica further comprises a cross-linking enzyme.

[0150] In some embodiments, said cross-linking enzyme is transglutaminase.

[0151] In some embodiments, one of said 1-3 isolated and purified proteins is moong bean 8S protein, pea albumin protein, or pea globulin protein.

[0152] In particular embodiments, said plant oil is rice bran oil or canola oil.

[0153] In some embodiments, the fat tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[0154] In particular embodiments, the fat tissue replica additionally contains less than 1% wheat gluten.

[0155] In a more particular embodiment, said fat tissue replica contains no wheat gluten.

[0156] In other particular embodiments, said fat tissue replica contains no soy protein isolate.

[0157] In other particular embodiments, said fat tissue replica contains no soy protein concentrate.

[0158] In other particular embodiments, said fat tissue replica contains no soy protein.

[0159] In a more particular embodiment, said fat tissue replica contains less than 5% carbohydrates.

[0160] In other particular embodiments, said fat tissue replica contains no tofu.

[0161] In some embodiments, said fat tissue replica contains no tofu, and no wheat gluten.

[0162] In some embodiments, said fat tissue replica contains no soy protein, and no wheat gluten.

Figure 8

[0163] In some embodiments, said fat tissue replica contains no animal products and less than 5% carbohydrates. [0164] In some embodiments, said fat tissue replica contains less than 1% cellulose.
[0165] In some embodiments, said fat tissue replica contains less than 5% insoluble carbohydrates.
[0166] In some embodiments, said fat tissue replica contains no soy protein, and less than 1% cellulose.
[0167] In some embodiments, said fat tissue replica contains no soy protein, and less than 5% insoluble carbohydrates.
[0168] In some embodiments, said fat tissue replica contains no wheat gluten, and less than 1% cellulose.
[0169] In some embodiments, said fat tissue replica contains no wheat gluten, and less than 5% insoluble carbohydrates.
[0170] In another aspect, the invention provides a connective tissue replica, comprising a protein content comprising one or more isolated, purified proteins, wherein said protein content has been assembled into structures approximating the texture and visual appearance of connective tissue or skin. [0171] In some embodiments, said protein content is derived from non-animal source.
[0172] In some embodiments, said non-animal source is a plant source.
[0173] In some embodiments, said non-animal source is a genetically modified yeast or bacteria.
[0174] In some embodiments, said one or more isolated proteins account for 50% or more of said protein content by weight.
[0175] In some embodiments, said one isolated and purified protein accounts for 90% or more of said protein content by weight.
[0176] In some embodiments, each of said one or more isolated proteins is isolated and purified separately.
[0177] In some embodiments, said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyano- globin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglo- bins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructoki- nases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-l,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-l,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.
[0178] In some embodiments, said one or more isolated and purified proteins is a prolamin family protein.
[0179] In some embodiments, said one or more isolated and purified proteins is zein.
[0180] In some embodiments, said protein content is suspended in a gel.
[0181] In some embodiments, said gel comprises an isolated, purified cross-linking enzyme.
[0182] In some embodiments, said isolated, purified cross- linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.
[0183] In some embodiments, said isolated, purified cross- linking enzyme is transglutaminase.
[0184] In some embodiments, said protein content is formed into a fiber.
[0185] In some embodiments, said fiber is produced by an extrusion process.
[0186] In some embodiments, said fiber is stabilized by protein crosslinks.
[0187] In some embodiments, fiber contains an isolated, purified cross-linking enzyme.
[0188] In some embodiments, said isolated, purified cross- linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.
[0189] In some embodiments, said isolated, purified cross- linking enzyme is transglutaminase.
[0190] In another aspect, the invention provides a meat substitute product, comprising a muscle replica; a fat tissue replica; and a connective tissue replica; wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat.
[0191] In some embodiments, the meat substitute product comprises two or more of said muscle replica, fat tissue replica, and connective tissue replica.
[0192] In some embodiments of the meat substitute product, said muscle replica accounts for 40-90% of said product by weight, said fat tissue replica accounts for 1-60% of said product by weight, and said connective tissue replica accounts for 1-30% of said product by weight.
[0193] In some embodiments, the meat substitute product comprises 60-90% water; 5-30% protein content; and 1-20% of a fat or fat replica; wherein said protein content comprises one or more isolated, purified plant proteins.
[0194] In some embodiments, said protein content is derived from non-animal source.
[0195] In some embodiments, non-animal source is a plant source.
[0196] In some embodiments, said non-animal source is a genetically modified yeast or bacteria.
[0197] In some embodiments, 50% or more of said protein content by weight are one or more isolated purified proteins. [0198] In some embodiments, each of said one or more isolated proteins is isolated and purified separately from different plant species.
[0199] In some embodiments, one or more of said isolated proteins is selected from the group consisting of: hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyano- globin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglo- bins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructoki- nases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases,

Figure 8

pyruvate decarboxylases, actins, translation elongation factors, ribulose-l,5-bisphosphate carboxylase oxygenase (mbisco), ribulose-l,5-bisphosphate carboxylase oxygenase activase (mbisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

[0200] In some embodiments, the meat substitute product further comprises one or more isolated and purified iron- containing proteins.

[0201] In some embodiments, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non- symbiotic hemoglobin, chlorocmorin, erythrocmorin, neuro- globin, cytoglobin, protoglobin, tmncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins. In some embodiments, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKA- NIPQYSWFYTSILEKAPAAKDLFS-FLANGVDPTNPKLTGHAE KLFALVRDSAGQLKAS- GTWADAALGSVHAQKAVTDPQFVWKEALLKTIKA AVGDKWSDE LSRAWEVAYDELAAAIKKA [0202] In some embodiments, the meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum. [0203] In particular embodiments, the meat substitute product additionally contains less than 1% wheat gluten. [0204] In a more particular embodiment, said meat substitute product contains no wheat gluten.

[0205] In other particular embodiments, said meat substitute product contains no soy protein isolate.

[0206] In other particular embodiments, said meat substitute product contains no soy protein concentrate.

[0207] In other particular embodiments, said meat substitute product contains no soy protein.

[0208] In a more particular embodiment, said meat substitute product contains less than 5% carbohydrates.

[0209] In other particular embodiments, said meat substitute product contains no tofu.

[0210] In some embodiments, said meat substitute product contains no tofu, and no wheat gluten.

[0211] In some embodiments, said meat substitute product contains no soy protein, and no wheat gluten.

[0212] In some embodiments, said meat substitute product contains no animal products and less than 5% carbohydrates. [0213] In some embodiments, said meat substitute product contains less than 1% cellulose.

[0214] In some embodiments, said meat substitute product contains less than 5% insoluble carbohydrates.

[0215] In some embodiments, said meat substitute product contains no soy protein, and less than 1% cellulose.

[0216] In some embodiments, said meat substitute product contains no soy protein, and less than 5% insoluble carbohydrates.

[0217] In some embodiments, said meat substitute product contains no wheat gluten, and less than 1% cellulose.

[0218] In some embodiments, said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.

[0219] In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by an animal.

[0220] In some embodiments, said one or more isolated, purified iron-containing proteins is derived from a non-animal source.

[0221] In some embodiments, said non-animal source is a plant source.

[0222] In some embodiments, said plant source comprises one or more plants of the legume family.

[0223] In some embodiments, said one or more plants of the legume family is a soy or pea plant.

[0224] In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

[0225] In some embodiments, said iron-containing protein is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chloro- cruorin, erythrocmorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

[0226] In one embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSV- VFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAE KLFALVRDSAGQLKASGTWADAALGS- VH AQKAVTDPQF VWKEALLKTIKAAV GDKWSDE LSRAWEVAYDELAAAIKKA

[0227] In some embodiments, the food product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[0228] In particular embodiments, the food product additionally contains less than 1% wheat gluten.

[0229] In a more particular embodiment, said food product contains no wheat gluten.

[0230] In other particular embodiments, said food product contains no soy protein isolate.

[0231] In other particular embodiments, said food product contains no soy protein concentrate.

[0232] In other particular embodiments, said food product contains no soy protein.

[0233] In a more particular embodiment, said food product contains less than 5% carbohydrates.

[0234] In other particular embodiments, said food product contains no tofu.

[0235] In some embodiments, said food product contains no tofu, and no wheat gluten.

[0236] In some embodiments, said food product contains no soy protein, and no wheat gluten.

[0237] In some embodiments, said food product contains no animal products and less than 5% carbohydrates.

[0238] In some embodiments, said food product contains less than 1% cellulose.

Figure 8

[0239] In some embodiments, said food product contains less than 5% insoluble carbohydrates.
[0240] In some embodiments, said food product contains no soy protein, and less than 1% cellulose.
[0241] In some embodiments, said food product contains no soy protein, and less than 5% insoluble carbohydrates. [0242] In some embodiments, said food product contains no wheat gluten, and less than 1% cellulose.
[0243] In some embodiments, said food product contains no wheat gluten, and less than 5% insoluble carbohydrates. [0244] In another aspect, the invention provides a method of making a meat substitute composition, comprising isolating and purifying one or more proteins; and assembling said one or more proteins into a physical organization that approximates the physical organization of meat.
[0245] In another aspect, the invention provides a method of making a muscle tissue replica, comprising isolating and purifying one or more proteins; and assembling said one or more proteins into a physical oiganization that approximates the physical organization of skeletal muscle.
[0246] In another aspect, the invention provides a method of making a fat tissue replica, comprising isolating and purifying one or more proteins; preparing a solution comprising one or more proteins; emulsifying one or more fats in said solution; and stabilizing said solution into a gelled emulsification with one or more cross-linking reagents.
[0247] In another aspect, the invention provides a method of making a connective tissue replica, comprising isolating and purifying one or more proteins; and precipitating said one or more proteins, wherein said precipitating results in said one or more proteins forming physical structures approximating the physical organization of connective tissue.
[0248] In some embodiments, said precipitating comprises solubilizing said one or more proteins in a first solution; and extruding said first solution into a second solution, wherein said one or more proteins is insoluble in said second solution, wherein said extruding induces precipitation of said one or more proteins.
[0249] In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by an animal.
[0250] In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by humans.
[0251] In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by an animal. In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by humans.
[0252] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0253] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.
[0254] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which: [0255] FIG. 1 depicts a portion of the root of a pea plant **{Pisum sativum)** with the root nodules sliced open to demonstrate the red color conferred by leghemoglobin contained therein. The sliced open rood nodule appears red.
[0256] FIG. 2 depicts leghemoglobin isolated from 1 oz of pea roots. The red color commonly attributed to meat is evident in the color photo.
[0257] FIG. 3 shows that leghemoglobins from different species are homologs and have similar color properties. In FIG. 3, panel A shows an SDS_PAGE gels of lysed root- nodules of three legume plant species (1) Fava bean (2) English Pea (3) Soybean. Arrows mark respective leghemoglobins. Panel B shows the similarity of UV-VIS spectral profile of leghemoglobins from two different plant species (Favabean and Soybean).
[0258] FIG. 4 shows a comparison of reduced (heme iron 2+) and oxidized (heme iron 3+) soybean leghemoglobin (FIG. 4 panel A) and equine heart muscle myoglobin (FIG. 4 panel B) showing similarity of UV-VIS absorption profiles of two proteins. We purified soybean leghemoglobin from soybean root-nodules using here described protocol. Purified equine myoglobin was purchased from SigmaAldrich. Soybean leghemoglobin (FIG. 4 panel A) and equine myoglobin (FIG. 4 panel B) were reduced with 1 mm sodium hydrosulfite. Shown are UV-VIS absorption spectra of heme Fe3+ (blue line – the higher peak in FIGS. 4 and 5) and heme Fe2+ (red line) of soybean leghemoglobin (FIG. 4 panel A) and equine myoglobin (FIG. 4 panel B). Insets show a zoom-in of UV-VIS spectra in 450 nm to 700 nm region. (FIG. 4 panel C) Images of 10 ul liquid droplet of a 40 mg/ml solution of soybean leghemoglobin in the heme-Fe3+ state (left droplet) showing characteristic rusty red color and a 40 mg/ml solution of soybean leghemoglobin solution in the heme-Fe2+ state (right droplet) showing characteristic red color of and (right image) corresponding samples of equine myoglobin. [0259] FIG. 5 depicts examples of successful reduction of leghemoglobin heme iron with sodium hydrosulfite and titanium citrate. In FIG. 5 the UV-VIS spectrogram of purified soybean leghemoglobin in which the heme iron is in the oxidized (+3) state is represented by the blue curves in each panel (the blue curves have the higher peaks on the main graphs). The red curves in

Figure 8

each panel represent the UV-VIS spectra of the same leghemoglobin species after reduction to the (+2) state (red lines) by addition of (Panel A) 1 mM sodium hydrosulfite or (Panel B) 0.24% (wt/v) titanium citrate in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride buffer. The Insets show a zoom-in of UV-VIS spectra in 450-700 nm region. For this example, leghemoglobin was purified from soybean root nodules using 60/90% ammonium sulfate fractionation and exchanged into 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride buffer. Sodium hydrosulfite stock solution was prepared by dissolving 100 mM sodium hydrosulfite in 1 mM sodium hydroxide in water. Titanium citrate stock solution was prepared from 20% (wt/v) Ti-chloride in hydrochloric acid by mixing it with 0.2M sodium citrate (1:10 v/v). pH was adjusted using sodium carbonate to pH 7.0.

[0260] FIG. 6 depicts an example of the leghemoglobin purification flow from soybean root nodules. The figure shows SDS-PAGE fractionation of different soybean leghemoglobin purification steps (Lane 1) Soybean root-nodule lysate; (Lane 2) Soybean root-nodule lysate purified by 60/90% (wt/v) ammonium sulfate fractionation. Shown is the protein content of 90% ammonium sulfate fractionated protein pellet resuspended in 20 mm potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mM EDTA buffer; Proteins from 90% ammonium sulfate pellet were further purified by anion-exchange chromatography (FFQ GE Healthcare) in 20 mM potassium phosphate ph 7.4, 100 mM sodium chloride. Leghemoglobin collected in the flowthrough is shown in Lane 3. Anion-exchange flowthrough was fractionated using size-exclusion chromatography (Sephacryl S-100 GE Healthcare) and resulting leghemoglobin fraction is shown in Lane 4. Leghemoglobin content at different purification steps was determined by determining the fraction of leghemoglobin band on SDS-PAGE gel in a respective sample using ImageDoc analysis software (BioRad). Purity (partial abundance) of leghemoglobin at respective steps in the purification steps was: lysate: 32.7% (lane 1), 60/90% (wt/v) ammonium sulfate fractionation 78% (lane 2), anion-exchange chromatography ~83% (lane 3), and size-exclusion chromatography to ~95% (lane 4).

[0261] FIG. 7 shows stained SDS-PAGE gel analysis of (A) soybean leghemoglobin expressed and purified using recombinant protein technology and (B) soybean leghemoglobin purified from soybean root nodules. (A) Recombinant Soybean leghemoglobin A carrying His-tag and TEY protease His-tag removal site was expressed inE. co//BL21 strain and purified using His-tag affinity chromatography (Talon resin, CloneTech). The left lane contains molecular weight standards, the right lane contains purified recombinant soybean leghemoglobin A (arrow). Expected molecular weight of the recombinant soybean leghemoglobin A is 17.1 kDa. (B) SDS- PAGE gel of purified Soybean leghemoglobin from root nodules. The left lane contains molecular weight standards, the right lane contains purified soybean leghemoglobin A (arrow). Mass spectrometry analysis of purified material determined that all four soybean leghemoglobin isoforms are present, and are full length (data not shown). Expected molecular weights (MW) of soybean leghemoglobin isoforms range from MW15.4 to 15.8 kDa.

[0262] FIG. 8 shows an example of 6 cubes of a commercial meat analog (Quom chicken analog), about 1 cm on a side, 4 of which (Left and lower right) have been soaked in a solution of about 10 mg/ml soybean leghemoglobin in 20 mM Potassium phosphate pH 7.4 and lOOmMNaCl; the remaining two (Upper right) were soaked in the same buffer without leghemoglobin. A deep pink color of the leghemoglobin-infused cubes is apparent in color photos contrasting the pale tan color of the un-infused cubes.

[0263] FIG. 9 shows the 4 leghemoglobin-infused cubes of Quom chicken analog in the process of cooking in a pan at 350° C. The two lower cubes have been turned over to expose the grilled surface, which has turned brown. In the upper two cubes that the heated portion has turned grey-brown, while the cooler top surface retains its pink color. In some embodiments the consumable is injected with a heme containing solution, for instance a leghemoglobin solution, until the consumable is the color of uncooked meat.

[0264] FIG. 10 depicts 43 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 37 ml of leghemoglobin solution (46.5 mg/ml leghemoglobin and 20 mg/ml of other soybean root nodule protein) in 20 mM potassium phosphate, 100 mM NaCl, pH 7.3). 20 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly mixed, divided into two 50 ml Falcon tubes and incubated overnight at room temperature. Final protein concentrations were 65 mg/ml for moong bean protein, 18 mg/ml of leghemoglobin, 91 mg/ml total protein.

[0265] FIG. 11 depicts "White" muscle analog prepared by mixing 43 ml moong bean protein solution (150 mg/ml) with 45 ml of 11.7 mg/ml solution of leghemoglobin and 0.8% (wt/v) of transglutaminase solution. Final protein concentrations were 63 mg/ml for moong bean protein, 5.2 mg/ml of leghemoglobin, 68 mg/ml total protein.

[0266] FIG. 12 depicts a fat tissue analog based on moong beans and prepared in eppendorf tubes formed an opaque gel of off-white color, smooth uniform texture, with no visible discernible liquid that was not incorporated into the gel. The gel was freely standing, elastic and springy. The gel has a slight, pleasant aroma and a mild and pleasant flavor. The taste is mildly salty.

[0267] FIG. 13 depicts at tissue analog based on pea globulin and prepared in eppendorf tubes very similar to moong bean-based fat analog, except that it gave up a little of oil upon compression.

[0268] FIG. 14 shows connective-tissue analog strands that were created using a 1:3 ratio in 70% ethanol, loaded into a syringe with a 23 gauge needle (ID 0.337 mm). The solution was slowly extruded from the bottom of a 5 inch-high vessel into an excess of 5 M NaCl solution. The ethanol-zein solution being less dense than the NaCl solution, floated upward, drawing out a fibrous stand of solidifying zein. The NaCl was constantly stirred as the strands began to develop to assist in the strand lengthening. The strands bunch together and become a hard, dense mass.

[0269] FIG. 15 depicts a ground beef prototype patty was made by combining 62% (wt/wt) muscle analog (62% (wt/ wt) "dark muscle analog" and 38% (wt/wt) "white muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (FIG. 15 panel A). A ground beef prototype patty was made by combining 62% muscle analog

Figure 8

(62% "dark muscle analog" and 38% "white muscle analog), 29% fat tissue analog (from moong bean seed 8S protein and rice bran oil), 5% connective tissue analog (FIG. 15 panel B). A ground beef prototype patty was made by combining 71% (wt/wt) muscle tissue analog (composed of 60% "white" muscle analog, 40% "dark" muscle analog), 23% fat tissue (from pea seed globulin proteins and canola oil) (FIG. 15 panel C). A ground beef prototype patty was made by combining 67% "White" muscle analog, with 28% fat tissue analog (from pea globulins and rice bran oil), (FIG. 15, panel D)

[0270] FIG. 16 depicts a ground beef patty analog was made by combining 62% (wt/wt) muscle tissue analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog. The panel on the left shows the patty before cooking and the panel on the right shows the same patty after cooking for about 2 minutes. Observers described the aroma of the cooking ground beef replica as distinctly "beefy".

DETAILED DESCRIPTION OF THE INVENTION

[0271] Methods and compositions for the production of consumables are described herein. The consumables can be for animal consumption. For example the consumable can be food fit for human consumption. The consumable can be approved by suitable regulatory authorities. The consumables can be sold in grocery stores or prepared in restaurants, schools, hospitals, military facilities, prisons, shelters, long-term care facilities, similar to already existing human foods. The consumables could also be food for domestic animals. For instance, dog food could be produced according to the present inventions. The consumables may also be food for wild animals. For instance, the consumables could be provided to non-domesticated predatory animals.

[0272] The consumables of the present invention can compete with, supplement or replace animal based foods. For instance the consumables can be meat replicas made entirely from plant sources. The consumables can be made to mimic the cut or appearance of meat as it is currently sold. For instance a consumable may be visually similar to or indistinguishable from ground beef or a particular cut of beef. Alternatively, the consumables can be made with a unique look or appearance. For instance the consumable could contain patterns or lettering that is based upon the structure of the consumable. In some instances the consumables look like traditional meat products after they are prepared. For example a consumable may be produced which is larger than a traditional cut of beef but which, after the consumable is sliced and cooked appears the same as a traditional cooked meet. In some embodiments the consumable may resemble a traditional meat shape in two dimensions, but not in a third. For example the consumable may resemble a cut of meat in two dimensions (for example when viewed from the top), but may be much longer (or thinker) than the traditional cut. So in some embodiments a composition that can be cut repeatedly into traditionally meat shaped products is provided.

[0273] The consumable may be made entirely from plant based sources. In some instances the consumable can be made from organic sources. The consumables may also be made from a combination of plant based sources and animal based sources. For instance, the consumable may be a ground beef product supplemented with plant based products of the invention.

[0274] The consumables can be made from local products. For instance the consumables can be made from plants grown within a certain radius of the eventual consumer. That radius could be 1, 10, 100, or 1000 miles for example. So, in some embodiments, the invention provides a method for producing a meat replica which does not contain products which have been shipped over 1,10,100, or 1000 miles prior to producing the meat replica.

[0275] The present invention provides methods for producing consistent properties from the consumables when they are produced from various sources. So, for example, a plant based meat replica produced from local plants in Iowa, USA, will have substantially similar taste, odor, and texture as a plant based meat replica produced from local plants in Lorraine, France. This consistency allows for methods for advertising locally grown foods with consistent properties. The consistency can arise from the concentration or purification of similar components at different locations. These components can be combined in predetermined ratios to insure consistency. In some embodiments a high degree of characteristic consistency is possible using components (e.g. isolated or concentrated proteins and fats) which come from the same plant species. In some embodiments a high degree of characteristic consistency is possible using components (e.g. isolated or concentrated proteins and fats) which come from the different plant species. In some embodiments the same proteins can be isolated from different plant species. In some embodiments the invention provides for a method comprising isolating similar plant constituents from plant sources in different locations, assembling in both locations compositions provided herein, and selling the compositions, wherein the compositions assembled and sold at different the geographic locations have consistent physical and chemical properties. In some embodiments the isolated constituents are from different plant populations in different locations. In some embodiments one or more of the isolated constituents are shipped to the separate geographic locations.

[0276] The consumables may require fewer resources to produce than consumables produced from domesticated animals. Accordingly, the present invention provides for meat replicates which require less water or energy to produce than meat. For example a consumable can require less than about 10, 50,100,200,300, 500, or 1000 gallons of water per pound of consumable. For comparison beef can require over 2000 gallons of water per pound of meat.

[0277] The consumable may require less land are to produce than a meat product with similar protein content. For example the consumable may require 30% or less of the land area required to produce a meat product with similar protein content.

[0278] The consumable may have health benefits compared to an animal product it replaces in the diet. For example it may have less cholesterol or lower levels of saturated fats than comparable meat products.

[0279] The consumable may have animal welfare benefits compared to an animal product it replaces in the diet. For instance it may

Figure 8

be produced without requiring confinement, forced feeding, premature weaning, disruption of maternal- offspring interactions, or slaughter of animals for their meat. [0280] The consumable may have a smaller "carbon footprint" than the meat products they replace. For example the consumable may result in net greenhouse gas emissions of 1%, 5%, 10%, 25%, 50% or 75% of the greenhouse gas emissions attributable to the animal product it replaces. [0281] The consumable may provide alternatives to animal products or combinations of animal products whose consumption is forbidden by religious beliefs. For example, the consumable may be a kosher pork chop.

[0282] The consumable can also be shipped in components and produced or assembled at a different location. When available local components can be used for production of the consumable. These can be supplemented with components which are not locally available. This allows for methods of producing consumables, for instance meat replicates, using less energy in shipment than is required for meat. For example, local water can be used in combination with a kit which provides other components of the consumable. Using local water will reduce shipping weight thereby reducing cost and environmental impact.

[0283] The consumables can be produced or assembled wholly or in part in areas where animal farming is not practical or is not allowed. The consumable can be produced or assembled within an urban environment. For example a kit may be provided to a user to enable the user to produce the consumable. The user could use local water or use plants from a rooftop garden, for instance in Shanghai. In another example, the consumables could be produced aboard a space craft, space station, or lunar base. Accordingly, the present invention provides methods and systems for the production of meat replicas for use in space travel or for training for the same. For instance the present invention could be used in earth based training for space travel. The consumables could also be produced on an island or upon a manmade platform at sea where the keeping of livestock is difficult or prohibited.

[0284] The consumables are, in some embodiments, designed to replicate the experience of eating meat. The look, texture, and taste of the consumable can be such that it is similar or indistinguishable from meat. The invention therefore provides in certain embodiments methods for determining whether an animal or human can distinguish the consumable from meat.

[0285] One method to determine whether the consumable is comparable to meat is to a) define the properties of meat and b) determine whether the consumable has similar properties. Properties of meat that can be tested include mechanical properties such as hardness, cohesiveness, brittleness, chewiness, gumminess, viscosity, elasticity, and adhesiveness. Properties of meat that can be tested also include geometric properties such as particle size and shape, and particle shape and orientation. Additional properties can include moisture content and fat content. These properties can be described using terms such as "soft," "firm" or "hard" describe hardness; "crumbly," "crunchy," "brittle," "chewy," "tender," "tough," "short," "mealy," "pasty," or "gummy," to describe cohesiveness; "thin" or "viscous" to describe viscosity; "plastic" or "elastic" to describe elasticity; "sticky," "tacky" or "gooey" to describe adhesiveness; "gritty," "grainy" or "course" to describe particle shape and size; "fibrous," "cellular" or "crystalline" to describe particle shape and orientation, "dry," "moist," "wet," or "watery" to describe moisture content; or "oily" or "greasy" to describe fat content. So, in one embodiment a group of people can be asked to rate a certain meat, for instance ground beef, according to properties which describe the meat. These ratings can be used as an indication of the properties of the meat. The consumables of the present invention can then be compared to the meat to determine how similar the consumable is to the meat. In some instances the properties of the consumables are then altered to make the consumable more similar to the meat. So, in some embodiments, the consumable is rated similar to meat according to human evaluation. In some embodiments the consumable is indistinguishable from real meat to a human.

[0286] In some embodiments, subjects asked to identify the consumable identify it as a form of meat. In some embodiments one property of the compositions of the invention is that an animal, for example a human, will identify the composition as a meat. In some embodiments the human identifies the composition of the invention as having properties equivalent to meat. In some embodiments one or more properties of meat are equivalent according to a human's perception. Such properties include the properties that can be tested. In some embodiments a human identifies a consumable of the present invention as more meat like than meat substitutes found in the art.

[0287] In embodiments an experiment can demonstrate that consumable s acceptable to consumers. A panel can be used to screen a variety of consumables described herein. A number of human panelists can tested multiple consumable samples, namely, natural meats vs. the consumable compositions described herein. Variables such as fat content can be standardized for example to 20% fat using lean and fat meat mixes. Fat content can be determined using the Babcock for meat method (S. S. Nielson, Introduction to the Chemical Analysis of Foods (Jones & Bartlett Publishers, Boston, 1994)). Mixtures of ground beef and consumables of the invention prepared according to the procedure described herein can be formulated.

[0288] Panelists can be served samples in booths, under red lights or under white light, in an open consumer panel. Samples can be assigned random three-digit numbers and rotated in ballot position to prevent bias. Panelists can be asked to evaluate samples for tenderness, juiciness, texture, flavor, and overall acceptability using a hedonic scale from 1=dislike extremely, to 9=like extremely, with a median of 5=neither like nor, dislike. Panelists can be encouraged to rinse their mouths with water between samples, and given opportunity to comment on each sample.

[0289] The results of this experiment can indicate significant differences ($p<0.05$) or similarities between the traditional meats and the compositions of the invention.

[0290] These results will demonstrate that the compositions of the invention are judged as acceptably equivalent to real meat products. Additionally these results can demonstrate that compositions of the invention are preferred by panelist over other -

Figure 8

commercially available meat substitutes. So, in some embodiments the present invention provides for consumables that are significantly similar to traditional meats.

[0291] Consumables of the invention can also have similar physical characteristics as traditional meat. In one embodiment the force required to pierce a 1 inch thick structure (e.g. a patty) made of a consumable of the invention with a fixed diameter steel rod is not significantly different than the force required to pierce a 1 inch thick similar meat structure (e.g. a ground beef patty) with a similar fixed diameter steel rod. Accordingly, the invention provides for consumables with similar physical strength characteristics to meat.

[0292] In some embodiments composition of the invention have a similar cook loss characteristic as meat. In one embodiment a consumable of the invention with a similar fat and protein content as ground beef has the same reduction in size when cooked as real ground beef. Similar similarities in size loss profiles can be achieved for various compositions of consumables described herein matched to various meats. [0293] In some embodiments the consumable is compared to real meat based upon olfactometer readings. In various embodiments the olfactometer can be used to assess odor concentration and odor thresholds, odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of appreciation, or relative intensity of odors. In some embodiments the olfactometer allows the training and automatic evaluation of expert panels. So in some embodiments the consumable is a product that causes similar or identical olfactometer readings. In some embodiments the similarity is sufficient to be beyond the detection threshold of human perception.

[0294] Gas chromatography-mass spectrometry (GCMS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to separate and identify different substances within a test sample. GCMS can, in some embodiments, be used to evaluate the properties of a consumable. For example volatile chemicals can be isolated from the head space around meat. These chemicals can be identified using GCMS. A profile of the volatile chemicals in the head- space around meat is thereby created. In some instances each peak of the GCMS can be further evaluated. For instance, a human could rate the experience of smelling the chemical responsible for a certain peak. This information could be used to further refine the profile. GCMS could then be used to evaluate the properties of the consumable. The GCMS profile could be used to refine the consumable.

[0295] Characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions molecules including amino acids, fats and sugars which are found in plants as well as meat. Therefore in some embodiments the consumable is tested for similarity to meat during or after cooking. In some embodiments human ratings, human evaluation, olfactometer readings, or GCMS measurements, or combinations thereof, are used to create an olfactory map of cooked meat. Similarly, an olfactory map of the consumable, for instance a meat replica, can be created. These maps can be compared to assess how similar the cooked consumable it so meat. In some embodiments the olfactory map of the consumable during or after cooking is similar to or indistinguishable from that of cooked or cooking meat. In some embodiments the similarity is sufficient to be beyond the detection threshold of human perception.

[0296] In one aspect, the invention provides a meat substitute product (alternatively referred to herein as "consumable") that is substantially or entirely composed of ingredients derived from non-animal sources, yet recapitulates key features associated with the cooking and consumption of an equivalent meat product derived from animals. The equivalent meat product can be a white meat or a dark meat. The equivalent meat product can be derived from any animal. Non-limiting examples of animals used to derive the equivalent meat product include farmed animals such as, e.g., cattle, sheep, pig, chicken, turkey, goose, duck, horse, dog or game animals (whether wild or farmed) such as, e.g., rabbit, deer, bison, buffalo, boar, snake, pheasant, quail, bear, elk, antelope, pigeon, dove, grouse, fox, wild pig, goat, kangaroo, emu, alligator, crocodile, turtle, groundhog, marmot, possum, partridge, squirrel, raccoon, whale, seal, ostrich, capybara, nutria, guinea pig, rat, mice, vole, any variety of insect or other arthropod, seafood such as, e.g, fish, crab, lobster, oyster, muscle, scallop, abalone, squid, octopus, sea urchin, tunicate and others. Many meat products are typically derived from skeletal muscle of an animal but it is understood that meat can also come from other muscles or organs of the animal. In some embodiments, the equivalent meat product is a cut of meat derived from skeletal muscle. In other embodiments, the equivalent meat product is an organ such as, e.g., a kidney, heart, liver, gallbladder, intestine, stomach, bone marrow, brain, thymus, lung, tongue. Accordingly, in some embodiments the compositions of the present invention are consumables similar to skeletal muscle or organs.

[0297] In some aspects, the present invention provides meat substitute products comprising one or more of a first composition comprising a muscle tissue replica, a second composition comprising an adipose tissue replica, and/or a third composition comprising a connective tissue replica, wherein the one or more compositions are combined in a manner that recapitulates the physical organization of meat. In other aspects, the present invention provides compositions for a muscle tissue replica (herein referred to as "muscle replica"), an adipose tissue replica (herein referred to as "fat replica"), and a connective tissue replica (herein referred to as "connective tissue replica"). In some embodiments, the compositions and meat substitute products are principally or entirely composed of ingredients derived from non-animal sources. In alternative embodiments, the muscle, fat, and/or connective tissue replica, or the meat substitute products comprising one or more of said replicas, are partially derived from animal sources but supplemented with ingredients derived from nonanimal sources. In yet other alternative embodiments, the invention provides meat products substantially derived from animal sources but which are supplemented with one or more of a muscle tissue replica, a fat replica, and/or a connective tissue replica, wherein said replicas are derived substantially or entirely from non-animal sources. A non-limiting example of such a meat product is an ultra-lean ground beef product supplemented with a non-animal derived fat replica which improves texture and mouthfeel while preserving the health benefits of a consumable low in animal fat. Such alternative embodiments result in products with properties that more closely recapitulate key features associated with preparing and consuming meat but which are less costly and associated with a lesser environmental impact, less animal welfare impact, or improved health

Figure 8

benefits for the consumer.

[0298] The physical organization of the meat substitute product can be manipulated by controlling the localization, organization, assembly, or orientation of the muscle, fat, and/ or connective tissue replicas described herein. In some embodiments the product is designed in such a way that the replicas described herein are associated with one another as in meat. In some embodiments the consumable is designed so that after cooking the replicas described herein are associated with one another as in cooked meat. In some embodiments, one or more of the muscle, fat, and/or connective tissue replicas are combined in a manner that recapitulate the physical organization of different cuts or preparations of meat. In an example embodiment, the replicas are combined in a manner that approximates the physical organization of natural ground meat. In other embodiments, the replicas are combined in a manner that approximates different cuts of beef, such as, e.g., ribeye, filet mignon, London broil, among others.

Proteins and Protein Sources

[0299] In some embodiments, any of the meat substitute products, muscle tissue replica, fat replica, or connective tissue replica, comprise one or more isolated, purified proteins. In some embodiments, the meat substitute products are comprised of one or more of a muscle replica, a fat replica, and/or connective tissue replica which comprise one or more isolated, purified proteins. In other embodiments, the muscle replica, fat replica, and/or connective tissue replica comprises one or more isolated, purified proteins. In some embodiments, about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component is comprised of one or more isolated, purified proteins. For the purposes of this document, "purified protein" will refer to a preparation in which the cumulative abundance by mass of protein components other than the specified protein, which can be a single monomeric or multimeric protein species, is reduced by a factor of 2 or more, 3 or more, 5 or more, 10 or more, 20 or more, 50 ormore, 100 ormore or 1000 ormore relative to the source material from which the specified protein was isolated. [0300] In some embodiments, the one or more isolated, purified proteins are derived from non-animal sources. Nonlimiting examples of non-animal sources include plants, funghi, bacteria, archaea, genetically modified organisms such as genetically modified bacteria or yeast, chemical or in vitro synthesis. In particular embodiments, the one or more isolated, purified proteins are derived from plant sources.

Non-limiting examples of plant sources include grains such as, e.g., com, maize, rice, wheat, barley, rye, triticale, teff, oilseeds including cottonseed, sunflower seed, safflower seed, rapeseed, leafy greens such as, e.g., lettuce, spinach, kale, collard greens, turnip greens, chard, mustard greens, dandelion greens, broccoli, cabbage, green matter not ordinarily consumed by humans, including biomass crops, including switchgrass, miscanthus, sorghum, other grasses, alfalfa, com stover, green matter ordinarily discarded from harvested plants, sugar cane leaves, leaves of trees, root crops such as cassava, sweet potato, potato, carrots, beets, turnips, plants from the legume family, such as, e.g., clover, peas such as cowpeas, englishpeas, yellow peas, green peas, beans such as, e.g., soybeans, fava beans, lima beans, kidney beans, garbanzo beans, mung beans, pinto beans, lentils, lupins, mesquite, carob, soy, and peanuts, vetch (vicia), stylo (sty- losanthes), arachis, indigofera, acacia, leucaena, cyamopsis, and sesbania. One of skill in the art will understand that proteins that can be isolated from any organism in the plant kingdom may be used in the present invention.

[0301] Proteins that are abundant in plants can be isolated in large quantities from one or more source plants and thus are an economical choice for use in any of the muscle, fat, connective tissue replicas, or meat substitute products. Accordingly, in some embodiments, the one or more isolated proteins comprises an abundant protein found in high levels in a plant and capable of being isolated and purified in laige quantities. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70% of the total protein content of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5-40%, about 10-50%, about 20-60%, or about 30-70% of the total protein content of the source plant. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50% of the total weight of the dry matter of the source plant. In some embodiments, the abundant protein comprises about 0.5-5%, about 1-10%, about 5-20%, about 10-30%, about 15-40%, about 20-50% of the total weight of the dry matter of the source plant.

[0302] In particular embodiments, the one or more isolated proteins comprises an abundant protein that is found in high levels in the leaves of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80% ofthe total protein content of the leaves of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%, about 20%-60%, or about 30-70% of the total protein content of the leaves of the source plant. In particular embodiments, the one or more isolated proteins comprise ribulose-l,5-bisphosphate carboxylase oxygenase (rubisco activase). Rubisco is a particularly useful protein for meat replicas because of its high solubility and an amino acid composition with close to the optimum proportions of essential amino acids for human nutrition. In particular embodiments, the one or more isolated proteins comprise ribulose-l,5-bisphosphate carboxylase oxygenase activase (rubisco activase). In particular embodiments, the one or more isolated proteins comprise a vegetative storage protein (VSP).

[0303] In some embodiments, the one or more isolated proteins includes an abundant protein that is found in high levels in the seeds of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or 90% or more of the total protein content of the seeds of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%,

Figure 8

about 20%- 60%, or about 30-70% or >70% of the total protein content of the seeds of the source plant. Non-limiting examples of proteins found in high levels in the seeds of plants are seed storage proteins, e.g., albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glu- tenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, triticeae gluten, zein, any seed storage protein, ole- osins, caloleosins, steroleosins or other oil body proteins [0304] In some embodiments, the one or more isolated proteins includes proteins that interact with lipids and help stabilize lipids in a structure. Without wishing to be bound by a particular theory, such proteins may improve the integration of lipids and/ or fat replicas with other components of the meat substitute product, resulting in improved mouthfeel and texture of the final product. A non-limiting example of a lipid-interacting plant protein is the oleosin family of proteins. Oleosins are lipid-interacting proteins that are found in oil bodies of plants. Other non-limiting examples of plant proteins that can stabilize emulsions include seed storage proteins from Great Northern Beans, albumins from peas, globulins from peas, 8S globulins from Moong bean, 8S globulins from Kidney bean.

Muscle Replicas

[0305] A laige number of meat products comprise a high proportion of skeletal muscle. Accordingly, the present invention provides a composition derived from non-animal sources which replicates or approximates key features of animal skeletal muscle. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources which replicates or approximates animal skeletal muscle. Such a composition will be labeled herein as "muscle replica". In some embodiments, the muscle replica and/or meat substitute product comprising the muscle replica are partially derived from animal sources. In some embodiments, the muscle replica and/or meat substitute product comprising the muscle replica are entirely derived from non-animal sources.

[0306] Many meat products comprise a high proportion of striated skeletal muscle in which individual muscle fibers are organized mainly in an isotropic fashion. Accordingly, in some embodiments the muscle replica comprises fibers that are to some extent organized isotropically. In some embodiments the fibers comprise a protein component. In some embodiments, the fibers comprise about 1%, about 2%, about 5%, about 10%, about 15%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99% or more of a protein component. [0307] In some embodiments, the protein component comprises one or more isolated, purified proteins. For example the one or more isolated, purified protein can comprise the 8S globulin from Moong bean seeds, or the albumin or globulin fraction of pea seeds. These proteins provide examples of proteins with favorable properties for constructing meat replicas because of their ability to form gels with textures similar to animal muscle or fat tissue. Examples and embodiments of the one or more isolated, purified proteins are described herein. The list of potential candidates here is essentially open and may include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microoiganisms, or synthesized in vitro. The proteins may be artificially designed to emulate physical properties of animal muscle tissue. The proteins may be artificially designed to emulate physical properties of animal muscle tissue. In some embodiments, one or more isolated, purified proteins accounts for about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component by weight.

[0308] Skeletal muscle of animals such as beef cattle typically contain substantial quantities of glycogen, which can comprise on the order of 1% of the mass of the muscle tissue at the time of slaughter. After slaughter, a fraction of this glycogen continues to be metabolized yielding products including lactic acid, which contributes to lowering the pH of the muscle tissue, a desirable quality in meat. Glycogen is a branched polymer of glucose linked together by alpha (1 ->4) glycosidic bonds in linear chains, with branch points comprising alpha (l->6) glycosidic bonds. Starches from plants, particularly amylopectins are also branched polymers of glu- cose linked together by alpha (l->4) glycosidic bonds in linear chains, with branch points comprising alpha (l->6) glycosidic bonds and can therefore be used as an analog of glycogen in constructing meat replicas. Thus in some embodiments, the muscle or meat replica includes a starch or pectin.

[0309] Additional components of animal muscle tissue include sodium, potassium, calcium, magnesium, other metal ions, lactic acid, other organic acids, free amino acids, peptides, nucleotides and sulfur compounds. Thus in some embodiments, the muscle replica can include sodium, potassium, calcium, magnesium, othermetal ions, lactic acid, other organic acids, free amino acids, peptides, nucleotides and sulfur compounds. In some embodiments the concentration of sodium, potassium, calcium, magnesium, othermetal ions, lactic acid, other organic acids, free amino acids, peptides, nucleotides and/or sulfur compounds in the muscle replica or consumable are within 10% of the concentrations found in a muscle or meat being replicated.

[0310] In another aspect, the invention provides methods for making a muscle replica. In some embodiments, the composition is formed into asymmetric fibers prior to incorporation into the consumable. In some embodiments these fibers replicate muscle fibers. In some embodiments the fibers are spun fibers. In other embodiments the fibers are extruded fibers. Accordingly, the present invention provides for methods for producing asymmetric or spun protein fibers. In some embodiments, the fibers are formed by extrusion of the protein component through an extruder. Methods of extrusion are well known in the art, and are described in U.S. Pat. No. 6,379,738, U.S. Pat. No. 3,693,533, US20120093994, which are herein incorporated by reference.

[0311] In some embodiments extrusion can be conducted using an MPF19 twin-screw extruder (APV Baker, Grand Rapids, Mich.) with a cooling die. The cooling die can cool the extrudate prior to return of the extrudate to atmospheric pressure, thus substantially inhibiting expansion or puffing of the final product. In the MPF19 apparatus, dry feed and liquid can be added separately and mixed in the barrel. Extrusion parameters can be, for example: screw speed of 200 rpm, product temperature at the die of 150 C., feedrate of

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Figure 8

23 g/min, and water flow rate of 11 g/min. Product temperature can be measured during extrusion by a thermocouple at the end of the extrusion barrel. Observations can be made on color, opacity, structure, and texture for each collected sample. Collected samples can be optionally dried at room temperature overnight, then ground to a fine powder (<60 mesh) using a Braun food grinder. The pH of samples can be measured in duplicate using 10% (w/v) slurries of powdered sample in distilled water.

[0312] Fat Replica

[0313] Animal fat is important for the experience of eating cooked meat. Accordingly, the present invention provides a composition derived from non-animal sources which recapitulates key features of animal fat. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources which recapitulates animal fat. Such a composition will be labeled herein as a "fat replica". In some embodiments, the fat replica and/or meat substitute product comprising the fat replica are partially derived from animal sources.

[0314] In some embodiments the meat substitute product has a fat component. In some embodiments the fat content of the consumable is 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, or 60% fat. In some embodiments, the fat replica comprises a gel with droplets of fat suspended therein. In some embodiments, the gel is a soft, elastic gel comprising proteins and optionally carbohydrates. In particular embodiments, the proteins used in the gel are plant or microbial proteins. In some embodiments, the proteins used in the fat replica might include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microoiganisms, or synthesized in vitro. The proteins may be artificially designed to emulate physical properties of animal fat. The proteins may be artificially designed to emulate physical properties of animal fat.

[0315] The fat droplets used in some embodiments of the present invention can be from a variety of sources. In some embodiments, the sources are non-animal sources. In particular embodiments, the sources are plant sources. Non-limiting examples of oils include corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phosphatidic acids, lysophosphatidic acids, oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelaigonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelaigonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelaigonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid.

[0316] In some embodiments, fat droplets are derived from pulp or seed oil. In other embodiments, the source may be yeast or mold. For instance, in one embodiment the fat droplets comprise triglycerides derived from **Mortierella isabellina.**

[0317] In some embodiments plant oils are modified to resemble animal fats. The plant oils can be modified with flavoring or other agents to recapitulate the taste and smell of meat during and after cooking. Accordingly, some aspects of the invention involve methods for testing the qualitative similarity between the cooking properties of animal fat and the cooking properties of plant oils in the consumable.

[0318] In some embodiments, the fat replica comprises a protein component comprising one or more isolated, purified proteins. The purified proteins contribute to the taste and texture of the meat replica. In some embodiments purified proteins can stabilize emulsified fats. In some embodiments the purified proteins can form gels upon denaturation or enzymatic crosslinking, which replicate the appearance and texture of animal fat. Examples and embodiments of the one or more isolated, purified proteins are described herein. In particular embodiments, the one or more isolated proteins comprise a protein isolated from the legume family of plants. Non-limiting examples of legume plants are described herein, although variations with other legumes are possible. In some embodiments, the legume plant is a pea plant. In some embodiments the isolated purified proteins stabilize emulsions. In some embodiments the isolated purified proteins form gels upon crosslinking or enzymatic crosslinking. In some embodiments, the isolated, purified proteins comprise seed storage proteins. In some embodiments, the isolated, purified proteins comprise albumin. In some embodiments, the isolated, purified proteins comprise globulin. In a particular embodiment, the isolated, purified protein is a purified pea albumin protein. In another particular embodiment, the isolated, purified protein is a purified pea globulin protein. In another particular embodiment the isolate purified protein is a Moong bean 8S globulin. In another particular embodiment, the isolated, purified protein is an oleosin. In another particular embodiment, the isolated, purified protein is a caloleosin. In another particular embodiment, the isolated, purified protein is Rubisco. In some embodiments, the protein component comprises about 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%ormoreofthefatreplicaby dry weight or total weight. In some embodiments, the protein component comprises about 0.1-5%, about 0.5-10%, about 1-20%, about 5-30%, about 10-50%, about 20-70%, or about 30-90% or more of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises a solution containing one or more isolated, purified proteins.

Figure 8

[0319] In some embodiments, the fat replica comprises cross-linking enzymes that catalyze reactions leading to covalent crosslinks between proteins. Cross-linking enzymes can be used to create or stabilize the desired structure and texture of the adipose tissue replica, to mimic the desired texture of an equivalent desired animal fat. Non-limiting examples of cross-linking enzymes include, e.g., transglutaminase, lysyl oxidases, or other amine oxidases (e.g. **Pichia pastoris** lysyl oxidase). In some embodiments, the cross-linking enzymes are isolated and purified from a nonanimal source, examples and embodiments of which are described herein. In some embodiments, the fat replica comprises at least 0.0001%, or at least 0.001%, or at least 0.01%, or at least 0.1%, or at least 1% (wt/vol) of a cross-linking enzyme. In particular embodiments, the cross-linking enzyme is transglutaminase.

[0320] In another aspect, the invention provides methods for making a fat replica. In some embodiments, the fat droplets are suspended in a gel. In some embodiments the present invention provides for methods for producing droplets of fat suspended in the gel. The fat can isolated and homogenized. For example an organic solvent mixture can be used to help mix a lipid. The solvent can then be removed. At this point the lipid can be frozen, lyophilized, or stored. So in some aspects the invention provides for a method for isolating and storing a lipid which has been selected to have characteristics similar to animal fat. The lipid film or cake can then be hydrated. The hydration can utilize agitation or temperature changes. The hydration can occur in a precursor solution to a gel. After hydration the lipid suspension can be sonicated or extruded to further alter the properties of the lipid in the solution.

[0321] In some embodiments, the fat replica is assembled to approximate the organization adipose tissue in meat. In some embodiments some or all of the components of the fat replica are suspended in a gel. In various embodiments the gel can be a proteinaceous gel, a hydrogel, an oiganogel, or a xerogel. In some embodiments, the gel can be thickened to a desired consistency using an agent based on polysaccharides or proteins. For example fecula, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, alginin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, cellulose, methylcel- lulose, hydroxymethylcellulose, acadia gum, konjac, starch, pectin, amylopectin orproteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, of fungi can be used alone or in combination to thicken the gel, forming an architecture or structure for the consumable.

[0322] In particular embodiments, the fat replica is an emulsion comprising a solution of one or more proteins and one or more fats suspended therein as droplets. In some embodiments, the emulsion is stabilized by one or more cross-linking enzymes into a gel. In more particular embodiments, the one or more proteins in solution are isolated, purified proteins. In yet more particular embodiments, the isolated, purified proteins comprise a purified pea albumin enriched fraction. In other more particular embodiments, the isolated, purified proteins comprise a purified pea globulin enriched fraction. In other more particular embodiments, the isolated, purified proteins comprise a purified Moong bean 8S globulin enriched fraction. In yet more particular embodiments, the isolated, purified proteins comprise a Rubisco enriched fraction. In other particular embodiments, the one or more fats are derived from plant-based oils. Inmore particular embodiments, the one or more fats are derived from one or more of: com oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phosphatidic acids, lysophosphatidic acids, oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pel- aigonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid. In yet even more particular embodiments, the one or more fats is a rice bran oil. In another particular embodiment, the one or more fats is a canola oil. In other particular embodiments, the cross-linking enzyme is transglutaminase, lysyl oxidase, or other amine oxidase. In yet even more particular embodiments, the cross-linking enzyme is transglutaminase. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of purified pea albumin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/ vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/ vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% canola oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of purified pea albumin emulsified with 40-80% rice bran oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% rice bran oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% canola oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel.

[0323] Connective Tissue Replica

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Figure 8

[0324] Animal connective tissue provides key textural features that are an important component of the experience of eating meat. Accordingly, the present invention provides a composition derived from non-animal sources which recapitulates key features of animal connective tissue. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources which recapitulates important textural and visual features of animal connective tissue. Such a composition will be labeled herein as "connective tissue replica". In some embodiments, the connective tissue replica and/or meat substitute product comprising the connective tissue replica are partially derived from animal sources.

[0325] Animal connective tissue can generally be divided into fascia-type and cartilage-type tissue. Fascia-type tissue is highly fibrous, resistant against extension (has high elastic modulus), and has a high protein content, a moderate water content (ca. 50%), and low-to-none fat and polysaccharide content. Accordingly, the present invention provides a connective tissue replica that recapitulates key features of fascia type tissue. In some embodiments, the connective tissue replica comprises about 50% protein by total weight, about 50% by liquid weight, and has a low fat and polysaccharide component.

[0326] The protein content of most fascia-type connective tissue is comprised mainly of collagen. Collagen is characterized by a high fraction of proline and alanine, and also is assembled into characteristic elongated fibrils or rod-like, flexible structures. Prolamins are one family of proteins found in non-animal sources, such as plant sources. Prolamins are highly abundant in plants and are similar in amino acid composition to collagen. Among proteins we tested for this purpose, prolamins were particularly favorable because of their low cost and their ability to readily form fibers or sheets when spun or extruded. Non-limiting examples of prolamin family proteins include, e.g., zein (found in corn), these include hordein from barley, gliadin from wheat, seca- lin, extensins from rye, kafirin from sorghum, avenin from oats. In fascia-type connective tissue, the prolamin family of proteins, individually or combinations thereof, demonstrates suitability for the protein component because they are highly abundant, similar in global amino acid composition to collagen (high fraction of proline and alanine), and amenable to processing into films and fibers. In addition to zein (found in corn), these include hordein from barley, gliadin from wheat, secalin, extensins from rye, kafirin from sorghum, avenin from oats. Other proteins may be necessary to supplement prolamins in order to achieve targets specifications for physi- cochemical and nutritional properties. The list of potential candidates here is essentially open and may include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microorganisms, or synthesized in vitro. The proteins may be artificially designed to emulate physical properties of animal connective tissue, animal-derived or recombinant collagen, extensins (hydroxyproline-rich glycoproteins abundant in cell walls e.g. **Arabidopsis thaliana,** monomers of which are "collagen-like" rod-like flexible molecules). The proteins may be artificially designed to emulate physical properties of animal connective tissue.

[0327] Methods for forming fascia-type connective tissue will be as those practiced in the art with a bias towards methods producing fibrous or fibrous-like structures by biological, chemical, or physical means, individually or in combination, serially or in parallel, before final forming. These methods may include extrusion or spinning.

[0328] Cartilage-type tissue is macroscopically homogenous, resistant against compression, has higher water content (up to 80%), lower protein (collagen) content, and higher polysaccharide (proteoglycans) contents (ca. 10% each).

[0329] Compositionally, cartilage-type connective tissue will be very similar to fascia-type tissue with the relative ratios of each adjusted to more closely mimic 'meat' connective tissue.

[0330] Methods for forming cartilage-type connective tissue will be similar to those for fascia-type connective tissue, but with a bias towards methods producing isotropically homogenous structures.

[0331] The fat can be suspended in a gel. In some embodiments the present invention provides for methods for producing droplets of fat suspended in the proteinaceous gel. The fat can be isolated from plant tissues and emulsified. The emulsification can utilize high-speed blending, homogenization, agitation or temperature changes. The lipid suspension can be sonicated or extruded to further alter the properties of the lipid in the solution. At this point, in some embodiments other components of the consumable are added to the solution followed by a gelling agent. In some embodiments crosslinking agents (e.g. transglutaminase or lysyl oxidase) are added to bind the components of the consumable. In other embodiments the gelling agent is added and the lipid/gel suspension is later combined with additional components of the consumable. In fascia-type connective tissue, the prolamin family of proteins, individually or combinations thereof, demonstrates suitability for the protein component because they are highly abundant, similar in global amino acid composition to collagen (high fraction of proline and alanine), and amenable to processing into films. In addition to zein (found in com), these include hordein from barley, gliadin from wheat, secalin, extensions from rye, kafirin from soighum, avenin from oats. Other proteins may be necessary to supplement prolamins in order to achieve targets specifications for physicochemical and nutritional properties. The list of potential candidates here is essentially open and may include any major seed storage proteins, animal-derived or recombinant collagen, extensins (hydroxyproline-rich glycoproteins abundant in cell walls e.g. **Arabidopsis thaliana,** monomers of which are "collagen-like" rod-like flexible molecules).

[0332] In some embodiments some or all of the components of the consumable are suspended in a gel. In various embodiments the gel can be a hydrogel, an organogel, or a xerogel, The gel can be made thick using an agent based on polysaccharides or proteins. For example fecula, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, algi- nin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, cellulose, methylcellulose, hydroxymethylcellulose, acadia gum, kon- jac, starch, pectin, amylopectin or proteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, of fungi can be used alone or in combination to thicken the gel, forming an architecture or structure for the consumable. Enzymes that catalyze reactions leading to covalent crosslinks between proteins can also be used alone or in combination to form an architecture or structure for the consumable. For example transclutaminase, lysyl oxidases, or other amine oxidases (e.g. **Pichia pastoris** lysyl

Figure 8

oxidase (PPLO)) can be used alone or in combination to form an architecture or structure forthe consumable. In some embodiments multiple gels with different components are combined to form the consumable. For example a gel containing a plant-based protein can be associated with a gel containing a plant-based fat. In some embodiments fibers or stings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

[0333] The compositions of the invention can be puffed or expanded by heating, such as frying, baking, microwave heating, heating in a forced air system, heating in an air tunnel, and the like, according to methods well known in the art. [0334] In some embodiments multiple gels with different components are combined to form the consumable. For example a gel containing a plant-based protein can be associated with a gel containing a plant-based fat. In some embodiments fibers or strings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

[0335] In some embodiments the meat replica contains no animal products, less than 1% wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[0336] In some embodiments the meat replica contains no animal products, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[0337] In some embodiments the meat replica contains no animal products, no soy protein isolate, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[0338] In some embodiments the meat replica contains no animal products, no soy protein concentrate, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[0339] In some embodiments the meat replica contains no animal products, no soy protein, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[0340] In some embodiments the meat replica contains no animal products, no tofu, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[0341] In some embodiments the meat replica contains no animal products, no tofu, and no wheat gluten.

[0342] In some embodiments the meat replica contains no animal products, no soy protein, and no wheat gluten.

[0343] In some embodiments the meat replica contains no methylcellulose, no carrageenan, no caramel color, no Konjac flour, no gum Arabic, and no acacia gum.

[0344] In some embodiments the meat replica contains no animal products and less than 5% carbohydrates.

[0345] In some embodiments the meat replica contains no animal products, no soy protein, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum and less than 5% carbohydrates.

[0346] In some embodiments the meat replica contains no animal products, and less than 1% cellulose.

[0347] In some embodiments the meat replica contains no animal products, and less than 5% insoluble carbohydrates. [0348] In some embodiments the meat replica contains no animal products, no soy protein, and less than 1% cellulose. [0349] In some embodiments the meat replica contains no animal products, no soy protein, and less than 5% insoluble carbohydrates.

[0350] In some embodiments the meat replica contains no animal products, no wheat gluten, and less than 1% cellulose. [0351] In some embodiments the meat replica contains no animal products, no wheat gluten, and less than 5% insoluble carbohydrates.

[0352] The percentage of different components may also be controlled. For example non-animal-based substitutes for muscle, fat tissue, connective tissue, and blood components can be combined in different ratios and physical organizations to best approximate the look and feel of meat. The various can also components can be arranged to insure consistency between bites of the consumable. The components can be arranged to insure that no waste is generated from the consumable. For example, while a traditional cut of meat may have portions that are not typically eaten, a meat replicate can improve upon meat by not including these inedible portions. Such an improvement allows for all of the product made or shipped to be consumed, which cuts down on waste and shipping costs. Alternatively, a meat replica may include inedible portions to mimic the experience of meat consumption. Such portions can include bone, cartilage, connective tissue, or other materials commonly referred to as gristle, or materials included simulating these components. In some embodiments the consumable may contain simulated inedible portions of meat products which are designed to serve secondary functions. For example a simulated bone can be designed to disperse heat during cooking, making the cooking of the consumable faster or more uniform than meat. In other embodiments a simulated bone may also serve to keep the consumable at a constant temperature during shipping. In other embodiments, the simulated inedible portions may be biodegradable.

[0353] In some embodiments the meat substitute compositions contains no animal protein, comprising between 10-30% protein, between 5-80% water, between 5-70% fat, comprising one or more isolated purified proteins. In particular embodiments, the meat substitute compositions comprise transglutaminase.

[0354] In some embodiments the consumable contains components to replicate the components of meat. The main component of meat is typically skeletal muscle. Skeletal muscle typically consists of roughly 75 percent water, 19 percent protein, 2.5 percent intramuscular fat, 1.2 percent carbohydrates and 2.3 percent other soluble non-protein substances. These include oiganic acids, sulfur compounds, nitrogenous compounds, such as amino acids and nucleotides, and inorganic substances such as minerals. Accordingly, some embodiments of the present invention provide for replicating approximations of this composition for the consumable. F or example, in some embodiments the consumable is a plant-based meat replica can comprise roughly 75% water, 19% protein, 2.5% fat, 1.2% carbohydrates; and 2.3 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 60-90% water, 10-30% protein, 1-20% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 60-90% water, 5-

Figure 8

10% protein, 1-20% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments the consumable is a plant- based meat replica comprising between 0-50% water, 5-30% protein, 20-80%% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments, the replica contains between 0.01 % and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin. Some meat also contains myoglobin, a heme protein, which accounts for most of the red color and iron content of some meat. In some embodiments, the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01 % and 5% by weight of leghemoglobin. It is understood that these percentages can vary in meat and the meat replicas can be produced to approximate the natural variation in meat. Additionally, in some instances, the present invention provides for improved meat replicas, which comprise these components in typically unnatural percentages. For example a meat replica can be produced with a higher than typical average fat content. The percentages of these components may also be altered to increase other desirable properties.

[0355] In some instances a meat replica is designed so that, when cooked, the percentages of components are similar to cooked meat. So, in some embodiments, the uncooked consumable has different percentages of components than uncooked meat, but when cooked the consumable is similar to cooked meat. For example, a meat replica may be made with a higher than typical water content for raw meat, but when cooked in a microwave the resulting product has percentages of components similar to meat cooked over a fire.

[0356] In some embodiments the consumable is a meat replica with a lower that typical water content for meat. In some embodiments the inventions provides for methods for hydrating a meat replica to cause the meat replica to have a content similar to meat. For example a meat replica with a water content that would be low for meat, for example 1%, 10%, 20%, 30%, 40% or 50% water, is hydrated to roughly 75% water. Once hydrated, in some embodiments, the meat replica is then cooked for human consumption.

[0357] The consumable can have a protein component. In some embodiments the protein content of the consumable is 10%, 20%, 30%, or 40%. In some embodiments the protein content of the consumable is similar to meat. In some embodiments the protein content in the consumable is greater than that of meat. In some embodiments the consumable has less protein than meat.

[0358] The protein in the consumable can come from a variety or combination of sources. Non-animal sources can provide some or all of the protein in the consumable. Nonanimal sources can include vegetables, fruits, nuts, grains, algae, bacteria, or fungi. The protein can be isolated or concentrated from one or more of these sources. In some embodiments the consumable is a meat replica comprising protein only obtained from non-animal sources.

[0359] In some embodiments protein is formed into asymmetric fibers for incorporation into the consumable. In some embodiments these fibers replicate muscle fibers. In some embodiments the protein are spun fibers. Accordingly, the present invention provides for methods for producing asymmetric or spun protein fibers. In some embodiments the consumable contains a protein or proteins that have all of the amino acids found in proteins that are essential for human nutrition. In some embodiments the proteins added to the consumable are supplemented with amino acids.

[0360] Indicators of Cooking Meat

[0361] The release of odorants upon cooking is an important aspect of meat consumption. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking beef. In some embodiments, the consumable when cooked generates an aroma recognizable by humans as typical of cooking pork. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking bacon. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking chicken. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking lamb. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking fish. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking turkey. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with an odorant that is released upon cooking. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with an odorant that is produced by chemical reactions that take place upon cooking. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing mixtures of proteins, peptides, amino acids, nucleotides, sugars and polysaccharides and fats in combinations and spatial arrangements that enable these compounds to undergo chemical reactions during cooking to produce odorants and flavor-producing compounds. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a volatile or labile odorant that is released upon cooking. In some embodiments the consumable is a method for preparing a meat replica where meat replicas principally or entirely composed of ingredients derived from non-animal sources are heated to release a volatile or labile odorant.

[0362] Odorants released during cooking of meat are generated by reactions that can involve as reactants fats, protein, amino acids, peptides, nucleotides, organic acids, sulfur compounds, sugars and other carbohydrates. In some embodiments the odorants that combine during the cooking of meat are identified and located near one another in the consumable, such that upon cooking of the consumable the odorants combine. So, in some embodiments, the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions involving amino acids, fats and sugars found in plants as well as meat. So, in some embodiments, the characteristic flavor and fragrance components are mostly produced during the cooking process by chemical

Figure 8

reactions involving one or more amino acids, fats, peptides, nucleotides, oiganic acids, sulfur compounds, sugars and other carbohydrates found in plants as well as meat.

[0363] Some reactions that generate odorants released during cooking of meat can be catalyzed by iron, in particular the heme iron of myoglobin. Thus in some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by iron. In some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by heme. In some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by the heme iron in leghemoglobin. In some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by the heme iron in a heme protein.

[0364] Evidence that the presence of leghemoglobin contributes favorably to aroma of meat replicas: A muscle replica comprising pea flour, sunflower oil, and glucose was heated for 10 minutes at 140 C in the presence of either reduced leghemoglobin (LHb) or a mixture of iron (Fe3+), sodium and EDTA (EES) in sealed containers carrying solid phase microextraction (SPME) fibers. These fibers contain poly- dimethylsiloxane (PDMS) which adsorbs volatile compounds for analysis by GC-MS. Analysis of GC-MS data from multiple replicas reveal consistent differences between the LElb and EPS samples. Non-limiting examples of compounds found exclusively or more abundantly in the LElb samples are: 2-octanone, 2-methyl furan, which are often associated with the aroma of cooked meat, and many other unidentified compounds.

[0365] Color Indicators

[0366] The color of meat is an important part the experience of cooking and eating meat. For instance, cuts of beef are of a characteristic red color in a raw state and gradually transition to a brown color during cooking. As another example, white meats such as chicken or pork have a characteristic pink color in their raw state and gradually transition to a white or brownish color during cooking. The amount of the color transition is used to indicate the cooking progression of beef and titrate the cooking time and temperature to produce the desired state of done-ness. In some aspects, the invention provides a non-meat based meat substitute product that provides a visual indicator of cooking progression. In some embodiments, the visual indicator is a color indicator that undergoes a color transition during cooking. In particular embodiments, the color indicator recapitulates the color transition of a cut of meat as the meat progresses from a raw to a cooked state. In more particular embodiments, the color indicator colors the meat substitute product a red color before cooking to indicate a raw state and causes the meat substitute product to transition to a brown color during cooking progression. In other particular embodiments, the color indicator colors the meat substitute product a pink color before cooking to indicate a raw state and causes the meat substitute product to transition to a white or brown color during cooking progression.

[0367] The main determinant of the nutritional definition of the color of meat is the concentration of iron carrying proteins in the meat. In the skeletal muscle component of meat products, one of the main iron-carrying proteins is myoglobin. It is estimated that the white meat of chicken has under 0.05%; pork and veal have 0.1-0.3%; young beef has 0.4-1.0%; and old beef has 1.5-2.0%. So, in some embodiments, the consumable is a meat replica which comprises an iron-carrying protein. In some embodiments, the meat replica comprises about 0.05%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, or more than about 2% of an iron-carrying protein by dry weight or total weight. In some cases, the iron carrying protein has been isolated and purified from a source. In other cases, the iron carrying protein has not been isolated and purified. In some cases, the source of the iron-carrying protein is an animal source, or a non-animal source such as a plant, fungus, or genetically modified organisms such as, e.g., bacteria or yeast. In some cases, the ironcarrying protein is myoglobin. In some embodiments the consumable is a plant based meat replica that has animal myoglobin added. So, for example a replica of young beef can have about 0.4-1% myoglobin. In some cases, the iron-carrying protein is leghemoglobin. In some embodiments the consumable is a plant based meat replica that has leghemoglobin added. So, for example a replica of young beef can have about 0.4-1% leghemoglobin. In some cases, the ironcarrying protein is a cytochrome. In some embodiments the consumable is a plant based meat replica that has a cytochrome added. So, for example a replica of young beef can have about 0.4-1% of a cytochrome.

[0368] Another example of iron-carrying proteins is hemoglobin, the iron-containing oxygen-binding protein in the red blood cells of vertebrates. Hemoglobin is similar in color to myoglobin. In some embodiments the invention provides methods of saving and recycling blood from animal farming to supplement the color of a consumable. For example blood is saved from a slaughter house, hemoglobin from the blood is used to enhance the color of a consumable. In some aspects the consumable is a plant-based meat replica containing hemoglobin.

[0369] Additional iron containing proteins exist in nature. In some embodiments the consumable comprises an iron containing protein that is not myoglobin. In some embodiments the consumable does not contain myoglobin. In some embodiments the consumable does not contain hemoglobin. In some embodiments the consumable is a meat replica that comprises an iron containing protein other than myoglobin or hemoglobin.

[0370] Examples ofiron containing proteins include hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins. In various embodiments these iron containing proteins are added to the consumable to alter the visual characteristics or iron content of the consumable. In some embodiments the consumable comprises a hemoprotein (e.g. hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins).

[0371] Leghemoglobin, similar in structure and physical properties to myoglobin, is readily available as an unused by-product of

Figure 8

commodity legume crops (eg., soybean, pea). The leghemoglobin in the roots of these crops in the US exceeds the myoglobin content of all the red meat consumed in the US. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, including a muscle tissue replica, an adipose tissue replica, a connective tissue replica, and leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a heme protein. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from nonanimal sources, containing a leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a member of the globin protein family. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from nonanimal sources, with a high iron content from a heme protein. In some embodiments the iron content is similar to meat. In some embodiments the consumable has the distinctive red color of meat, such color provided by leghemoglobin.

[0372] Leghemoglobin is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.

[0373] A heme protein is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.

[0374] A heme protein from the group of: Hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non- symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.

Food Products Comprising Isolated, Purified Leghemoglobin

[0375] In some embodiments leghemoglobin is added to meat to enhance the properties of meat. For example, a leghemoglobin containing solution can be injected into raw or cooked meat. In another example a leghemoglobin solution is dripped over meat or a consumable of the invention to enhance appearance. In one embodiment advertising, photography, or videography of food products such as meat or a meat substitute is enhanced with leghemoglobin.

Sources of Leghemoglobin

[0376] In some embodiments the present invention provides methods for obtaining leghemoglobin from plants. Leghemoglobin can be obtained from a variety of plants. Various legumes species and their varieties, for example, Soybean, Fava bean, Lima bean, Cowpeas, English peas, Yellow peas, Lupine, Kidney bean, Garbanzo beans, Peanut, Alfalfa, Vetch hay, Clover, Lespedeza and Pinto bean, contain nitrogen-fixing root nodules in which leghemoglobin has a key role in controlling oxygen concentrations (for example root nodules from a pea plant, FIG. 1). FIG. 2 shows 100 mis of leghemoglobin solution isolated from 30 grams of pea root nodules. Leghemoglobins from different species are homologs and have similar color properties (FIG. 3). In FIG. 3, panel A shows an SDS_PAGE gels of lysed root-nodules of three legume plant species (1) Fava bean (2) English Pea (3) Soybean. Arrows mark respective leghemoglobins. Note that leghemoglobin is the most abundant soluble protein in each lysate. Panel B shows the similarity of UV-VIS spectral profile of leghemoglobins from two different plant species (Fava- bean and Soybean). We purified leghemoglobin from fava bean (green curve) and Soybean (red curve) root nodules using the protocol described elsewhere in the specification. UV-VIS spectra of both proteins shows that the heme iron is in the reduced (+2) state. Note that they are almost perfectly superimposed, consistent with their visually identical red color. The heme iron in the respective leghemoglobins was reduced to the +2 oxidation state by incubating Fava bean and Soybean leghemoglobin with 10 mM sodium hydrosulfite in 20 mM potassium-phosphate pFl 7.4, 100 mM sodium chloride buffer. Sodium hydrosulfite was then removed from the leghemoglobin solution using gel-exclusion chromatography. Inset shows a zoom-in of UV-VIS spectra in 450 nm to 700 nm region. Some plant species express several leghemoglobin isoforms (for example soybean has four leghemoglobin isoforms). Minor variations in precise amino acid sequence can modify overall charge of the protein at a particular pFl and can modify precise structural conformation of iron containing heme group in leghemoglobin. Differences in structural conformation of heme group of different leghemoglobins can influence oxidation and reduction rates of the heme iron. These differences may contribute to color and flavor generation properties of different leghemoglobins. [0377] Leghemoglobin has a virtually identical absorbance spectrum and visual appearance to myoglobin from animal muscle. FIG. **4** shows a comparison of reduced (heme iron 2+) and oxidized (heme iron 3+) soybean leghemoglobin (FIG. **4** panel A) and equine heart muscle myoglobin (FIG. **4** panel B) showing similarity of UV-VIS absorption profiles of two proteins. We purified soybean leghemoglobin from soybean root-nodules using here described protocol. Purified equine myoglobin was purchased from SigmaAldrich. Soybean leghemoglobin (FIG. **4** panel A) and equine myoglobin (FIG. **4** panel B) were reduced with 1 mm sodium hydrosulfite. Shown are UV-VIS absorption spectra of heme Fe3+ (blue line) and heme Fe2+ (red line) of soybean leghemoglobin (FIG. **4** panel A) and equine myoglobin (FIG. **4** panel B). Insets show a zoom-in of UV-VIS

Figure 8

spectra in 450 nm to 700 nm region. (FIG. **4** panel C) Images of 10 ul liquid droplet of a 40 mg/ml solution of soybean leghemoglobin in the heme- Fe3+ state (left droplet) showing characteristic rusty red color and a 40 mg/ml solution of soybean leghemoglobin solution in the heme-Fe2+ state (right droplet) showing characteristic red color of and (right image) corresponding samples of equine myoglobin.

[0378] In other embodiments, leghemoglobin can be sourced from non-plant sources, such as from organisms such as bacteria or yeast which have been genetically modified to express high levels of leghemoglobin.

[0379] The oxidation state of the iron ion in leghemoglobin is important for its color. Leghemoglobin with the heme iron in the +2 oxidation state appears vivid red in color, while leghemoglobin with the heme iron in the +3 oxidation state appears brownish red. Thus, in using leghemoglobin as a source of red color in a meat replica, it is desirable to reduce the heme iron from the +3 state to the +2 state. Heme iron in leghemoglobin can be switched from oxidized (+3) state to reduced (+2) state with reducing reagents. Examples of successful reduction of leghemoglobin heme iron with sodium hydrosulfite and titanium citrate are illustrated in FIG. **5**. In FIG. **5** the UV-VIS spectrogram of purified soybean leghemoglobin in which the heme iron is in the oxidized (+3) state is represented by the blue curves in each panel. The red curves in each panel represent the UV-VIS spectra of the same leghemoglobin species after reduction to the (+2) state (red lines) by addition of (Panel A) 1 mM sodium hydrosulfite or (Panel B) 0.24% (wt/v) titanium citrate in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride buffer. The Insets showazoom-in ofUV-VIS spectra in450-700nm region. For this example, leghemoglobin was purified from soybean root nodules using 60/90% ammonium sulfate fractionation and exchanged into 20 mM potassium phosphate pH 7.4,100 mM sodium chloride buffer. Sodium hydrosulfite stock solution was prepared by dissolving 100 mM sodium hydrosulfite in 1 mM sodium hydroxide in water. Titanium citrate stock solution was prepared from 20% (wt/v) Ti-chloride in hydrochloric acid by mixing it with 0.2M sodium citrate (1:10 v/v). pH was adjusted using sodium carbonate to pH 7.0.

[0380] Leghemoglobin can be purified from legume root nodules, such as the root nodules of peas or soybeans (FIG. **1** shows Leghemoglobin isolated from pea root nodules). Root nodules from soy beans were thoroughly cleaned to remove soil and extraneous root tissues prior to root nodule lysis in 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mm EDTA and 1 mM ascorbic acid. Root nodules were lysed by grinding root-nodules using a Vitamix blender. For some samples Polyvinylpyrrolidone polymer was added at 30% wt/v to aid in removal of plant phenolic small molecules that mediate oxidation of leghemoglobin heme-iron. Root nodule lysate was fractionated using ammonium sulfate in two steps, first ammonium sulfate was added to 60% wt/v. Pellet was discarded and supernatant brought to 90% wt/v. ammonium sulfate. Leghemoglobin was collected as a precipitated pellet in 90% ammonium sulfate fraction. Ammonium sulfate precipitated leghemoglobin was resuspended in 20 mM potassium phosphate, 1 mM EDTA, 50 mM sodium chloride and ammonium sulfate was removed using dialysis or size-exclusion chromatography in the same buffer. In some instances this was the last purification step, while in other instances leghemoglobin was further purified using anion- exchange chromatography (FFQ GE Healthcare), which was sometimes followed by size-exclusion chromatography (Sephacryl S-100, GE Healthcare). Soybean leghemoglobin from 90% ammonium sulfate pellet was loaded on anion exchange columns (FFQ or DEAE, GE Healthcare) in different buffers (20 mM potassium phosphate pH 7.4, containing 1—to 100 mM sodium chloride, 20 mM Tris pH 8 containing 0 to 100 mM sodium chloride, 20 mM sodium borax pH 9.8,20 mM sodium chloride, 20 mM sodium carbonate pH 9,20 mM sodium chloride) and purified either in flow-through or using sodium chloride (0-1M salt gradient). An example of the leghemoglobin purification flow from soybean root nodules is represented in FIG. 6. The figure shows SDS-PAGE fractionation of different soybean leghemoglobin purification steps (Lane 1) Soybean root-nodule lysate; (Lane 2) Soybean root- nodule lysate purified by 60/90% (wt/v) ammonium sulfate fractionation. Shown is the protein content of 90% ammonium sulfate fractionated protein pellet resuspended in 20 mm potassium phosphate pH 7.4, 100 mM sodium chloride,

2—mM EDTA buffer; Proteins from 90% ammonium sulfate pellet were further purified by anion-exchange chromatography (FFQ GE Healthcare) in 20 mM potassium phosphate ph 7.4, 100 mM sodium chloride. Leghemoglobin collected in the flowthrough is shown in Lane 3. Anion-exchange flowthrough was fractionated using size-exclusion chromatography (Sephacryl S-100 GE Healthcare) and resulting leghemoglobin fraction is shown in Lane 4. Leghemoglobin content at different purification steps was determined by determining the fraction of leghemoglobin band on SDS- PAGE gel in a respective sample using ImageDoc analysis software (BioRad). Purity (partial abundance) of leghemoglobin at respective steps in the purification steps was: lysate: 32.7% (lane 1), 60/90% (wt/v) ammonium sulfate fractionation 78% (lane 2), anion-exchange chromatography -83% (lane 3), and size-exclusion chromatography to -95% (lane 4).

[0381] Leghemoglobin can also be produced by genetically engineering a bacterium or fungus to produce it. One illustrative example is shown in FIG. 7. FIG. 7 shows stained SDS-PAGE gel analysis of (A) soybean leghemoglobin expressed and purified using recombinant protein technology and (B) soybean leghemoglobin purified from soybean root nodules. (A) Recombinant Soybean leghemoglobin A carrying Flis-tag and TEV protease Flis-tag removal site was expressed in ***E. coli*** BL21 strain and purified using Flis-tag affinity chromatography (Talon resin, CloneTech). The left lane contains molecular weight standards, the right lane contains purified recombinant soybean leghemoglobin A (arrow) . Expected molecular weight of the recombinant soybean leghemoglobin A is 17.1 kDa. (B) SDS-PAGE gel of purified Soybean leghemoglobin from root nodules. The left lane contains molecular weight standards, the right lane contains purified soybean leghemoglobin A (arrow). Mass spectrometry analysis of purified material determined that all four soybean leghemoglobin isoforms are present, and are full length (data not shown). Expected molecular

Figure 8

weights (MW) of soybean leghemoglobin isoforms range from MW 15.4 to 15.8 kDa.

[0382] Leghemoglobin purified from soybean and fava root nodules, respectively was tasted by a panel of volunteers and in each case described as tasting like blood.

[0383] Leghemoglobin can be isolated from the roots nodules of legumes such as soy beans, fava beans, cow peas, lima beans, garbanzo beans, peas, lupine, lotus japonicum or other legumes. The root nodule (for example root nodules from a pea plant) is obtained and homogenized in an aqueous solution, soluble proteins including leghemoglobin are recovered after insoluble matter is removed by precipitation or filtration. Leghemoglobin can be purified by selective precipitation and/or chromatography and/or the use of molecules with specific affinity for leghemoglobin. (FIG. 1, showing 100 mis of solution of leghemoglobin isolated from 30 grams of pea root nodules).

[0384] Heme proteins, for example leghemoglobin, can be combined with other plant based meat replica components. In some embodiments the heme proteins are captured in a gel which contains other components, for example lipids and or proteins. In some aspects a multiple gels are combined with non-gel based heme proteins. In some embodiments the combination of the heme proteins and the other compounds of the consumable are done to insure that the heme proteins are able to diffuse through the consumable. In some embodiments the consumable is ed in a heme-protein containing solution, for instance a leghemoglobin solution. In some embodiments the consumable is soaked in a heme protein containing solution, for instance a leghemoglobin solution for 1,5,10,15,20 or 30 hours. In some embodiments the consumable is soaked in a heme containing solution, for instance a leghemoglobin solution for 1, 5, 10, 15, 30, or 45 minutes.

[0385] FIG. 8 shows an example of 6 cubes of a commercial meat analog (Quom chicken analog), about 1 cm on a side, 4 of which (Left and lower right) have been soaked in a solution of about 10 mg/ml soybean leghemoglobin in 20 mM Potassium phosphate pH 7.4 and 100 mM NaCl; the remaining two (Upper right) were soaked in the same buffer without leghemoglobin. Note the deep pink color of the leghemoglobin- infused cubes in contrast to the pale tan color of the uninfused cubes.

[0386] FIG. 9 shows the 4 leghemoglobin-infused cubes of Quorn chicken analog in the process of cooking in a pan at 350° C. The two lower cubes have been turned over to expose the grilled surface, which has turned brown. Note in the upper two cubes that the heated portion has turned grey-brown, while the cooler top surface retains its pink color. In some embodiments the consumable is injected with a heme containing solution, for instance a leghemoglobin solution, until the consumable is the color of uncooked meat.

[0387] Given the usefulness of heme proteins for coloring consumables it will be useful to detect whether a product contains a particular heme protein. Accordingly the present invention includes in some embodiments methods to determine whether a product contains a heme protein. Methods for detecting proteins are well known in the art. For example an ELISA or proximity-ligation assay or luminex assay or western blot analysis can be performed to determine whether leghemoglobin is present in a food product such as meat or a meat replica. In one embodiment the detection methods are performed to determine whether meat has been altered with leghemoglobin.

EXAMPLES

[0388] An exemplary muscle replica composition comprising one or more isolated, purified plant proteins is described herein.

[0389] Protein Purification for Components of the Replica [0390] Moong bean seeds, Green Pea dry seed were purchased as milled flour and used for purification of respective seed storage proteins. Rubsico was purified from fresh alfalfa plant. Protein composition at individual fractionation steps was monitored by SDS-PAGE and protein concentrations were measured by standard UV-VIS and Pierce assay methods.

[0391] Moong bean 8S globulins: Moong bean flour was resuspended in in 50 mM potassium phosphate buffer pH 7 and 0.5M NaCl at 1:4 (wt/v) ratio, and mixture was incubated for 1 hr. Unsoluble material was separated by centrifugation and proteins in the supernatant were fractionated by addition of ammonium sulfate in 2 steps: 50% (wt/v) followed by 90% (wt/v). Protein precipitated in 90% fraction contained the moong bean 8S globulins and was stored at -20 C until further use.

[0392] Pea-albumins: Green pea dry seed flour was resuspended at 1:10 (wt/v) ratio in 50 mM sodium acetate buffer pH 5 and incubated for 1 hr. Unsoluble material was separated by centrifugation and proteins in the supernatant were fractionated by ammonium sulfate precipitation in two steps: 50% (wt/v) followed by 90% (wt/v). Ammonium sulfate solutions were stirred for 1 hour and ammonium sulfate precipitated proteins removed by centrifugation. Proteins of interest precipitated in 90% (wt/v) ammonium sulfate. Pellet was stored at -20 C until further use.

[0393] Pea-globulins: Green pea dry seed flour was resuspended at 1:10 (wt/v) ratio in 20 mM potassium phosphate buffer pH 8, 0.4M sodium chloride and stirred for 1 hr. After centrifugation, the supernatant was subjected to ammonium sulfate fractionation. First, supernatant was brought to 50% (wt/v) ammonium sulfate, and precipitated proteins removed. Second, 50% (wt/v) ammonium sulfate supernatant was brought to 80% (wt/v) ammonium sulfate saturation. The 80% (wt/v) ammonium sulfate pelleted protein contained globulins of interest. Pellet was stored at -20° C. until further use.

[0394] RuBisCO: RuBisCO was fractionated from alfalfa greens (or other green plants eg soybean plants, spinach etc) by first grinding leaves with 4 volumes of cold 50 mM KPhos- phate buffer pH 7.4 buffer (with (in lab) or without (in field) 0.5M NaCl+2 mM DTT+1 mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (crude lysate) was used in further purification steps. Proteins in the crude lysate were fractionated by addition of ammonium sulfate to 30% (wt/v) saturation. The solution was stirred for 1 hr and then centrifuged. The pellet from this step was discarded and additional ammonium sulfate was added to the supernatant to 50% (wt/v) ammonium sulfate saturation. The solution was centrifuged again after stirring for 1 hr. The pellet from this step contains RuBisCO, and was kept at -20 C until used.

Figure 8

[0395] Obtaining Plant Proteins.
[0396] Moong bean seed 8S protein was purified by ammonium sulfate fractionation as described. Pellet was resuspended in 20 mM potassium phosphate pH pH 7.4 and 0.5M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16000 g, 10 min and protein concentrated to desired concentration. Pea globulins purified by ammonium sulfate fractionation as described. Protein pellet was resuspended in 20 mM potassium phosphate pH pH 7.4 and 0.4M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16000 g, 10 min and protein concentrated to desired concentration. Pea albumin purified by ammonium sulfate fractionation as described. Protein pellet was resuspended in 20 mM potassium phosphate pH pH 7.4 and 0.1M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16000 g, 10 min and protein concentrated to desired concentration.
[0397] Constructing a Muscle Tissue Analog [0398] Moong bean seed 8S protein was purified by ammonium sulfate fractionation as described above, For preparation of gels, 200 g of pellet was dissolved in 400 ml of dialysis buffer (20 mM potassium phosphate, 400 mM NaCl, pH 7.3) and the resulting solution dialyzed for 6 hours against 5l of dialysis buffer, replaced twice with fresh buffer. Protein solution was centrifuged at 12,000 g for 15 min to remove debris. Protein was concentrated by dialyzing for 3-6 hours against 5l of 30% w/w solution of PEG 8000 (polyethylene glycol, molecular weight 8000) in dialysis buffer. Final protein concentration was 150 mg/ml.
[0399] Leghemoglobin was purified from soybean root nodules. Legume root nodules were cleaned to remove soil and extraneous root tissues prior to root nodule lysis in 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mm EDTA and 1 mM ascorbic acid. Root nodules were lysed by grinding root nodules using juicer blender. Unsoluble material was separated by centrifugation. Root nodule lysate was fractionated using ammonium sulfate in two steps, first ammonium sulfate was added to 60% wt/v and solution incubated for 1 hr, 4° C. Pellet was discarded and supernatant brought to 90% wt/v ammonium sulfate and incubated for 12 hr, 4° C. Leghemoglobin was collected as a precipitated pellet in 90% ammonium sulfate fraction and resuspended in 20 mM potassium phosphate, 1 mM EDTA, 100 mM sodium chloride. SDS-PAGE gel analysis determined that protein solution contains 70% leghemoglobin and 30% other root nodule proteins. Ammonium sulfate was removed using size-exclusion chromatography in the same buffer. Leghemoglobin was concentrated by dialyzing for 48 hr against 30% PEG 8000 (polyethylene glycol, molecular weight 8000) in 20 mM potassium phosphate pH 7.3,100 mM sodium chloride. Total protein concentration was 57 mg/ml. UV-VIS spectra suggested that leghemoglobin was in heme- iron oxidized state. Thus, leghemoglobin was incubated with 5 mM sodium hydrosulfite for 5 min and sodium hydrosulfite was removed using size-exclusion chromatography in 20 mM potassium phosphate, 100 mM sodium chloride buffer. Leghemoglobin was further concentrated to 35.4 mg/ml. UV- VIS spectra analysis confirmed that leghemoglobin is in heme-iron reduced state.
[0400] Transglutaminase was obtained commercially from (Activa TI, Ajimoto). Stock solution (20% wt/v) was made in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride buffer.
[0401] To prepare "dark" muscle tissue analog (FIG. 10), 43 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 37 ml of leghemoglobin solution (46.5 mg/ml leghemoglobin and 20 mg/ml of other soybean root nodule protein) in 20 mM potassium phosphate, 100 mM NaCl, pH 7.3). 20 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly mixed, divided into two 50 ml Falcon tubes and incubated overnight at room temperature. Final protein concentrations were 65 mg/ml for moong bean protein, 18 mg/ml of leghemoglobin, 91 mg/ml total protein.
[0402] "White" muscle analog (FIG. 11) was prepared by mixing 43 ml moong bean protein solution (150 mg/ml) with 45 ml of 11.7 mg/ml solution of leghemoglobin and 0.8% (wt/v) of transglutaminase solution. Final protein concentrations were 63 mg/ml for moong bean protein, 5.2 mg/ml of leghemoglobin, 68 mg/ml total protein.
[0403] The "dark" muscle tissue analog formed an opaque gel of dark chocolate color, smooth uniform texture, with glistening surface, and a small amount (<1 ml) of dark red, venous blood colored liquid on top. The gel was freely standing, elastic but fragile, similar in appearance to thin Jell-O. The gel has a medium aroma with notes of beans and blood clearly discernible. The flavor is dominated by notes of beans and iron/blood, with weaker grassy and medicinal/chemical flavors. The taste is salty, with a long aftertaste of blood. [0404] The "white" muscle tissue analog was very similar, but with much lighter, cappuccino-like, color. It was also more fragile, 2-3-fold less strong against compression. [0405] Fat Tissue Analog
[0406] Fat tissue analog using moong bean 8S globulin fraction was prepared as follows: 15 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 15 ml of rice bran oil. 6 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly emulsified using a homogenizer (VWR) at speed #2. Emulsion was aliquoted into 1.6 eppendorf tubes and incubated overnight at room temperature. After that, tubes were heated at 95° C. for 5 min in a heat block, and allowed to cool down to room temperature on a bench. Final concentrations were 75 mg/ml for moong bean protein, 50% w/w oil.
[0407] Fat tissue analog using pea globulin (100 mg/ml) was prepared by the same method. Additionally, fat tissue analog was prepared from pea globulin, and either rice bran or canola oil, in bulk by the same method, but without aliquoting emulsions into eppendorf tubes. Instead, emulsions in 50 ml Falcon tubes were rotated overnight on a nutator, and were subsequently incubated at 90° C. for 30 min.
[0408] Fat tissue analog based on moong beans (FIG. 12) and prepared in eppendorf tubes formed an opaque gel of off-white color, smooth uniform texture, with no visible discernible liquid that was not incorporated into the gel. The gel was freely standing, elastic and springy. The gel has a slight, pleasant aroma and a mild and pleasant flavor. The taste is mildly salty.
[0409] Fat tissue analog based on pea globulin (FIG. 13) and prepared in eppendorf tubes was very similar to moong bean-based fat

Figure 8

~~analog, except that it gave up a little of oil upon compression. Fat tissue analog prepared in 50 ml Falcon tubes were similar in appearance, texture and aromas, but substantially softer (2-fold softer for canola oil, and 3-fold softer for rice bran oil, according to compressibility measurements).~~

~~[0410] Connective Tissue Analog~~

~~[0411] Connective tissue analog prototypes were developed using zein protein sourced from 100% yellow corn gluten meal, or from commercial sources, such as Amazein (Prairie Gold, Bloomington, 111.). Zein proteins were solubilized in 70-90% ethanol with desired ratios at 1:3 to 1:5 (solids:solution). By precipitating zein proteins, for example by a change in pH, in a controlled manner, large zein structures result with physicochemical properties that can be manipulated as desired. For example, FIG. 14 shows connective-tissue analog strands that were created using a 1:3 ratio in 70% ethanol, loaded into a syringe with a 23 gauge needle (ID 0.337 mm). The solution was slowly extruded from the bottom of a 5 inch-high vessel into an excess of 5 M NaCl solution. The ethanol-zein solution being less dense than the NaCl solution, floated upward, drawing out a fibrous stand of solidifying zein. The NaCl was constantly stirred as the strands began to develop to assist in the strand lengthening. The strands bunch together and become a hard, dense mass. [0412] Ground Beef Replica Prototypes Made from Gels of Plant Proteins and Plant Oils.~~

~~[0413] A ground beef prototype patty was made by combining 62% (wt/wt) muscle analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "white muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (FIG. 15 panel A). A ground beef prototype patty was made by combining 62% muscle analog (62% "dark muscle analog" and 38% "white muscle analog), 29% fat tissue analog (from moong bean seed 8S protein and rice bran oil), 5% connective tissue analog (FIG. 15 panel B). A ground beef prototype patty was made by combining 71% (wt/wt) muscle tissue analog (composed of 60% "white" muscle analog, 40% "dark" muscle analog), 23% fat tissue (from pea seed globulin proteins and canola oil) (FIG. 15 panel C). A ground beef prototype patty was made by combining 67% "White" muscle analog, with 28% fat tissue analog (from pea globulins and rice bran oil), (FIG. 15, panel D)~~

~~[0414] In a further test, the effect of cooking the ground beef replica patties was evaluated by grilling on a 350° F. pan. A ground beef patty analog was made by combining 62% (wt/wt) muscle tissue analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (FIG. 16). The panel on the left shows the patty before cooking and the panel on the right shows the same patty after cooking for about 2 minutes. Observers described the aroma of the cooking ground beef replica as distinctly "beefy".~~

~~SEQUENCE LISTING~~

~~<160> NUMBER OF SEQ ID NOS: 3~~

~~<210> SEQ ID NO 1~~
~~<211> LENGTH: 144~~
~~<212> TYPE: PRT~~
~~<213> ORGANISM: Glycine max~~

~~<400> SEQUENCE: 1~~

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~~20 25 30~~
~~Ser Ile Leu Glu Lys Ala Pro Ala Ala Lys Asp Leu Phe Ser Phe Leu~~
~~35 40 45~~
~~Ala Asn Gly Val Asp Pro Thr Asn Pro Lys Leu Thr Gly His Ala Glu~~
~~50 55 60~~
~~Lys Leu Phe Ala Leu Val Arg Asp Ser Ala Gly Gln Leu Lys Ala Ser~~
~~65 70 75 80~~
~~Gly Thr Val Val Ala Asp Ala Ala Leu Gly Ser Val His Ala Gln Lys~~
~~85 90 95~~
~~Ala Val Thr Asp Pro Gln Phe Val Val Val Lys Glu Ala Leu Leu Lys~~
~~100 105 110~~

~~1.-162. (canceled)~~

~~inued~~

1. ~~163. A meat substitute product,~~ A food product comprising:

~~Thr Ile Lys Ala Ala Val Gly Asp Lys Trp Ser Asp Glu Leu Ser Arg~~
~~115 120 125~~
Ala Trp Glu Val Ala Tyr Asp Glu Leu Ala Ala Ala Ile Lys Lys Ala
130 135 140

~~<210> SEQ ID NO 2 <211> LENGTH: 133 <212> TYPE: PRT~~

~~<213> ORGANISM: Methylacidiphilum infernorum <400> SEQUENCE: 2~~

~~Ser Trp Lys Arg~~
~~15~~

100
~~Figure 8~~

EXHIBIT 1C

~~a) a muscle replica;~~
~~b) a fat tissue replica; and~~
~~c) a connective tissue replica;~~
~~wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat.~~
~~164. The meat substitute product of claim 163, comprising two or more of said muscle replica, fat tissue replica, and connective tissue replica.~~
~~165. The meat substitute product of claim 163, wherein (i) said muscle replica accounts for 40-90% of said product by weight, (ii) said fat tissue replica accounts for 1-60% of said product by weight, and (iii) said connective tissue replica accounts for 1 -30% of said product by weight.~~

a) a highly conjugated heterocyclic ring complexed to iron ion; and

b) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, com oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone.

2. The food product of claim 1, wherein said highly conjugated heterocyclic ring complexed to an iron ion is selected from the group consisting of a heme moiety, and a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochorophyll, a corphin, a chlorophyllin, a bacteriochlorin, and an isobacteriochlorin moiety complexed to iron ion.

3. The food product of claim 2, wherein said heme moiety is a heme-containing protein, a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support.

~~Figure 8~~

4. ~~166.The meat substitute~~ The food product of claim ~~163~~2, ~~comprising:~~wherein said highly conjugated heterocyclic ring complexed to iron is ferrous chlorin.

5. A food product comprising:

a) a heme-containing protein; and

b) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a hydrolyzed protein, a malt extract, a yeast extract, and a peptone.

6. The food product of any one of claims 1-5, wherein said food product comprises two to fifty flavor precursors.

7. The food product of any one of claims 1-5, wherein said food product comprises two to forty flavor precursors.

8. The food product of any one of claims 1-5, wherein said food product comprises two to thirty-five flavor precursors.

9. The food product of any one of claims 1-5, wherein said food product comprises two to ten flavor precursors.

10. The food product of any one of claims 1-5, wherein said food product comprises two to six flavor precursors.

~~Figure 8~~

11. The food product of any one of claims 1-10, wherein said one or more flavor precursor molecules are selected from the group consisting of glucose, ribose, sucrose, maltose, cysteine, a cysteine derivative, thiamine, lysine, a lysine derivative, glutamic acid, a glutamic acid derivative, IMP, GMP, lactic acid, maltodextrin, creatine, alanine, arginine, asparagine, aspartate, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, linoleic acid, lecithin, and mixtures thereof.

12. The food product of any one of claims 3-11, wherein the heme-containing protein is a plant heme-containing protein.

13. The food product of any one of claims 3-11, wherein the heme-containing protein is a mammalian heme-containing protein.

14. The food product of any one of claims 3-11, wherein the heme-containing protein is a yeast or filamentous fungus heme-containing protein.

15. The food product of any one of claims 3-11, wherein the heme-containing protein is a bacterial or archeal heme-containing protein.

16. The food product of any one of claims 3-12, wherein the heme-containing protein is a non-symbiotic hemoglobin.

17. The food product of any one of claims 3-12, wherein the heme-containing protein is a leghemoglobin.

18. The food product of claim 17, wherein the leghemoglobin is a from soybean, alfalfa, lupin, fava bean, pea, cow pea, or lupine.

Figure 8

19. The food product of any one of claims 12-18, wherein the heme-containing protein is recombinantly produced, chemically synthesized, or transcribed and translated *in vitro*.

a) 60-90% water;
b) 5-30% protein content;
c) 1-20% of a fat or fat replica;
wherein said protein content comprises one or more isolated, purified plant proteins.
167. The meat substitute product of claim 166, wherein said protein content is derived from a non-animal source.
168. The meat substitute product of claim 167, wherein said non-animal source is a plant source, or a genetically modified yeast or bacteria.
169. (canceled)
170. The meat substitute product of claim 163, wherein 50% or more of said protein content by weight are one or more isolated purified proteins.
171. The meat substitute product of claim 170, wherein each of said one or more isolated proteins is isolated and purified separately from different plant species.
172. The meat substitute product of claim 170, wherein one or more of said isolated proteins is selected from the group consisting of: hemoglobin, myoglobin, chlorocruorin, eryth- rocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexoki- nase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phos- phoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-l,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-l,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglyci- nins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteino- plast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.
173. The meat substitute product of claim 163, further comprising one or more isolated and purified iron-containing proteins.
174. The meat substitute product of claim 173, wherein said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, and flavohemoglobins.

20. 175.The meat substitute composition of claim 174 wherein said iron-containing The food product of any one of claims 3-19, wherein the heme-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1.having at least 80% sequence identity to a polypeptide set forth in SEQ ID NOs:l-26.

21. The food product of any one of claims 1-20, wherein said heme-containing protein is isolated and purified.

22. The food product of any one of claim 1-21, further comprising a food-grade oil, a seasoning agent, a flavoring agent, a protein, a protein concentrate, an emulsifier, a gelling agent, or a fiber.

23. The food product of any one of claims 1-18, wherein said food product is a meat substitute.

Figure 8

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24. The food product of any one of claim 1-19, wherein said food product is a soup base, stew base, snack food, bouillon powder, bouillon cube, or a flavor packet.

25. The food product of any one of claims 1-20, wherein said food product is free of animal products.

26. The food product of any one of claims 1-21, wherein said food product is a frozen food product.

27. The food product of any one of claims 1-22, wherein said food product is sealed within a packet or shaker.

28. A method for producing a flavor compound, said method comprising:

a) combining a highly conjugated heterocyclic ring complexed to iron ion with one or more flavor precursor molecules to form a mixture, said one or more flavor precursor molecules selected from the group consisting of glucose, ribose, fructose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, cottonseed oil, soybean oil, com oil, palm fruit oil, palm seed oil, rice bran oil, safflower oil, olive oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and

b) heating the mixture to form one or more flavor compounds selected from the group consisting of phenylacetaldehyde, l-octen-3-one, 2-n-heptylfuran, 2-thiophenecarboxaldehyde, 3-thiophenecarboxaldehyde, butyrolactone, 2-undecenal,

methyl-pyrazine, furfural, 2-decanone, pyrrole, l-octen-3-ol, 2-acetylthiazole, 2-(E)-octenal, decanal, benzaldehyde, (E)-2-nonenal, pyrazine, 1-hexanol, 1-heptanol, dimethyl trisulfide, 2-nonanone, 2-pentanone, 2-heptanone, 2,3-butanedione, heptanal, nonanal, 2-octanone, 1-octanol, 3-ethylcyclopentanone, 3-octen-2-one, (E,E)-2,4-heptadienal, (Z)-2-heptenal, 6-methyl-2-heptanone, (Z)-4-heptenal, (E,Z)-2,6-nonadienal, 3-methyl-2-butenal, 2-pentyl-furan, thiazole, (E,E)-2,4-decadienal, hexanoic acid, l-ethyl-5-methylcyclopentene, (E,E)-2,4-nonadienal, (Z)-2-decenal, dihydro-5-pentyl-2(3H)-furanone, trans-3-nonen-2-one, (E,E)-3,5-octadien-2-one, (Z)-2-octen-l-ol, 5-ethyldihydro-2(3H)-furanone, 2-butenal, l-penten-3-ol, (E)-2-hexenal, formic acid, heptyl ester, 2-pentyl-thiophene, (Z)-2-nonenal, 2-hexyl-thiophene, (E)-2-decenal, 2- ethyl-5-methyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine, 2-ethyl-1-hexanol, thiophene, 2- methyl-furan, pyridine, butanal, 2-ethyl-furan, 3-methyl-, butanal, 2-methyl-, butanal, methacrolein, 2-methyl-propanal, propanal, acetaldehyde, 2-propyl-furan, dihydro-5-propyl-2(3H)-furanone, 1,3-hexadiene, 4-decyne, pentanal, 1-propanol, heptanoic acid, ethanethiol, 1-butanol, l-penten-3-one, dimethyl sulfide, 2-ethyl furan, 2-pentyl-thiophene, 2-Propenal, 2-Tridecen-l-ol, 4-Octene, 2-methyl thiazole,, methyl-pyrazine, 2-butanone, 2-pentyl-furan, 2-methyl-propanal, butyrolactone,, 3-methyl-butanal, methyl-thiirane, 2-hexyl-furan, butanal,, 2-methyl-butanal, trichloromethane, 2-methyl-furan, furan, octanal, 2-Heptenal, 1-Octene, heptyl ester formic acid, 3-pentyl-furan, and 4-penten-2-one.

29. The method of claim 28, wherein said highly conjugated heterocyclic ring complexed to an iron ion is selected from the group consisting of a heme moiety, and a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochorophyll, a corphin, a chlorophyllin, a bacteriochlorin, or an isobacteriochlorin moiety complexed to iron ion.

30. The method of claim 29, wherein said heme moiety is a heme-containing protein, a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support.

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31. The method of claim 30, wherein said heme moiety is a heme-containing protein.

32. The method of claim 29, wherein said highly conjugated heterocyclic ring complexed to an iron ion is ferrous chlorin.

33. The method of any one of claims 28-32, said method comprising combining cysteine, ribose, lactic acid, lysine, and/or thiamine with said highly conjugated heterocyclic ring complexed to iron.

34. A method for producing a flavor compound, said method comprising:

a) combining a heme-containing protein, cysteine, and a sugar, and one or more optional flavor precursor molecules to form a mixture, said one or more optional flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, cottonseed oil, soybean oil, com oil, palm fruit oil, palm seed oil, rice bran oil, safflower oil, olive oil, canola oil, coconut oil, mango oil, a free fatty acid, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and

b) heating the mixture to form one or more flavor compounds set forth in Table 3, 8,9, 12, 14, 16, or 17.

35. A method for imparting a meat-like flavor to a food product comprising contacting said food product with a flavoring composition, said flavoring composition comprising i) a heme-containing protein; and ii) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6- phosphate, fructose

EXHIBIT 1C

6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, cottonseed oil, soybean oil, com oil, palm fruit oil, palm seed oil, rice bran oil, safflower oil, olive oil, sunflower oil, canola oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a hydrolyzed protein, a malt extract, a yeast extract, and a peptone; wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product.

36. The method of claim 35, wherein said meat-like flavor is a beef-like, chicken-like, pork-like, lamb-like flavor, or turkey-like.

37. The method of claim 35 or claim 36, wherein said flavoring composition further comprises a seasoning agent, a flavoring agent, a protein, a protein concentrate, or an emulsifier.

38. The method of any one of claims 35-37, wherein said flavoring composition is sealed within a packet or shaker.

39. A method of making a food product, said method comprising combining an isolated heme-containing protein and one or more flavor precursor molecules to form a mixture, said one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, sunflower oil,

Figure 8

canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a hydrolyzed protein, a malt extract, a yeast extract, and a peptone; and heating the mixture.

40. A method for producing a flavor compound, said method comprising:

a) incubating a heme-moiety bound to a solid support with one or more flavor precursor molecules, said one or more flavor precursor molecules selected from the group consisting of cysteine, ribose, glucose, fructose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, cottonseed oil, soybean oil, com oil, palm fruit oil, palm seed oil, rice bran oil, safflower oil, olive oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and b) heating the heme-moiety bound to said solid support and one or more flavor precursors to form one or more flavor compounds selected from the group consisting of phenylacetaldehyde, l-octen-3-one, 2-n-heptylfuran, 2-thiophenecarboxaldehyde, 3-thiophenecarboxaldehyde, butyrolactone, 2-undecenal, methyl-pyrazine, 2-furfural, decanone, pyrrole, l-octen-3-ol, 2-acetylthiazole, (E)-2-octenal, decanal, benzaldehyde, (E)-2-nonenal, pyrazine, 1-hexanol, 1-heptanol, dimethyl trisulfide, 2-nonanone, 2-pentanone, 2-heptanone, 2,3-butanedione, heptanal, nonanal, 2-octanone, 1-octanol, 3-ethylcyclopentanone, 3-octen-2-one, (E,E)-2,4-heptadienal, (Z)-, 2-heptenal, 6-methyl-2-heptanone, (Z)-4-heptenal, (E,Z)-2,6-nonadienal, 3-methyl-2-butenal, 2-pentyl-furan,

Figure 8

thiazole, (E,E)-2,4-decadienal, hexanoic acid, 1-ethyl-5-methylcyclopentene, (E,E)-2,4-nonadienal, (Z)-2-decenal, dihydro-5-pentyl-2(3H)-furanone, trans-3-nonen-2-one, (E,E)-3,5-octadien-2-one, (Z)-2-octen-l-ol, 5-ethyldihydro-2(3H)-furanone, 2-butenal, 1- penten-3-ol, (E)-2-hexenal, formic acid, heptyl ester, 2-pentyl-thiophene, (Z)-2-nonenal, 2- hexyl-thiophene, (E)-2-decenal, 2-ethyl-5-methyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine, 2-ethyl-1-hexanol, thiophene, 2-methyl-furan, pyridine, butanal, 2-ethyl-furan, 3- methyl-butanal, 2-methyl-butanal, methacrolein, 2-methyl-propanal, propanal, acetaldehyde, 2-propyl-furan, dihydro-5-propyl-2(3H)-furanone, 1,3-hexadiene, 4-decyne, pentanal, 1-propanol, heptanoic acid, ethanethiol, 1-butanol, l-penten-3-one, dimethyl sulfide, 2-ethyl furan, 2-pentyl-thiophene, 2-propenal, 2-tridecen-l-ol, 4-octene, 2-methyl thiazole, methyl-pyrazine, 2-butanone, 2-pentyl-furan, 2-methyl-propanal, butyrolactone, 3-methyl-butanal, methyl-thiirane, 2-hexyl-furan, butanal,, 2-methyl-butanal, trichloromethane, 2-methyl-furan, furan, octanal, 2-heptenal, 1-octene, heptyl ester formic acid, 3-pentyl-furan, and 4-penten-2-one.

~~Figure 8~~

176. The meat substitute product of claim 163, wherein said meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arable, and no acacia gum.

177. The meat substitute product of claim 174, wherein said meat substitute product contains less than 1% wheat gluten.

178. The meat substitute product of claim 175, wherein said meat substitute product contains no wheat gluten.

179. The meat substitute product of claim 176, wherein said meat substitute product is characterized by one or more of the following contains no soy protein isolate, no soy protein concentrate, or no tofu.

180. (canceled)

181. (canceled)

182. The meat substitute product of claim 179, wherein said meat substitute product contains less than 5% carbohydrates.

183. (canceled)

184. The meat substitute product of claim 163, wherein said meat substitute product is characterized by one or more of the following: contains no tofu, no soy protein, contains less than 1% cellulose, contains less than 5% insoluble carbohydrates, or no wheat gluten.

185. (canceled)

186. The meat substitute product of claim 163, wherein said meat substitute product contains no animal products and less than 5% carbohydrates.

187. (canceled)

188. (canceled)

189. (canceled)

190. (canceled)

191. (canceled)

192. The meat substitute product of claim 163, wherein said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.

193-222. (canceled)

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(54) Title: METHODS AND COMPOSITIONS FOR CONSUMABLES

(57) Abstract: Methods and compositions for the production of non-meat consumable products are described herein. A meat substitute is described which is constructed from a muscle analog, a fat analog, and a connective tissue analog.

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METHODS AND COMPOSITIONS FOR CONSUMABLES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 13/941,211, filed July 12, 2013, U.S. Application Serial No. 61/908,634, filed November 25, 2013, and to U.S. Application Serial No. 61/751,816, filed January 11, 2013; and is related to the following co-pending patent applications: Application Serial No. PCT/US12/46560; Application Serial No PCT/US12/46552; Application Serial No. 61,876,676, filed September 11, 2013; Application Serial No. 61/751,818, filed January 11, 2013, and Application Serial No. 61/611,999, filed March 16, 2012, all of which are incorporated herein by reference.

TECHNICAL FIELD

This invention relates to consumable products and more particularly, to non-animal based replicas of animal-based food products, that can be produced, in some embodiments, by breaking down non-animal materials into their constituent parts and reassembling those parts into the consumables.

BACKGROUND

Animal farming has a profound negative environmental impact. Currently it is estimated that 30% of Earth's land surface is dedicated to animal farming and that livestock account for 20% of total terrestrial animal biomass. Due to this massive scale, animal farming accounts for more than 18% of net greenhouse gas emissions. Animal farming may be the largest human source of water pollution, and animal farming is by far the world's largest threat to biodiversity. It has been estimated that if the world's human population could shift from a meat containing diet to a diet free of animal products, 26% of Earth's land surface would be freed for other uses. Furthermore the shift to a vegetarian diet would massively reduce water and energy consumption.

The consumption of meat has a profound negative impact on human health. The health benefits of a vegetarian diet are well established. If the human population would shift to a more vegetarian diet, there would be a decrease in health care costs.

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Hunger is a worldwide problem, yet the world's 4 major commodity crops (soybeans, maize, wheat, and rice) already supply more than 100% of the human population's requirements for calories and protein, including every essential amino acid.

Plant based meat substitutes have largely failed to cause a shift to a vegetarian diet. The current state of the art for meat substitute compositions involves the extrusion of soy/grain mixture, resulting in products which largely fail to replicate the experience of cooking and eating meat. Common limitations of these products are a texture and mouthfeel that are more homogenous than that of equivalent meat products. Furthermore, as the products must largely be sold pre-cooked, with artificial flavors and aromas built in, they fail to replicate aromas, flavors, and other key features associated with cooking meat. As a result, these products appeal mainly to a limited consumer base that is already committed to vegetarianism/veganism, but have failed to appeal to the larger consumer segment accustomed to eating meat.

Food is any substance that is either eaten or drunk by any animal, including humans, for nutrition or pleasure. It is usually of plant or animal origin, and contains essential nutrients, such as carbohydrates, fats, proteins, vitamins, or minerals. The substance is ingested by an organism and assimilated by the organism's cells in an effort to produce energy, maintain life, or stimulate growth.

Food typically has its origin in a photosynthetic organism, typically from plants. Some food is obtained directly from plants; but even animals that are used as food sources are raised by feeding them food derived from plants. Edible fungi and bacteria are used to transform materials from plants or animals into other food products, mushrooms, bread, yogurt and the like.

In most cases, the plant or animal is fractionated into a variety of different portions, depending upon the purpose of the food. Often, certain portions of the plant, such as the seeds or fruits, are more highly prized by humans than others and these are selected for human consumption whilst other less desirable portions, such as the stalks of grasses, are typically used for feeding animals.

Animals are typically butchered into smaller cuts of meat with specific flavor and handling properties before consumption.

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While many foods can be eaten raw, many also undergo some form of preparation for reasons of safety, palatability, texture, or flavor. At the simplest level, this may involve washing, cutting, trimming, or adding other foods or ingredients. It may also involve mixing, heating or cooling or fermentation and individual foods may be combined with other food products to achieve the desired mix of properties.

In recent years, attempts have been made to bring scientific rigor to the process of food preparation, under the fields of food science and molecular gastronomy. Food science broadly studies the safety, microbiology, preservation, chemistry, engineering and physics of food preparation, whereas molecular gastronomy focuses on the use of scientific tools such as liquid nitrogen, emulsifying agents such as soy lecithin and gelling agents such as calcium alginates to transform food products into unexpected forms.

However, the raw material is typically an entire organism (plant or animal) or an isolated tissue such as a steak, the fruiting body of a fungus, or the seed of a plant. In some cases, the isolated tissue is modified before food preparation, such as making flour or isolating oils and bulk proteins from seeds.

Despite that fact that all of these items comprise a mixture of proteins, carbohydrates, fats, vitamins and minerals, the physical arrangement of these materials in the original plant or animal determines the use to which the plant or animal tissue will be put. Disclosed herein are improved methods and composition for the production of consumables.

SUMMARY

Provided herein are consumable products and methods of making the same. The consumables can be non-animal based consumable goods, e.g., containing mainly plant or entirely-plant based proteins and/or fats, and can be in the form of a beverage (e.g., an alcoholic beverage such as cream liquor, or a protein drink), a protein supplement, a baked good (e.g., a bread or a cookie), a condiment (e.g., a mayonnaise, a mustard), a meat product, or a meat substitute product (e.g., a ground beef product). For example, the protein drink can be a meal replacement beverage, a beer supplemented with the

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protein, or a distilled alcoholic beverage (e.g., vodka or rum) supplemented with the protein. The condiment can be mayonnaise. The meat product can be a pate, a sausage, or a meat substitute that can include a muscle replica, plant-based adipose and/or connective tissue. Coacervates that include one or more proteins can be used to help bind the ingredients to each other in the consumable products (e.g., a ground beef product).

Accordingly, provided herein is a consumable product comprising an isolated and purified plant protein, wherein the isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2°C and about 32°C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90°C and 110°C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM. In some embodiments, the consumable product is a beverage, a protein supplement, a baked good, a condiment, a meat product, or a meat substitute product. In some embodiments, the beverage is an alcoholic beverage or a protein drink. In some embodiments, the alcoholic beverage is a cream liquor. The cream liquor can further include a non-dairy lipid emulsion, where the cream liquor is free of animal products. In some embodiments, the protein drink is a meal replacement beverage, a beer supplemented with said protein, or a distilled alcoholic beverage supplemented with the protein. A condiment can be a mayonnaise replica. In some embodiments, the meat product can be a pate, a sausage replica, or a meat substitute. In some embodiments, the isolated and purified plant protein is at least 10 kDa in size. In some embodiments, the isolated and purified plant protein is not fully denatured. In some cases, the isolated and purified plant protein is not derived from soy. In some embodiments, the isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin.

In some embodiments, the isolated and purified plant protein comprises a dehydrin, a hydrophilin, an intrinsically disordered protein, or a protein identified based on its ability to stay soluble after boiling at a pH and a salt concentration comparable to a food. In some embodiments, the consumable product further comprises a plant derived

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lipid or a microbial-derived lipid. In some embodiments, the consumable product further includes a second isolated and purified protein, and/or a seasoning agent, a flavoring agent, an emulsifier, a gelling agent, a sugar, or a fiber.

The disclosure also provides a consumable product comprising a coacervate comprising one or more isolated and purified proteins. In some embodiments, the consumable product is a meat replica. In some embodiments, the consumable product further includes a plant derived lipid or a microbial-derived lipid. The plant derived lipid or microbial-derived lipid can comprise lecithin and/or an oil. The product can include up to about 1% lecithin by weight. The product can include lecithin and the oil. In some embodiments, the oil is canola oil, palm oil, or cocoa butter. The product can include about 1% to about 9% of the oil. The one or more isolated and purified proteins can comprise plant proteins. The one or more plant proteins can comprise one or more pea proteins, chickpea proteins, lentil proteins, lupine proteins, other legume proteins, or mixtures thereof. In some embodiments, the one or more pea proteins are legumins, vicilin, a convicilin, or a mixture thereof.

The disclosure also provides a meat replica comprising a muscle replica, a connective tissue replica, an adipose tissue replica, and a coacervate comprising one or more isolated and purified proteins. The coacervate further can comprise a plant-derived lipid or microbial-derived lipid. The plant derived lipid or microbial-derived lipid can be lecithin and/or an oil. The meat replica can be a ground beef replica.

Also provided is a consumable product comprising a comprising a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt. In some embodiments, the isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin. In some embodiments, the isolated and purified plant protein comprises a dehydrin, a hydrophilin, or an intrinsically disordered protein. In some embodiments, the cold set gel further comprises a plant derived lipid or microbial derived lipid. In some embodiments, the plant derived lipid or microbial derived lipid is lecithin and/or oil.

The disclosure further provides an adipose tissue replica comprising one or more isolated plant proteins, one or more plant or algal derived oils, and optionally a

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phospholipid. In some embodiments, the phospholipid is lecithin. In some embodiments, the plant based oils are selected from the group consisting of corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, and combinations thereof. In some embodiments, the fat release temperature of the adipose tissue replica is between 23°C to 33°C, 34°C to 44°C, 45°C to 55°C, 56°C to 66°C, 67°C to 77°C, 78°C to 88°C, 89°C to 99°C, 100°C to 110°C, 111°C to 121°C, 122°C to 132°C, 133°C to 143°C, 144°C to 154°C, 155°C to 165°C, 166°C to 167°C, 168°C to 169°C, 170°C to 180°C, 181°C to 191°C, 192°C to 202°C, 203°C to 213°C, 214°C to 224°C, 225°C to 235°C, 236°C to 246°C, 247°C to 257°C, 258°C to 268°C, 269°C to 279°C, 280°C to 290°C, or 291°C to 301°C. In some embodiments, the percent fat release of the adipose tissue replica is 0 to 10%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, 50% to 60%, 60% to 70%, 70% to 80%, 80% to 90%, or 90% to 100% upon cooking. In some embodiments, the isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin.

In some embodiments, the adipose tissue replica comprises from about 40% to about 90% of the oil. In some embodiments, the adipose tissue replica comprises from about 1% to about 6% of the isolated and purified plant protein. In some embodiments, the adipose tissue replica comprises from about 0.05 to about 2% of the phospholipid. In some embodiments, the firmness of the adipose tissue replica is similar to that of beef adipose tissue.

Also provided is a consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, wherein the consumable product does not comprise meat. In some embodiments, the heme-containing protein accounts for at least 0.01% of the composition. In some embodiments, the consumable product further comprises one or more ammonium, sodium, potassium, or calcium salts. In some embodiments, the isolated and purified proteins are crosslinked.

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Further provided is a consumable product comprising a gelled emulsion, wherein the gelled emulsion comprises:

a) an isolated and purified protein;

b) a first lipid that when not in the consumable product is solid at a selected temperature range; and

c) a second lipid that when not in the consumable product is liquid at the selected temperature range; wherein the melting temperature of the mixture of the first and second lipids is similar to the melting temperature of lipids found in meat, and wherein the first and second lipids are plant derived lipids or microbial derived lipids.

The disclosure also provides a method for making a consumable product comprising:

a) preparing a solution comprising an isolated and purified plant protein, wherein the isolated and purified plant protein has (i) a solubility in the solution of at least 25 at a temperature between about 2°C and 32°C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in the solution of at least 1 mg/ml at a temperature of between 90°C and 110°C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM; and

b) adding the solution to a beverage.

In some embodiments, the solution comprises two or more isolated and purified plant proteins. In some embodiments, the beverage is clear. In some embodiments, the isolated and purified plant protein is at a concentration of at least 1% by weight in the solution. In some embodiments, the isolated and purified plant protein is selected from the group consisting of RuBisCo, a moong globulin, a soy globulin, a pea globulin, a pea albumin, a prolamin, a lentil protein, a dehydrin, a hydrophilin, and an intrinsically disordered protein. In some embodiments, the isolated and purified plant protein is lyophilized prior to making said solution. In some embodiments, the beverage has an improved mouthfeel compared to a corresponding beverage without the isolated and purified protein.

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Also provided is a method for extending the shelf-life of a meat-free consumable product, the method comprising adding a heme-containing protein to the consumable product, wherein the heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions. In some embodiments, the heme-containing protein comprises an amino acid sequence with at least 70% homology to an amino acid sequence set forth in any one of SEQ ID NOs: 1-27.

Further provided is a method for making a meat replica comprising a cold set gel, wherein the method includes:

a) denaturing a solution comprising at least one isolated and purified protein from a non-animal source under conditions wherein the isolated and purified protein does not precipitate out of the solution;

b) optionally adding any heat-labile components to the solution of denatured protein;

c) gelling the solution of denatured protein at about 4°C to about 25°C by increasing the ionic strength of the solution to form a cold set gel; and

d) incorporating the cold set gel into a meat replica.

In some embodiments, the gelling is induced using 5 to 100 mM sodium or calcium chloride. In some embodiments, the heat-labile components are proteins or lipids, or mixtures thereof. In some embodiments, the protein is a heme-containing protein. In some embodiments, the cold set gel is formed in a matrix comprising a freeze-aligned plant protein.

In some embodiments, the isolated and purified protein from a non-animal source is a plant protein. In some embodiments, the plant protein is selected from the group consisting of RuBisCo, a moong globulin, a soy globulin, a pea globulin, a pea albumin, a prolamin, a lentil protein, a dehydrin, a hydrophilin, and an intrinsically disordered protein.

Further provided is an adipose tissue replica, comprising

a) an isolated and purified non-animal protein;

b) a non-animal lipid; and

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c. a three-dimensional matrix comprising fibers derived from non-animal sources, wherein the lipid and the protein are dispersed in the three-dimensional matrix, and wherein the three-dimensional matrix stabilizes the structure of the adipose tissue replica.

Also provided is a connective tissue replica comprising one or more isolated and purified proteins assembled into fibrous structures by a solution spinning process. In some embodiments, the fibrous structures are stabilized by a cross-linking agent.

Provided herein is a method for imparting a beef like flavor to a consumable product, comprising adding to the consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to the consumable composition.

Also provided is a method for making a poultry or a fish composition taste like beef, the method comprising adding a heme protein to the poultry or fish composition, respectively.

In some embodiments, the heme-containing protein has an amino acid sequence with at least 70% homology to any one of the amino acid sequences set forth in SEQ ID NOs: 1-27.

Further provided is a method of making a coacervate, the method comprising

a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, wherein the solution comprises 100 mM or less of sodium chloride; and

b) isolating the coacervate from the solution. In some embodiments, the pH is between 4 and 5. In some embodiments, the plant proteins comprise one or more pea proteins, chickpea proteins, lentil proteins, lupine proteins, other legume proteins, or mixtures thereof. In some embodiments, the pea proteins comprise isolated and purified legumins, isolated and purified vicilins, isolated and purified convicilins, or combinations thereof. In some embodiments, the isolated and purified pea proteins comprise isolated and purified vicilins and isolated and purified convicilins. In some embodiments, the acidifying step is done in the presence of a plant derived lipid or microbial derived lipid. In some embodiments, the plant derived lipid or microbial derived lipid comprises oils and/or phospholipids.

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Provided herein is a method of making an adipose tissue replica, the method comprising forming an emulsion comprising one or more isolated plant proteins, one or more plant or algal derived oils, and optionally a phospholipid. In some embodiments, when the phosoholipid is included, it is lecithin. In some embodiments, the plant based oils are selected from the group consisting of corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, and combinations thereof. In some embodiments, the fat release temperature of the adipose tissue replica is between 23°C to 33°C, 34°C to 44°C, 45°C to 55°C, 56°C to 66°C, 67°C to 77°C, 78°C to 88°C, 89°C to 99°C, 100°C to 110°C, 111°C to 121°C, 122°C to 132°C, 133°C to 143°C, 144°C to 154°C, 155°C to 165°C, 166°C to 167°C, 168°C to 169°C, 170°C to 180°C, 181°C to 191°C, 192°C to 202°C, 203°C to 213°C, 214°C to 224°C, 225°C to 235°C, 236°C to 246°C, 247°C to 257°C, 258°C to 268°C, 269°C to 279°C, 280°C to 290°C, or 291°C to 301°C. In some embodiments, the percent fat release of the adipose tissue replica is 0 to 10%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, 50% to 60%, 60% to 70%, 70% to 80%, 80% to 90%, or 90% to 100% upon cooking. In some embodiments, the isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin. In some embodiments, the emulsion comprises from about 40% to about 90% of the oil. In some embodiments, the emulsion comprises from about 1% to about 4% of the isolated and purified plant protein. In some embodiments, the adipose tissue replica comprises from about 0.05 to about 1% of the phospholipid. In some embodiments, the emulsion is formed by high-pressure homogenization, sonication, or hand homogenization.

Further provided is a method of minimizing undesirable odors or flavors in a composition comprising plant proteins, the method comprising contacting the composition with a ligand having affinity for one or more lipoxygenases.

Also provided is a method of minimizing undesirable odors or flavors in a composition comprising plant proteins, the method comprising contacting the

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composition with activated carbon then removing the activated carbon from the composition.

Also provided is a method of minimizing undesirable odors or flavors in a composition comprising plant proteins, the method comprising adding a lipoxygenase inhibitor and/or an antioxidant to the composition.

The disclosure further provides a chocolate flavored spread comprising:

a) sugar

b) a chocolate flavoring, and

c) a cream fraction from a plant based milk.

Provided herein is a method for altering the texture of a consumable during or after cooking comprising incorporating within the consumable one or more plant proteins with a low denaturation temperature. In some embodiments, at least one of the one or more plant proteins is isolated and purified. In some embodiments, the one or more plant proteins are selected from the group consisting of rubisco, pea proteins, lentil proteins, or other legume proteins. In some embodiments, the pea proteins comprise pea albumin proteins. In some embodiments, the consumable becomes firmer during or after cooking.

Also provided is a tissue replica, which comprises a freeze-aligned non-animal protein. In some embodiments, the non-animal protein is a plant protein. In some embodiments the non-animal protein is isolated and purified. In some embodiments the tissue replica is amuscle tissue replica.

The disclosure also provides a meat replica which includes a tissue replica comprising a freeze-aligned non-animal protein.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

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The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims. The word "comprising" in the claims may be replaced by "consisting essentially of" or with "consisting of," according to standard practice in patent law.

DESCRIPTION OF THE DRAWING

FIG. 1 contains amino acid sequences of exemplary heme-containing proteins.

FIG. 2A is a bar graph depicting the percent fat release based on the amount of lecithin.

FIG. 2B is a bar graph depicting the temperature of the fat release based on the amount of lecithin.

FIG. 2C is a bar graph depicting the firmness of adipose replicas based on the amount of lecithin.

FIG. 3 is a bar graph depicting the percent fat release of adipose replicas containing different oils (canola oil, cocoa butter, coconut oil, or rice bran oil).

FIG. 4 is a bar graph depicting the fat release temperature of adipose replicas containing different oils (canola oil, cocoa butter, coconut oil, or rice bran oil).

DETAILED DESCRIPTION

I. Consumables

Methods and compositions for producing consumables are described herein. In some cases, the consumables are non-animal based replicas of animal-based food products that can be produced by breaking down non-animal materials into their constituent parts and reassembling those parts into the consumables. In certain instances, the consumables are not intended to replicate an animal-based food and instead have their own unique characteristics desirable as a food. Additionally, the consumables may, in some instances, act as nutraceuticals or carriers for pharmaceutical compositions rather than serving a primary function as food.

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The advantages of the consumables described herein can include, for example, the using less energy or water in the production of the consumable compared to similar food products, using no animals in the production of the consumable, making a healthier product, using raw materials that would otherwise be discarded, or allowing for the elimination (or lack of incorporation) of certain components (e.g., allergens) from the consumables. The consumables also may have a higher degree of production consistency, allowing for improved quality control of the products. Another advantage is that the consumables can be designed intentionally to have desirable characteristics for food preparation that are superior to traditional food products.

The consumables can be for animal consumption, including human consumption. The consumables can be food for domestic animals (e.g., dog food could be produced according to the present inventions) or wild animals (e.g., food for non-domesticated predatory animals).

The consumables can be sold in grocery stores, convenience stores, mass merchandisers, and club stores or prepared in restaurants, including fast food restaurants, schools, event locations, hospitals, military facilities, prisons, shelters, or long-term care facilities, similar to already existing human foods.

The consumable can be approved by suitable regulatory authorities. For example, the consumable could be prepared to be suitable for the U.S. Food and Drug Administration. Methods of the invention can include steps necessary to satisfy regulatory agencies.

The consumables of the present invention can replicate, compete with, supplement or replace conventional food products (herein referred to as "food products"). Food products can be any foods which presently exist. The consumables of the invention can be made to replicate the food products, e.g., an equivalent meat product. The equivalent meat product can be a white meat or a dark meat. The equivalent meat product can be derived from any animal. Non-limiting examples of animals used to derive the equivalent meat product include farmed animals such as, e.g., cattle, sheep, pig, chicken, turkey, goose, duck, horse, dog or game animals (whether wild or farmed) such as, e.g., rabbit, deer, bison, buffalo, boar, snake, pheasant, quail, bear, elk, antelope,

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pigeon, dove, grouse, fox, wild pig, goat, kangaroo, emu, alligator, crocodile, turtle, groundhog, marmot, possum, partridge, squirrel, raccoon, whale, seal, ostrich, capybara, nutria, guinea pig, rat, mice, vole, any variety of insect or other arthropod, or seafood such as, e.g., fish, crab, lobster, oyster, muscle, scallop, abalone, squid, octopus, sea urchin, tunicate and others.

Many meat products are typically derived from skeletal muscle of an animal but it is understood that meat can also come from other muscles or organs of the animal. In some embodiments, the equivalent meat product is a cut of meat derived from skeletal muscle. In other embodiments, the equivalent meat product is an organ such as, e.g., kidney, heart, liver, gallbladder, intestine, stomach, bone marrow, brain, thymus, lung, or tongue. Accordingly, in some embodiments, the compositions of the present invention are consumables similar to skeletal muscle or organs.

A consumable (e.g., a meat substitute) can comprise one or more of a first composition comprising a muscle tissue replica, a second composition comprising an adipose tissue replica, and/or a third composition comprising a connective tissue replica, wherein the one or more compositions are combined in a manner that recapitulates the physical organization of meat. The present invention also provides distinct compositions for a muscle tissue replica (herein referred to as "muscle replica"), an adipose tissue replica (herein referred to as an "adipose replica" or "fat replica"), and a connective tissue replica (herein referred to as "connective tissue replica"). In some embodiments, these compositions are principally or entirely composed of ingredients derived from non-animal sources (e.g., 10% or less of the ingredients are from animal sources). In alternative embodiments, the muscle, fat, and/or connective tissue replica, or the meat substitute products comprising one or more of the replicas, are partially derived from animal sources but supplemented with ingredients derived from non-animal sources. In some embodiments, as much as 90% of the food product is derived from animal sources. In some embodiments about 75% of the food product is derived from animal sources. In some embodiments, about 50% of the food product is derived from animal sources. In some embodiments about 10% of the food product is derived from animal sources. In yet other alternative embodiments, the invention provides meat products substantially

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derived from animal sources (e.g., a beef, chicken, turkey, or a pork product) that are supplemented with one or more of a muscle tissue replica, a fat replica, and/or a connective tissue replica, wherein the replicas are derived substantially or entirely from non-animal sources. A non-limiting example of such a meat product is an ultra-lean ground beef product supplemented with a non-animal derived fat replica which improves texture and mouthfeel while preserving the health benefits of a consumable low in animal fat. Such alternative embodiments can result in products with properties that more closely recapitulate key features associated with preparing and consuming meat but which are less costly and associated with a lesser environmental impact, less animal welfare impact, or improved health benefits for the consumer.

Examples of other food products which the consumable can replicate or replace include: beverages (e.g., cream liquor or milk), protein drinks (e.g., RuBisCo can be used as a protein supplement in beer, distilled alcohol beverages such as vodka, fruit juices, meal replacement beverages, or water), pastes (e.g. Nutella™, cream, nacho cheese or mayonnaise replicas), pate, blood sausage, meat extenders, eggs, fish, sausage, tenders , spam or chilled foods (e.g., ice cream , yogurt, kefir, sour cream or butter replicas).

The consumables can be a meat replica. The consumables can be made to mimic the cut or appearance of meat. For instance, a consumable may be visually similar to or indistinguishable from ground beef or a particular cut of beef. In an example embodiment, the replicas are combined in a manner that approximates the physical organization of natural ground meat (e.g., ground beef, ground chicken, or ground turkey). In other embodiments, the replicas are combined in a manner that approximates different cuts of beef, such as, e.g., rib-eye, filet mignon, London broil, among others. Alternatively, the consumables can be a made with a unique look or appearance. For instance, the consumable could contain patterns (e.g., lettering or pictures) that are formed from the structure of the consumable. In some instances, the consumables look like traditional food products after they are prepared. For example, a consumable may be produced which is larger than a traditional cut of beef but which, after the consumable is sliced and cooked, appears the same as a traditional cooked meet. In some embodiments the consumable may resemble a traditional food product shape in two dimensions, but not

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in a third. For example the consumable may resemble a cut of meat in two dimensions (for example when viewed from the top), but may be much longer (or thicker) than the traditional cut. In this example the composition can be cut repeatedly into traditionally meat shaped products.

The consumables can be made from local products. For instance the consumables can be made from plants grown within a certain radius of the eventual consumer. That radius could be 1, 10, 100, or 1000 miles for example. Thus, in some embodiments, the invention provides method for producing a consumable which does not contain products which have been shipped over 1, 10, 100, or 1000 miles.

The present invention provides methods for producing consistent properties from the consumables when they are produced from various sources. For example, a plant based meat replica produced from local plants in Iowa, USA, will have substantially similar taste, odor, and texture as a plant based meat replica produced from local plants in Lorraine, France. This consistency allows for methods for advertising locally grown foods with consistent properties. The consistency can arise from the concentration or purification of similar components at different locations. These components can be combined in predetermined ratios to insure consistency. In some embodiments, a high degree of characteristic consistency is possible using components (e.g. isolated or concentrated proteins and fats) which come from the same plant species. In some embodiments, a high degree of characteristic consistency is possible using components (e.g., isolated or concentrated proteins and fats) which come from the different plant species. In some embodiments, the same proteins can be isolated from different plant species (i.e. homologous proteins). In some embodiments, the invention provides for a method comprising isolating similar plant constituents from plant sources in different locations, assembling in both locations compositions provided herein, and selling the compositions, wherein the compositions assembled and sold at different the geographic locations have consistent physical and chemical properties. In some embodiments, the isolated constituents are from different plant populations in different locations. In some embodiments one or more of the isolated constituents are shipped to the separate geographic locations.

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The consumables may require fewer resources to produce than consumables produced from domesticated animals. Accordingly, the present invention provides for meat replicates which require less water or energy to produce than meat. For example a consumable described herein can require less than about 10, 50, 100, 200, 300, 500, or 1000 gallons of water per pound of consumable. For comparison producing beef can require over 2000 gallons of water per pound of meat.

The consumable may require less land area to produce than a meat product with similar protein content. For example, a consumable described herein may require 30% or less of the land area required to produce a meat product with similar protein content.

The consumable may have health benefits compared to an animal product it replaces in the diet. For example it may have less cholesterol or lower levels of saturated fats than comparable meat products. The American Heart Association and the National Cholesterol Education Program recommends limiting cholesterol intake from food to 300 mg per day, which is equivalent to the consumption of 12 ounces of beef or two egg yolks. Consumables described herein that are indistinguishable to animal products such as ground beef, and having a reduced cholesterol content or no cholesterol, can help maintain a low cholesterol diet. In another example, a consumable described herein may contain no cholesterol, or higher levels of poly-unsaturated fatty acids compared to the animal product it replaces.

The consumable may have animal welfare benefits compared to an animal product it replaces in the diet. For instance, it may be produced without requiring confinement, forced feeding, premature weaning, disruption of maternal-offspring interactions, or slaughter of animals for their meat.

The consumable may have a smaller "carbon footprint" than the meat products they replace. For example the consumable may result in net greenhouse gas emissions of 1%, 5%, 10%, 25%, 50% or 75% of the greenhouse gas emissions attributable to the animal product it replaces. By way of example, according to the Environmental Working Group (2011) "meat eaters guide to Climate Change and Health," the production of beef causes the emission of 27 kg equivalents of carbon dioxide per kilogram of beef that is

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consumed, and the production of lamb causes the emission of 39 kg equivalents of carbon dioxide per kilogram of beef that is consumed.

The consumable described herein may provide alternatives to animal products or combinations of animal products whose consumption is forbidden by religious beliefs. For example, the consumable may be a kosher replica pork chop.

The consumable can also be shipped in components and produced or assembled at a different location. When available, local components can be used for production of the consumable. The local components can be supplemented with components that are not locally available. This allows for methods of producing consumables, for instance meat replicates, using less energy in shipment than is required for meat. For example, local water can be used in combination with a kit which provides other components of the consumable. Using local water reduces shipping weight, thereby reducing cost and environmental impact.

The consumables described herein can be produced or assembled wholly or in part in areas where animal farming is not practical or is not allowed. The consumable can be produced or assembled within an urban environment. For example, a kit may be provided to a user to enable the user to produce the consumable. The user could use local water or use plants from a rooftop garden, for instance in Shanghai. In another example, the consumables could be produced aboard a space craft, space station, or lunar base. Accordingly, the present invention provides methods and systems for the production of meat replicas for use in space travel or for training for the same. For instance the present invention could be used in earth based training for space travel. The consumables could also be produced on an island or upon a manmade platform at sea where the keeping of livestock is difficult or prohibited.

II. Properties of the Consumable

The consumables described herein are typically designed to replicate the experience of eating a food product, e.g., meat. The look, texture, and taste of the consumable can be such that it is similar to, or indistinguishable from, a food product, e.g., meat. The consumable can also be produced to have the desirable characteristics of

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food products without incorporating other undesirable characteristics. For example, a consumable can be a replica steak that does not have gristle or other components not typically consumed in a predicate food product.

The invention provides, in certain embodiments, methods for determining the suitability for a consumable to qualify as a replica of a food product, for example, by determining whether an animal or human can distinguish the consumable from a predicate food product, e.g., a particular meat. One method to determine whether the consumable is comparable to a food product (e.g. meat) is to a) define the properties of meat and b) determine whether the consumable has similar properties.

Properties that can be tested or used to compare or describe a food product or the consumable include mechanical properties such as hardness, cohesiveness, brittleness, chewiness, gumminess, viscosity, elasticity, and adhesiveness. Properties of food products that can be tested also include geometric properties such as particle size and shape, and particle shape and orientation. The three dimensional organization of particles may also be tested. Additional properties can include moisture content and fat content. These properties can be described using terms such as "soft," "firm" or "hard" describe hardness; "crumbly," "crunchy," "brittle," "chewy," "tender," "tough," "short," "mealy," "pasty," or "gummy," to describe cohesiveness; "thin" or "viscous" to describe viscosity; "plastic" or "elastic" to describe elasticity; "sticky," "tacky" or "gooey" to describe adhesiveness; "gritty," "grainy" or "course" to describe particle shape and size; "fibrous," "cellular" or "crystalline" to describe particle shape and orientation, "dry," "moist," "wet," or "watery" to describe moisture content; or "oily" or "greasy" to describe fat content. Accordingly, in one embodiment, a group of people can be asked to rate a certain food product, for instance ground beef, according to properties which describe the food product. A consumable described herein can be rated by the same people to determine equivalence.

Flavor of the food product can also be assessed. Flavors can be rated according to similarity to food products, e.g., "eggy," "fishy," "buttery," "chocolaty," "fruity", "peppery," "baconlike," "creamy," "milky," "or "beefy." Flavors can be rated according to the seven basic tastes, i.e., sweet, sour, bitter, salty, umami (savory), pungent (or

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piquant), and metallic. Flavors can be described according to the similarity to an experience caused by a chemical, e.g., diacetyl (buttery), 3-hydroxy-2 butanone (buttery), nona-2E-enal (fatty), 1-octene-3-ol (mushroom), hexanoic acid (sweaty), 4-hydroxy-5-methyl furanone (HMF, meaty), pyrazines (nutty), bis(2-methyl-3-furyl) disulfide (roast meat), decanone (musty/fruity), isoamyl acetate (banana), benzaldehyde (bitter almond), cinnamic aldehyde (cinnamon), ethyl propionate (fruity), methyl anthranilate (grape), limonene (orange), ethyl decadienoate (pear), allyl hexanoate (pineapple), ethyl maltol (sugar, cotton candy), ethylvanillin (vanilla), butanoic acid (rancid), 12-methyltridecanal (beefy), or methyl salicylate (wintergreen). These ratings can be used as an indication of the properties of the food product. The consumables of the present invention can then be compared to the food product to determine how similar the consumable is to the food product. In some instances the properties of the consumables are then altered to make the consumable more similar to the food product. Accordingly, in some embodiments, the consumable is rated similar to a food product according to human evaluation. In some embodiments the consumable is indistinguishable from real meat to a human.

The consumables can be made to eliminate properties associated with the source of the components of the consumables. For example a consumable can be made from components obtained from beans but can be made to lack a "beany" flavor or texture. One way this can be achieved is by breaking down the component source materials into isolated and purified components and not using components that cause undesired characteristic properties of the source. In addition, as described herein, off-flavors or aromas (e.g., undesirable flavors or aromas) in isolated and/or purified components can be minimized by deodorizing with activated charcoal or by removing enzymes such as lipoxygenases (LOX), which can be present in trace amounts and which can convert unsaturated triacylglycerides (such as linoleic acid or linolenic acid) into smaller and more volatile molecules. LOX are naturally present in legumes such as peas, soybeans, and peanuts, as well as rice, potatoes, and olives. When legume flours are fractionated into separate protein fractions, LOX can act as undesirable "time-bombs" that can cause undesirable flavors or aromas on aging or storage. As shown in Example 34, compositions containing plant proteins (e.g., from ground plant seeds) can be subjected to

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purification to remove LOX using, for example, an affinity resin that binds to LOX and removes it from the protein sample. The affinity resin can be linoleic acid, linolenic acid, stearic acid, oleic acid, propyl gallate, or epigalloccatechin gallate attached to a solid support such as a bead or resin. See, e.g., WO2013138793. In addition, depending on the protein component, certain combinations of antioxidants and/or LOX inhibitors can be used as effective agents to minimize off-flavor or off-odor generation in protein solutions, especially in the presence of fats and oils. Such compounds can include, for example, one or more of β-carotene, α-tocopherol, caffeic acid, propyl gallate, or epigallocatechin gallate. These may be included during purification of the proteins or during subsequent food processing steps to mitigate generation of off-flavors or off-odors in protein-based foods.

In some compositions, subjects asked to identify the consumable identify it as a form of a food product, or as a particular food product, e.g., a subject will identify the consumable as meat. For example, in some compositions a human will identify the consumable as having properties equivalent to meat. In some embodiments one or more properties of the consumable are equivalent to the corresponding properties of meat according to a human's perception. Such properties include the properties that can be tested. In some embodiments a human identifies a consumable of the present invention as more meat like than any meat substitutes found in the art.

Experiments can demonstrate that a consumable is acceptable to consumers. A panel can be used to screen a variety of consumables described herein. A number of human panelists can test multiple consumable samples, namely, natural meats vs. the consumable compositions described herein, or a meat substitute vs. a consumable composition described herein. Variables such as fat content can be standardized, for example to 20% fat using lean and fat meat mixes. Fat content can be determined using the Babcock for meat method (S. S. Nielson, Introduction to the Chemical Analysis of Foods (Jones & Bartlett Publishers, Boston, 1994)). Mixtures of ground beef and consumables of the invention prepared according to the procedure described herein can be formulated.

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Panelists can be served samples (e.g., in booths), under red lights or under white light, in an open consumer panel. Samples can be assigned random three-digit numbers and rotated in ballot position to prevent bias. Panelists can be asked to evaluate samples for tenderness, juiciness, texture, flavor, and overall acceptability using a hedonic scale from 1=dislike extremely, to 9=like extremely, with a median of 5=neither like nor dislike. Panelists can be encouraged to rinse their mouths with water between samples, and given opportunity to comment on each sample.

The results of this experiment can indicate significant differences or similarities between the traditional meats and the compositions of the invention.

These results can demonstrate that the compositions described herein are judged as acceptably equivalent to real meat products. Additionally, these results can demonstrate that compositions described herein are preferred by panelist over other commercially available meat substitutes. Thus, in some embodiments the present invention provides for consumables that are similar to traditional meats and are more meat like than previously known meat alternatives.

Consumables of the invention can also have similar physical characteristics as food products, e.g., traditional meat. In one embodiment, the force required to pierce a 1 inch thick structure (e.g., a patty) made of a consumable of the invention with a fixed diameter steel rod is not significantly different than the force required to pierce a 1 inch thick similar food product structure (e.g., a ground beef patty) with a similar fixed diameter steel rod. Accordingly, the invention provides for consumables with similar physical strength characteristics to meat. In another embodiment, the force required to tear a sample of the invention with a cross-sectional area of 100 mm^2 is not significantly different than the force required to tear a sample of animal tissue (muscle, fat or connective tissue) with a cross-sectional area 100 mm^2 measured the same way. Force can be measured using, for example, TA.XT Plus Texture Analyzer (Textrue Technologies Corp.). Accordingly, the invention provides for consumables with similar physical strength characteristics to meat.

Consumables described herein can have a similar cook loss characteristic as a food product, e.g., meat. For example a consumable can have a similar fat and protein

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content to ground beef and have the same reduction in size when cooked as real ground beef. Similarities in size loss profiles can be achieved for various compositions of consumables described herein matched to various meats. The cook loss characteristics of the consumable also can be engineered to be superior to food products. For example a consumable can be produced that has less loss during cooking but achieves similar tastes and texture qualities as the cooked products. One way this is achieved is by altering the proportions of lipids based on melting temperatures in the consumable composition. Another way this is achieved is by altering the protein composition of the consumable by controlling the concentration of protein or by the mechanism by which the tissue replica is formed.

In some embodiments, the consumable is compared to an animal based food product (e.g., meat) based upon olfactometer readings. In various embodiments the olfactometer can be used to assess odor concentration and odor thresholds, or odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of appreciation, or relative intensity of odors. In some embodiments, the olfactometer allows the training and automatic evaluation of expert panels. So in some embodiments the consumable is a product that causes similar or identical olfactometer readings. In some embodiments the differences are sufficiently small to be below the detection threshold of human perception.

Gas chromatography–mass spectrometry (GCMS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to separate and identify different substances within a test sample. GCMS can, in some embodiments, be used to evaluate the properties of a consumable. For example volatile chemicals can be isolated from the head space around meat. These chemicals can be identified using GCMS. A profile of the volatile chemicals in the headspace around meat is thereby created. In some instances each peak of the GCMS can be further evaluated. For instance, a human could rate the experience of smelling the chemical responsible for a certain peak. This information could be used to further refine the profile. GCMS could then be used to evaluate the properties of the consumable. The GCMS profile can be used to refine the consumable.

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Characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions molecules including amino acids, fats and sugars which are found in plants as well as meat. Therefore, in some embodiments, the consumable is tested for similarity to meat during or after cooking. In some embodiments human ratings, human evaluation, olfactometer readings, or GCMS measurements, or combinations thereof, are used to create an olfactory map of cooked meat. Similarly, an olfactory map of the consumable, for instance a meat replica, can be created. These maps can be compared to assess how similar the cooked consumable is to meat. In some embodiments the olfactory map of the consumable during or after cooking is similar to or indistinguishable from that of cooked or cooking meat. In some embodiments the similarity is sufficient to be beyond the detection threshold of human perception. The consumable may be created so its characteristics are similar to a food product after cooking, but the uncooked consumable may be have properties that are different from the predicate food product prior to cooking.

Shelf life is the length of time that consumables are given before they are considered unsuitable for sale, use, or consumption. Generally, it is important to maintain a meat product at about 2°C as the shelf life decreases with exposure to higher temperatures.

The shelf life of meat is determined through research into the meat products' sensory cues over time (odor, visual appearance of the package, color, taste and texture), and through laboratory analysis under controlled conditions to determine how long a product remains safe, wholesome and enjoyable. Ground beef is being used as an example, but similar conditions would apply to steaks, chops and roasts from other meat types. Beef in its natural state is dark bluish-purple. However, oxygen can permeate into the meat and cause a chemical reaction with the myoglobin in meat, leading to a red color. Ongoing exposure to oxygen causes oxidation of myoglobin and causes red meat to become brown and develop "off" flavors. To control this oxidation, there has been significant research into different methods of storing and displaying meat products to increase the shelf life of meat products. These include the use of vacuum packing,

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modified atmosphere packing (high oxygen), modified atmosphere packaging (low oxygen with carbon monoxide), and/or High Pressure Pasteurization (HPP).

The main determinant of the color of meat is the concentration of iron carrying proteins in the meat. In the skeletal muscle component of meat products, one of the main iron-carrying proteins is myoglobin. It is estimated that the white meat of chicken has under 0.05% myoglobin; pork and veal have 0.1-0.3% myoglobin; young beef has 0.4-1.0% myoglobin; and old beef has 1.5-2.0% myoglobin. Normally, myoglobin in meat exists in three states: Oxymyoglobin (Fe^{2+}) (oxygenated = bright red); myoglobin (Fe^{2+}) (non-oxygenated = purplish/magenta); and metmyoglobin (Fe^{3+}) (oxidized = brown). The transition of oxymyoglobin to metmyoglobin in the presence of oxygen is thought to be the cause of the color change of ground meat from red to brown. Meat shelf life extenders have been developed to extend the lifetime of the red color of meat products including but not limited to carbon monoxide, nitrites, sodium metabisulfite, Bombal, vitamin E, rosemary extract, green tea extract, catechins and other anti-oxidants.

However an intrinsically more stable heme protein such a hemoglobin isolated from *Aquifex aeolicus* (SEQ ID NO:3) or *Methylacidiphilum infernorum* (SEQ ID NO: 2) will oxidize more slowly than a mesophilic hemoglobin such as myoglobin. The heme proteins described herein (see, e.g., FIG. 1) also may have the lifetime of the reduced heme-Fe^{2+} state extended by meat shelf life extenders such as carbon monoxide and sodium nitrite. Heme proteins may be selected for the desired color retention properties. For example for low temperature *sous-vide* cooking, a relatively unstable heme protein such as one from *Hordeum vulgare* may provide a brown product that appears cooked under conditions wherein myoglobin would retain its red, uncooked appearance. In some embodiments the heme protein may be selected to have increased stability where for example the meat replica may retain an attractive medium rare appearance despite being thoroughly cooked for food safety.

The main determinant of rancidity and production of off flavors or off odors is the oxidation of components of the consumable, including but not limited to, the fats. For example, oxidation of unsaturated fatty acids is a known cause of rancid odors. In some embodiments, meat replicas have extended shelf life because the makeup of the chemical

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properties of the meat replica are controlled such that the taste, texture, smell, and chemical properties do not react with oxygen to create off flavors or off odors. In some embodiments the meat replicas are less sensitive to oxidation due to the presence of higher degree of unsaturated fatty acids than present in beef. In some embodiments the meat replica contains no unsaturated fatty acids. In other embodiments the meat replica contains higher levels of anti-oxidants such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases than are present in meat. In other embodiments, off flavor or off odor generating components such as lipoxygenase are not present.

In some embodiments, a consumable described herein shows increased stability under commercial packaging conditions. In some embodiments, the improved shelf life is improved by using components with increased oxidative stability such as lipids with reduced levels of unsaturated fatty acids, and/or by using a more stable heme protein such a hemoglobin isolated from *Aquifex aeolicus* (SEQ ID NO:3) or *Methylacidiphilum infernorum* (SEQ ID NO: 2). In some embodiments, the improved shelf life is due to the combination of components used in the consumable. In some embodiments, the consumable is designed specifically for the desired packaging method.

III. Composition of the Consumables

A consumable described herein includes one or more isolated and purified proteins. "Isolated and purified protein" refers to a preparation in which the cumulative abundance by mass of protein components other than the specified protein, which can be a single monomeric or multimeric protein species, is reduced by a factor of 2 or more, 3 or more, 5 or more, 10 or more, 20 or more, 50 or more, 100 or more or 1000 or more relative to the source material from which the specified protein was isolated. For clarity, the isolated and purified protein is described as isolated and purified relative to its starting material (e.g., plants or other non-animal sources). In some embodiments, the term "isolated and purified" can indicate that the preparation of the protein is at least 60% pure, e.g., greater than 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% pure. The fact that a consumable may comprise materials in addition to the isolated and purified protein

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does not change the isolated and purified nature of the protein as this definition typically applies to the protein before addition to the composition.

In some embodiments, the one or more isolated and purified proteins accounts for at least 1%, at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, or at least 50% of the protein content of the consumable by weight. In some embodiments, each of the one or more isolated proteins is isolated and purified separately.

A consumable described herein may be substantially or entirely composed of ingredients derived from non-animal sources, e.g., plant, fungal, or microbial based sources. The plant sources can be organically grown sources. Proteins can be extracted from the source material (e.g., extracted from animal tissue, or plant, fungal, algal, or bacterial biomass, or from the culture supernatant for secreted proteins) or from a combination of source materials (e.g., multiple plant species). The consumables also can be made from a combination of plant based and animal based sources. For instance, the consumable may be a ground beef product supplemented with plant based products of the invention.

A. SOURCES OF COMPONENTS OF THE CONSUMABLE

As described above, isolated and purified proteins can be derived from non-animal sources such as plants, algae, fungi (e.g., yeast or filamentous fungi), bacteria, or Archaea. In some embodiments, the isolated and purified proteins can be obtained from genetically modified organisms such as genetically modified bacteria or yeast. In some embodiments, the isolated and purified proteins are chemically synthesized or obtained via *in vitro* synthesis.

In some embodiments, the one or more isolated and purified proteins are derived from plant sources. The isolated and purified proteins can be isolated from a single plant source or, alternatively, multiple plant sources can serve as the starting material for the isolation and purification of proteins. As described herein, isolated and purified plant proteins are soluble in solution. The solution can comprise EDTA (0 – 0.1M), NaCl (0-1M), KCl (0-1M), $NaSO_4$ (0 – 0.2M), potassium phosphate (0-1M), sodium citrate (0-1M), sodium carbonate (0-1M), sucrose (0-50%), Urea (0-2M) or any combination

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thereof. The solution can have a pH of 3 to 11. In some embodiments, plant proteins can have a solubility in a solution of >25 g/L (e.g., at least 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, or 225 g/L) at a temperature between about 2°C and about 32°C (e.g., between 3°C and 8°C, 10°C and 25°C, or 18°C and 25°C), wherein the solution has a pH between 3 and 8 (e.g., pH of 3-6, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, or 8), and has a sodium chloride content of 0 to 300 mM (e.g., 50, 100, 150, 200, 250, or 300 mM). In some embodiments, the isolated and purified proteins are soluble in solution at greater than 10, 15, 20, 25, 50, 100, 150, 200, or 250g/L.

One of skill in the art will understand that proteins that can be isolated from any organism in the plant kingdom may be used to produce the consumables described herein. Non-limiting examples of plant sources include grain crops such as, e.g., maize, oats, rice, wheat, barley, rye, millet, sorghum, buckwheat, amaranth, quinoa, triticale (a wheat rye hybrid), teff (*Eragrostis tef*); oilseed crops including cottonseed, sunflower seed, safflower seed, *Crambe*, *Camelina*, mustard, rapeseed (*Brassica napus*); *Acacia*, or plants from the legume family, such as, e.g., clover, *Stylosanthes*, *Sesbania*, vetch (*Vicia*), *Arachis*, *Indigofera*, *Leucaena*, *Cyamopsis*, peas such as cowpeas, english peas, yellow peas, or green peas, or beans such as, e.g., soybeans, fava beans, lima beans, kidney beans, garbanzo beans, mung beans, pinto beans, lentils, lupins, mesquite, carob, soy, and peanuts (*Arachis hypogaea*); leafy greens such as, e.g., lettuce, spinach, kale, collard greens, turnip greens, chard, mustard greens, dandelion greens, broccoli, or cabbage; or green matter not ordinarily consumed by humans, including biomass crops such as switchgrass (*Panicum virgatum*), *Miscanthus*, *Arundo donax*, energy cane, *Sorghum*, or other grasses, alfalfa, corn stover, kelp or other seaweeds, green matter ordinarily discarded from harvested plants, sugar cane leaves, leaves of trees, root crops such as cassava, sweet potato, potato, carrots, beets, or turnips; or coconut.

Protein can be isolated from any portion of the plant, including the roots, stems, leaves, flowers, or seeds. For example, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) can be isolated from, for example, alfalfa, carrot tops, corn stover, sugar cane leaves, soybean leaves, switchgrass, *Miscanthus*, energy cane, *Arundo donax*, seaweed, kelp, algae or mustard greens.

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Proteins that are abundant in plants can be isolated in large quantities from one or more source plants and thus are an economical choice for use in any of compositions provided herein (e.g., the muscle, fat, or connective tissue replicas, meat substitute products or others). Accordingly, in some embodiments, the one or more isolated and purified proteins comprise an abundant protein found in high levels in a plant and capable of being isolated and purified in large quantities. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 70% of the total protein content of the source plant material. In some embodiments, the abundant protein comprises about 0.5-10%, about 5-40%, about 10-50%, about 20-60%, or about 30-70% of the total protein content of the source plant material. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the total weight of the dry matter of the source plant material. In some embodiments, the abundant protein comprises about 0.5-5%, about 1-10%, about 5-20%, about 10-30%, about 15-40%, or about 20-50% of the total weight of the dry matter of the source plant material.

The one or more isolated and purified proteins can comprise an abundant protein that is found in high levels in the leaves of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% of the total protein content of the leaves of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%, about 20%-60%, or about 30-70% of the total protein content of the leaves of the source plant. In some embodiments, the one or more isolated proteins comprise RuBisCo, which is a particularly useful protein for meat replicas because of its high solubility and an amino acid composition that is close to the optimum proportions of essential amino acids for human nutrition. In particular embodiments, the one or more isolated proteins comprise ribulose-1,5-bisphosphate carboxylase oxygenase activase (RuBisCo activase). In some embodiments, the one or more isolated and purified proteins comprise a vegetative storage protein (VSP).

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The one or more isolated proteins can comprise an abundant protein that is found in high levels in the seeds of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or 90% or more of the total protein content of the seeds of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%, about 20%-60%, or about 30-70% or >70% of the total protein content of the seeds of the source plant. Non-limiting examples of proteins found in high levels in the seeds of plants include seed storage proteins, e.g., albumins, glycinins, conglycinins, legumins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamins, phaseolin (protein), proteinoplast, secalin, triticeae gluten, or zein, or oil body proteins such as oleosins, caloleosins, or steroleosins.

The one or more isolated and purified proteins can include highly soluble proteins such as dehydrins, hydrophilins, natively unfolded proteins (also referred to as intrinsically disordered proteins), or other proteins of the late-embryogenesis abundant (LEA) family. LEA proteins have been found in animals, plants and microorganisms and are thought to act as osmoprotectants and stress response proteins. See, e.g., Battaglia, *et al., Plant Physiol.*, 148:6-24 (2008). Such proteins also are heat stable. Such LEA protiens can have a solubility in a solution of at least 1 g/L (e.g., 2, 4, 6, 8, 10, 15, 20, 25, 50, 100, 150, 200, or 250g/L) at a temperature of between 90°C and 110°C (e.g., between 95°C and 105°C, 95°C, or 100°C), wherein the solution has a pH between 5 and 8 (e.g., pH of 5, 5.5., 6, 6.5, 7, 7.5, or 8) and has a sodium chloride content of 0 to 300 mM (e.g., 50, 100, 150, 200, 250, or 300 mM). In some cases, the LEA proteins may be isolated by heating a protein extract to 90°C-110°C (e.g., 95°C or 100°) and, after centrifugation or filtration of insoluble material, concentrating the LEA protein fraction by, for example, ultrafiltration. In some cases, isoionic pH precipitation, trichloroacetic acid precipitation, and/or ammonium sulfate precipitation steps can be done before or after the heating step to additionally remove non-LEA proteins. Heating the solution to 90°C-110°C denatures most proteins, allows the majority of the proteins to be removed from solution.

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B. PROTEINS

Without being bound by theory, it is believed that by isolating and purifying non-animal proteins (e.g., plant proteins), consumables can be made with greater consistency and greater control over the properties of the consumable. In some embodiments, about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component of the consumable is comprised of one or more isolated and purified proteins. The isolated and purified protein may be greater than 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% pure.

The isolated and purified proteins can be isolated from one or more other components of a non-animal source. For example, a protein fraction may be isolated from an isolate of a plant. The isolated proteins may in some cases be purified, wherein a certain kind of protein is separated from other components found in the non-animal source. Proteins can be separated on the basis of their molecular weight, for example, by size exclusion chromatography, ultrafiltration through membranes, or density centrifugation. In some embodiments, the proteins can be separated based on their surface charge, for example, by isoelectric precipitation, anion exchange chromatography, or cation exchange chromatography. Proteins also can be separated on the basis of their solubility, for example, by ammonium sulfate precipitation, isoelectric precipitation, surfactants, detergents or solvent extraction. Proteins also can be separated by their affinity to another molecule, using, for example, hydrophobic interaction chromatography, reactive dyes, or hydroxyapatite. Affinity chromatography also can include using antibodies having specific binding affinity for the protein of interest, nickel NTA for His-tagged recombinant proteins, lectins to bind to sugar moieties on a glycoprotein, or other molecules which specifically binds the protein of interest.

Isolating proteins allows for the elimination of unwanted material. In some embodiments, an isolated and purified protein is a protein that has been substantially separated from unwanted material (e.g., nucleic acids such as RNA and DNA, lipid membranes, phospholipids, fats, oils, carbohydrates such as starch, cellulose, and

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glucans, phenolic compounds, polyphenolic compounds, aromatic compounds, or pigments) in the seeds, leaves, stems, or other portion of the plant.

The isolated and purified proteins also can be recombinantly produced using polypeptide expression techniques (e.g., heterologous expression techniques using bacterial cells, insect cells, fungal cells such as yeast cells, plant cells, or mammalian cells). In some cases, standard polypeptide synthesis techniques (e.g., liquid-phase polypeptide synthesis techniques or solid-phase polypeptide synthesis techniques) can be used to produce proteins synthetically. In some cases, cell-free translation techniques can be used to produce proteins synthetically.

The protein or proteins incorporated into the consumable can serve a nutritional function. In some instance, the protein also serves to alter the properties of the consumable, e.g., the flavor, color, odor, and/or texture of the consumable. For example, a meat substitute product can comprise a protein indicator that indicates cooking progression from a raw state to a cooked state, wherein the meat substitute product is derived from non-animal sources.

Examples of proteins that can be isolated and purified, and used in the consumables described herein include ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, proteases, lipases, amylases, glycoproteins, lectins, mucins, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, histones, ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo), ribulose-1,5-bisphosphate carboxylase oxygenase activase (RuBisCo activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, collagens, zein, kafirin, avenin, dehydrins, hydrophilins, late embyogenesis abundant proteins, natively unfolded proteins, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin, globulin, pea globulins, and pea albumins.

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In some embodiments, an isolated and purified protein can be a protein that interacts with lipids and helps to stabilize lipids in a structure, a protein that binds lipids and helps crosslink lipid structures, or a protein that binds lipids and helps crosslink lipid structures and non-lipid interacting proteins. Without wishing to be bound by a particular theory, using such proteins in a consumable described herein may improve the integration of lipids and/or fat replicas with other components of the meat substitute product, resulting in improved mouthfeel and texture of the final product. A non-limiting example of a lipid-interacting plant protein includes proteins in the oleosin family. Oleosins are lipid-interacting proteins that are found in oil bodies of plants. Other non-limiting examples of plant proteins that can interact with lipids and stabilize emulsions include seed storage proteins from Great Northern beans, albumins from peas, globulins from peas, 8S globulins from moong bean, 8S globulins from Kidney bean, prolamin and lipid transfer proteins.

In some embodiments, one or more of the isolated and purified proteins can be an iron-carrying protein such as a heme-containing protein. As used herein, the term "heme containing protein" can be used interchangeably with "heme containing polypeptide" or "heme protein" or "heme polypeptide" and includes any polypeptide that can covalently or noncovalently bind a heme moiety. In some embodiments, the heme-containing polypeptide is a globin and can include a globin fold, which comprises a series of seven to nine alpha helices. Globin type proteins can be of any class (e.g., class I, class II, or class III), and in some embodiments, can transport or store oxygen. For example, a heme-containing protein can be a non-symbiotic type of hemoglobin or a leghemoglobin. A heme-containing polypeptide can be a monomer, i.e., a single polypeptide chain, or can be a dimer, a trimer, tetramer, and/or higher order oligomers. The life-time of the oxygenated Fe^{2+} state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption. The life-time of the unoxygenated Fe^{2+} state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or

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more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption

Non-limiting examples of heme-containing polypeptides can include an androglobin, a cytoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a leghemoglobin, a flavohemoglobin, Hell's gate globin I, a myoglobin, an erythrocruorin, a beta hemoglobin, an alpha hemoglobin, a protoglobin, a cyanoglobin, a cytoglobin, a histoglobin, a neuroglobins, a chlorocruorin, a truncated hemoglobin (e.g., HbN or HbO), a truncated 2/2 globin, a hemoglobin 3 (e.g., Glb3), a cytochrome, or a peroxidase.

Heme-containing proteins that can be used in the consumables described herein can be from mammals (e.g., farms animals such as cows, goats, sheep, horses, pigs, ox, or rabbits), birds, plants, algae, fungi (e.g., yeast or filamentous fungi), ciliates, or bacteria. For example, a heme-containing protein can be from a mammal such as a farm animal (e.g., a cow, goat, sheep, pig, ox, or rabbit) or a bird such as a turkey or chicken. Heme-containing proteins can be from a plant such as *Nicotiana tabacum* or *Nicotiana sylvestris* (tobacco); *Zea mays* (corn), *Arabidopsis thaliana*, a legume such as *Glycine max* (soybean), *Cicer arietinum* (garbanzo or chick pea), *Pisum sativum* (pea) varieties such as garden peas or sugar snap peas, *Phaseolus vulgaris* varieties of common beans such as green beans, black beans, navy beans, northern beans, or pinto beans, *Vigna unguiculata* varieties (cow peas), *Vigna radiata* (Mung beans), *Lupinus albus* (lupin), or *Medicago sativa* (alfalfa); *Brassica napus* (canola); *Triticum sps.* (wheat, including wheat berries, and spelt); *Gossypium hirsutum* (cotton); *Oryza sativa* (rice); *Zizania sps*. (wild rice); *Helianthus annuus* (sunflower); *Beta vulgaris* (sugarbeet); *Pennisetum glaucum* (pearl millet); *Chenopodium sp.* (quinoa); *Sesamum sp.* (sesame); *Linum usitatissimum* (flax); or *Hordeum vulgare* (barley). Heme-containing proteins can be isolated from fungi such as *Saccharomyces cerevisiae*, *Pichia pastoris*, *Magnaporthe oryzae*, *Fusarium graminearum*, or *Fusarium oxysporum*. Heme-containing proteins can be isolated from bacteria such as *Escherichia coli*, *Bacillus subtilis, Bacillus megaterium, Synechocistis sp., Aquifex aeolicus, Methylacidiphilum infernorum*, or thermophilic bacteria (e.g, that grow at temperatures greater than 45°C) such as *Thermophilus*. Heme-containing proteins can be isolated from algae such as *Chlamydomonas eugametos*. Heme-

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containing proteins can be isolated from protozoans such as *Paramecium caudatum* or *Tetrahymena pyriformis*. In some embodiments, the bacterial hemoglobins are selected from the group consisting of *Aquifex aeolicus*, *Thermobifida fusca*, *Methylacidiphilum infernorum* (Hells Gate), *Synechocystis SP,* or *Bacillus subtilis*. The sequences and structure of numerous heme-containing proteins are known. See for example, Reedy, *et al.*, *Nucleic Acids Research*, 2008, Vol. 36, Database issue D307–D313 and the Heme Protein Database available on the world wide web at http://hemeprotein.info/heme.php.

For example, a non-symbiotic hemoglobin can be from a plant selected from the group consisting of soybean, sprouted soybean, alfalfa, golden flax, black bean, black eyed pea, northern, garbanzo, moong bean, cowpeas, pinto beans, pod peas, dried peas, quinoa, sesame, sunflower, wheat berries, spelt, barley, wild rice, or rice.

Any of the heme-containing proteins described herein that can be used for producing consumables can have at least 70% (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%) sequence identity to the amino acid sequence of the corresponding wild-type heme-containing protein or fragments thereof that contain a heme-binding motif. For example, a heme-containing protein can have at least 70% sequence identity to an amino acid sequence set forth in FIG. 1, including a non-symbiotic hemoglobin such as that from *Vigna radiata* (SEQ ID NO:1), *Hordeum vulgare* (SEQ ID NO:5), *Zea mays* (SEQ ID NO:13), *Oryza sativa subsp. japonica (rice)* (SEQ ID NO:14), or *Arabidopsis thaliana* (SEQ ID NO:15), a Hell's gate globin I such as that from *Methylacidiphilum infernorum* (SEQ ID NO:2), a flavohemoprotein such as that from *Aquifex aeolicus* (SEQ ID NO:3), a leghemoglobin such as that from *Glycine max* (SEQ ID NO:4), *Pisum sativum* (SEQ ID NO:16), or *Vigna unguiculata* (SEQ ID NO:17), a heme-dependent peroxidase such as from *Magnaporthe oryzae*, (SEQ ID NO:6) or *Fusarium oxysporum* (SEQ ID NO:7), a cytochrome c peroxidase from *Fusarium graminearum* (SEQ ID NO:8), a truncated hemoglobin from *Chlamydomonas moewusii* (SEQ ID NO:9), *Tetrahymena pyriformis* (SEQ ID NO:10, group I truncated), *Paramecium caudatum* (SEQ ID NO:11, group I truncated), a hemoglobin from *Aspergillus niger* (SEQ ID NO:12), or a mammalian myoglobin protein such as the *Bos taurus* (SEQ ID NO:18) myoglobin, *Sus scrofa* (SEQ ID NO:19) myoglobin, or *Equus*

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caballus (SEQ ID NO:20) myoglobin, a heme-protein from *Nicotiana benthamiana* (SEQ ID NO:21), *Bacillus subtilis* (SEQ ID NO:22), *Corynebacterium glutamicum* (SEQ ID NO:23), Synechocystis PCC6803 (SEQ ID NO:24), Synechococcus sp. PCC 7335 (SEQ ID NO:25), *Nostoc commune* (SEQ ID NO:26), or *Bacillus megaterium* (SEQ ID NO:27). See FIG. 1.

The percent identity between two amino acid sequences can be determined as follows. First, the amino acid sequences are aligned using the BLAST 2 Sequences (Bl2seq) program from the stand-alone version of BLASTZ containing BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from Fish & Richardson's web site (e.g., www.fr.com/blast/) or the U.S. government's National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov). Instructions explaining how to use the Bl2seq program can be found in the readme file accompanying BLASTZ. Bl2seq performs a comparison between two amino acid sequences using the BLASTP algorithm. To compare two amino acid sequences, the options of Bl2seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two amino acid sequences: C:\Bl2seq –i c:\seq1.txt –j c:\seq2.txt –p blastp –o c:\output.txt. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology, then the designated output file will not present aligned sequences. Similar procedures can be following for nucleic acid sequences except that blastn is used.

Once aligned, the number of matches is determined by counting the number of positions where an identical amino acid residue is presented in both sequences. The percent identity is determined by dividing the number of matches by the length of the full-length polypeptide amino acid sequence followed by multiplying the resulting value by 100. It is noted that the percent identity value is rounded to the nearest tenth. For example, 78.11, 78.12, 78.13, and 78.14 is rounded down to 78.1, while 78.15, 78.16,

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78.17, 78.18, and 78.19 is rounded up to 78.2. It also is noted that the length value will always be an integer.

It will be appreciated that a number of nucleic acids can encode a polypeptide having a particular amino acid sequence. The degeneracy of the genetic code is well known to the art; i.e., for many amino acids, there is more than one nucleotide triplet that serves as the codon for the amino acid. For example, codons in the coding sequence for a given enzyme can be modified such that optimal expression in a particular species (e.g., bacteria or fungus) is obtained, using appropriate codon bias tables for that species.

Heme-containing proteins can be extracted from the source material (e.g., extracted from animal tissue, or plant, fungal, algal, or bacterial biomass, or from the culture supernatant for secreted proteins) or from a combination of source materials (e.g., multiple plant species). Leghemoglobin is readily available as an unused by-product of commodity legume crops (e.g., soybean, alfalfa, or pea). The amount of leghemoglobin in the roots of these crops in the United States exceeds the myoglobin content of all the red meat consumed in the United States.

In some embodiments, extracts of heme-containing proteins include one or more non-heme-containing proteins from the source material (e.g., other animal, plant, fungal, algal, or bacterial proteins) or from a combination of source materials (e.g., different animal, plant, fungi, algae, or bacteria).

In some embodiments, heme-containing proteins are isolated and purified from other components of the source material (e.g., other animal, plant, fungal, algal, or bacterial proteins) using techniques described above. As used herein, the term "isolated and purified" indicates that the preparation of heme-containing protein is at least 60% pure, e.g., greater than 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% pure.

Heme-containing proteins also can be recombinantly produced using polypeptide expression techniques (e.g., heterologous expression techniques using bacterial cells, insect cells, algal cells, fungal cells such as yeast cells, plant cells, or mammalian cells). For example, the heme-containing protein can be expressed in *E. coli* cells. The heme-containing proteins can be tagged with a heterologous amino acid sequence such as FLAG, polyhistidine (e.g., hexahistidine, HIS tag), hemagluttinin (HA), glutathione-S-

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transferase (GST), or maltose-binding protein (MBP) to aid in purifying the protein. In some embodiments, a recombinant heme containing protein including a HIS-tag and a protease (e.g., TEV) site to allow cleavage of the HIS-tag, can be expressed in *E.coli* and purified using His-tag affinity chromatography (Talon resin, CloneTech). In some cases, standard polypeptide synthesis techniques (e.g., liquid-phase polypeptide synthesis techniques or solid-phase polypeptide synthesis techniques) can be used to produce heme-containing proteins synthetically. In some cases, cell-free translation techniques can be used to produce heme-containing proteins synthetically..

In some embodiments, the isolated and purified protein is substantially in its native fold and water soluble. In some embodiments, the isolated and purified protein is more than 50, 60, 70, 80, or 90% in its native fold. In some embodiments, the isolated and purified protein is more than 50, 60, 70, 80, or 90% water soluble.

The proteins used in the consumable can be altered (e.g., hydrolyzed, cleaved, crosslinked, denatured, polymerized, extruded, electrospun, spray dried or lyophilized, or derivatized or chemically modified). For example, the proteins can be modified by covalent attaching sugars, lipids, cofactors, peptides, or other chemical groups including phosphate, acetate, methyl, and other natural or unnatural molecule. For example, the peptide backbones of the proteins can be cleaved by exposure to acid or proteases or other means. For example, the proteins can be denatured, i.e., their secondary, tertiary, or quaternary structure can be altered, by exposure to heat or cold, changes in pH, exposure to denaturing agents such as detergents, urea, or other chaotropic agents, or mechanical stress including shear. The alignment of proteins in a solution, colloid, or a solid assembly can be controlled to affect the mechanical properties including tensile strength, elasticity, deformability, hardness, or hydrophobicity.

Proteins also can be assembled into fibers that can form a matrix for a structure for the compositions. A 3-dimensional matrix of protein fibers can, for example, contain chemicals that promote formation of inter-molecular disulfide cross-links (mixed glutathione, dithiothreitol (DTT), beta-mercaptoethanol (BME)). In some embodiments, the chemicals are proteins (thioredoxin, glutaredoxin). In some embodiments, the proteins are enzymes (disulfide isomerase). In some embodiments, fibers are crosslinked

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by chemical cross-linkers with two reactive groups selected from the group consisting of N-hydroxysuccinimide (NHS) esters, imidoesters, aryl fluorides, aldehydes, maleimides, pyridyldithiols, haloacetyls, aryl azides, diazirines, carbodiimides, hydrazides and isocyanates.

In some embodiments, coacervates comprising one or more plant proteins can be formed and used, for example, as binding agents in meat or other replicas. Coacervation is the process during which a homogeneous solution of charged polymers undergoes a phase separation to result in a polymer-rich dense phase (the 'coacervate') and a solvent-rich phase (supernatant). Protein-polysaccharide coacervates have been used in the development of biomaterials. See, for example, Boral and Bohidar (2010) *Journal of Physical Chemistry B*. Vol 114 (37): 12027-35; and Liu *et al.*, (2010) *Journal of Agricultural and Food Chemistry*, Vol 58:552-556. Formation of such coacervates is driven by associative interactions between oppositely charged polymers. However, as described herein, coacervates can be formed using proteins (e.g., plant proteins comprise one or more pea proteins, chickpea proteins, lentil proteins, lupine proteins, other legume proteins, or mixtures thereof). In general, a coacervate can be formed by acidifying a low ionic strength solution (e.g., a buffered solution at or below100 mM sodium chloride) comprising one or more isolated and purified plant proteins such as pea legumins or vicilins (e.g., a vicilin fraction comprising convicilins), a combination of both vicilins and legumins, or unfractionated pea proteins to a pH of 3.5 to 5.5. (e.g., pH 4 to 5). Under these conditions, the proteins separate out of solution and the mixture can be centrifuged to cleanly separate out the coacervate. This coavervate, unlike a precipitate, is a viscous material that can be stretched by pulling and that melts on heating. The process can be carried out in the presence of oils (up to 70%, e.g., palm or other oil), to form a creamy material. By varying the composition of the solution (ratio of vicilin: legumin, type and amount of oil used), the binding properties of the coacervate can be tuned as desired. In some embodiments, one or more gums (e.g., acacia gum or xanthan gum) can be used to form a coacervate. Coacervates can be used as binding agents in beef patty replicas to bind and hold together the adipose-, muscle- and connective tissue replicas.

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Binding materials with different adhesive and cooking characteristics can be prepared by combining wheat gluten (0-20%) and pea protein fractions (0-50%) in the presence of a plasticizer such as glycerol (0-30%) or polyethylene glycol. Leghemoglobin or other heme-containing protein can be added to the mixture if necessary. Upon mixing to remove any clumps, the material may be incorporated into beef patty replicas.

In some embodiments, proteins can be subjected to freeze alignment to texturize the proteins without extrusion. The method involves slow freezing of protein comprising materials to allow for formation of ice crystals. When cooled from one side, ice crystals form preferentially in a direction perpendicular to the cooled side. After freezing, the ice can be removed from the material in a freeze-dryer, leaving behind material with several layers. The structure can then be stabilized by heating under pressurized, moist conditions to produce a material that can be used in meat replicas. Freeze-alignment of soy proteins has been described by Lugay and Kim (1981) (see Freeze alignment: A novel method for protein texturization. Page 177-187, Chapter 8 in: D.W. Stanley, E.D. Murray and D.H. Lees eds. 1981. Utilization of Protein Resources. Westport, CT: Food & Nutrition Press, Inc). The freeze-aligned proteins can be subjected to further processing (by soaking in solutions comprising beef flavors and/or leghemoglobin) and used in combination with adipose- and connective-tissue replicas to form beef replicas. The replicas may also be used as structures around which cold-set gels (comprising, for example, pea proteins and myoglobin) or crosslinked gels (comprising, for example, pea proteins and leghemoglobin) can be formed prior to their combination with adipose- and connective-tissues.

C. LIPIDS

Consumables described herein can include a lipid component. Lipids can be isolated and/or purified and can be in the form of triglycerides, monoglycerides, diglycerides, free fatty acids, sphingosides, glycolipids, phospholipids, or oils, or assemblies of such lipids (e.g., membranes, lecithin, lysolecithin, or fat droplets containing a small amount of lipid in a bulk water phase). In some embodiments, the lipid sources are oils obtained from non-animal sources (e.g., oils obtained from plants,

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algae, fungi such as yeast or filamentous fungi, seaweed, bacteria, or Archae), including genetically engineered bacteria, algae, archaea or fungi. Non-limiting examples of plant oils include corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, or rice bran oil; or margarine. The oils can be hydrogenated (e.g., a hydrogenated vegetable oil) or non-hydrogenated.

In some embodiments, the lipid can be triglycerides, monoglycerides, diglycerides, free fatty acids, sphingosides, glycolipids, lecithin, lysolecithin, phospholipids such as phosphatidic acids, lysophosphatidic acids, phosphatidyl cholines, phosphatidyl inositols, phosphatidyl ethanolamines, or phosphatidyl serines; sphingolipids such as sphingomyelins or ceramides; sterols such as stigmasterol, sitosterol, campesterol, brassicasterol, sitostanol, campestanol, ergosterol, zymosterol, fecosterol, dinosterol, lanosterol, cholesterol, or episterol; lipid amides, such as N-palmitoyl proline, N-stearoyl glycine, N-palmitoyl glycine, N-arachidonoyl glycine, N-palmitoyl taurine, N-arachidonoyl histidine, or anandamide; free fatty acids such as palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid (C18:2), eicosanoic acid (C22:0), arachidonic acid (C20:4), eicosapentanoic acid (C20:5), docosapentaenoic acid (C22:5), docosahexanoic acid (C22:6), erucic acid (C22:1), conjugated linoleic acid, linolenic acid (C18:3), oleic acid (C18:1), elaidic acid (trans isomer of oleic acid), trans-vaccenic acid (C18:1 *trans* 11), or conjugated oleic acid; or esters of such fatty acids, including monoacylglyceride esters, diacylglyceride esters, and triacylglyceride esters of such fatty acids.

The lipids can comprise phospholipids, lipid amides, sterols or neutral lipids. The phospholipids can comprise a plurality of amphipathic molecules comprising fatty acids (e.g., see above), glycerol and polar groups. In some embodiments, the polar groups are, for example, choline, ethanolamine, serine, phosphate, glycerol-3-phosphate, inositol or inositol phosphates. In some embodiments, the lipids are, for example, sphingolipids,

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ceramides, sphingomyelins, cerebrosides, gangliosides, ether lipids, plasmalogens or pegylated lipids.

In some embodiments, the lipids used in the consumable are the cream fraction created from seeds, nuts, and legumes, including but not limited to sunflower seeds, safflower seeds, sesame seeds, rape seeds, almonds, macadamia, grapefruit, lemon, orange, watermelon, pumpkin, cocoa, coconut, mango, butternut squash, cashews, brazilnuts, chestnuts, hazelnuts, peanuts, pecans, walnuts and pistachios. As used herein, the term "cream fraction" can refer to an isolated emulsion comprising lipids, proteins and water.

To obtain a cream fraction from seeds, nuts, or legumes, one or more of the following steps can be performed. Seeds, nuts or legumes can be blended from 1 minute up to 30 minutes. For example, the seeds, nuts, or legumes can be blended by increasing the speed gradually to maximum speed over 4 minutes, then blending at maximum speed for 1 minute. The seeds, nuts or legumes can be blended in water or solutions that contain all or some of the following: EDTA (0 – 0.1M), NaCl (0-1M), KCl (0-1M), $NaSO_4$ (0 – 0.2M), potassium phosphate (0-1M), sodium citrate (0-1M), sodium carbonate (0-1M), and/or sucrose (0-50%), from pH of 3 to 11 to obtain a slurry. The slurry can be heated to 20°C to 50°C and centrifuged to obtain the cream fraction (the top layer, also referred to as the "cream"). Further purification of the cream fraction may be achieved by washing the cream fraction with 0.1M to 2M urea solution before re-isolating the cream fraction by centrifugation. The residual liquid (referred to as the "skim" layer) that is a solution comprising proteins in water can also be used.

The "cream" can be used as is, or subjected to further purification steps. For example, washing and heating can remove color and flavor molecules (e.g., unwanted molecules), or unwanted grainy particles to improve the mouth feel and creaminess. In particular, washing with a high pH buffer (pH >9) can remove bitter tasting compounds and improve mouth feel, washing with urea can remove storage proteins, washing below pH 9, followed by washing with a pH above pH 9 can remove unwanted color molecules, and/or washing with salts can decrease taste compounds. Heating can increase the removal of grainy particles, color and flavor compounds. For example, the cream

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fraction can be heated from 0-24 hours, at temperatures ranging from 25°C to 80°C. In some embodiments, the resulting creamy fraction comprises seed storage proteins. In some embodiments, the seed storage proteins are substantially removed from the resulting creamy fraction.

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D. FIBER

Fiber can be isolated and/or purified for inclusion in the consumables described herein. Fiber can refer to non-starch polysaccharides such as arabinoxylans, cellulose, and other plant components such as resistant starch, resistant dextrins, inulin, lignin, 10 waxes, chitins, pectins, beta-glucans, and oligosaccharides from any plant source.

Fibers can refer to extruded and solution spun proteins as described herein.

E. SUGARS

In some embodiments, the consumable also can comprise sugars. For example 15 the consumable can comprise: monosaccharides, including but not limited to glucose (dextrose), fructose (levulose), galactose, mannose, arabinose, xylose (D- or L-xylose), and ribose, disaccharides including but not limited to sucrose, lactose, melibiose, trehalose, cellobiose, or maltose, sugar alcohols such as arabitol, mannitol, dulcitol, or sorbitol, sugar acids such as galacturonate, glucuronate, or gluconate, oligosaccharides 20 and polysaccharides such as glucans, starches such as corn starch, potato starch, pectins such as apple pectin or orange pectin, raffinose, stachyose, or dextrans; plant cell wall degradation products such as salicin, and/or sugar derivatives such as N-acetylglucosamine.

25 **F. GEL FORMATION**

The components of the composition can be formed into a gel. In some embodiments, gels comprise protein, where the protein is derived from non-animal source (e.g., a plant source or other non-animal source such as a genetically modified yeast or bacteria). Gels can be formed using a variety of methods. The protein

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concentration, enzyme concentration, pH, and/or process temperature will affect the rate of gel formation and quality of the final tissue replica.

Gels can be stabilized entirely by physical cross-links between the components. In some embodiments, gels can be produced by heat/cool cycles, in which case the gel is stabilized by physical interactions (entanglements, hydrophobic interactions) between protein molecules. For example, a gel can be formed by heating a protein solution to a temperature of at least 40°C, 45°C, 50°C, 60°C, 70°C, 80°C, 90°C, or 100°C and then cooling to room temperature, or to a temperature below 40°C.

In some embodiments, a gel can be formed by subjecting the composition containing the protein and any other components (e.g., a lipid) to high pressure processing.

In some embodiments, gels can be produced by adjusting the pH of the solution. For example, the pH of a concentrated protein solution can be adjusted to near the isolectric pH of the main protein component by adding hydrochloric acid or other acid, or sodium hydroxide or other base.

In some embodiments, gels can be produced by soaking protein powders in solutions. For example, protein powder can be soaked with at least 1%, 5%, 10%, 20% (wt/v) or more of a concentrated sodium hydroxide solution. In other examples, protein powder can be soaked in mixed water/ethanol solutions.

In some embodiments, a cold set gel is formed to avoid denaturing or the breakdown of any heat-labile components (e.g., oxidizing the iron in a heme moiety or generating undesirable flavors). See, Ju and Kilara A. (1998) *J. Food Science*, Vol 63(2): 288-292; and Maltais *et al.*, (2005) *J. Food Science*, Vol 70 (1): C67-C73) for general methodologies for forming cold set gels. In general, cold set gels are formed by first heat denaturing a protein solution below its minimum gelling concentration (dependent on pH and type of protein, typically <8% (w/v) at pH 6-9 for globular plant proteins such as pea proteins). The protein solution can heated to a temperature above the denaturation temperature of the protein under conditions where it does not precipitate out of solution (e.g., 0-500 mM sodium chloride, pH 6-9). The solution can be cooled back to room temperature or below, and any heat-labile components (e.g., heme-containing proteins

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and/or oils) can be mixed in when the solution is sufficiently cool, but before gelling.. Gelation can be induced by adding sodium chloride or calcium chloride (e.g., 5 to 100 mM), and the solution can be incubated at or below room temperature to allow for gel formation (typically minutes-hours). The resulting gel can be used as-is in meat replicas or processed further (e.g., stabilized) before incorporation in meat replicas.

In some embodiments, gels can comprise or be produced (e.g., stabilized by) at least in part by a cross-linking enzyme. The cross-linking enzyme can be, for example, a transglutaminase, a tyrosinase, a lipoxygenase, a protein disulfide reductase, a protein disulfide isomerase, a sulfhydryl oxidase, a peroxidase, a hexose oxidase, a lysyl oxidase, or an amine oxidase.

In some cases, gels can comprise chemicals that promote formation of inter-molecular disulfide cross-links between the proteins. In some embodiments, the chemicals are proteins (e.g., thioredoxin, glutaredoxin). In some embodiments, the proteins are enzymes (disulfide isomerase).

Gels can be stabilized by chemical crosslinking by chemical cross-linkers with two reactive groups selected from the group consisting of N-hydroxysuccinimide (NHS) esters, imidoesters, aryl fluorides, aldehydes, maleimides, pyridyldithiols, haloacetyls, aryl azides, diazirines, carbodiimides, hydrazides and isocyanates.

In some embodiments, gels can be stabilized by the addition of starches and gums.

In some embodiments, more than one of these approaches are used in combination. For example, a transglutaminase cross-linked gel can be further stabilized by a heat/cool treatment.

G. MUSCLE REPLICAS

A large number of meat products comprise a high proportion of skeletal muscle. Accordingly, the present invention provides a composition, which can be derived from non-animal sources which replicates or approximates key features of animal skeletal muscle. A composition derived from non-animal sources, which replicates or approximates animal skeletal muscle can be used as a component of a consumable, for

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example, a meat replica. Such a composition will be labeled herein as "muscle replica." In some embodiments, the muscle replica and/or meat substitute product comprising the muscle replica are partially derived from animal sources. In some embodiments, the muscle replica and/or meat substitute product comprising the muscle replica are entirely derived from non-animal sources.

The muscle tissue replica can comprise a protein content, wherein the protein content comprises one or more isolated and purified proteins, wherein the muscle tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source.

Many meat products comprise a high proportion of striated skeletal muscle in which individual muscle fibers are organized mainly in an anisotropic fashion. Accordingly, in some embodiments, the muscle replica comprises fibers that are to some extent organized anisotropically. The fibers can comprise a protein component. In some embodiments, the fibers comprise about 1% (wt/wt), about 2%, about 5%, about 10%, about 15%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99% (wt/wt) or more of a protein component.

The connective tissue component of skeletal muscle substantially contributes to texture, mouth feel and cooking behavior of meat products. Connective tissue is composed of protein (collagen, elastin) fibers in the range of 0.1 – 20 microns. In some embodiments, a mixture of fibers of diameters of <1-10 microns, and 10-300 microns is produced to replicate the fiber composition of animal connective tissue. In some embodiments, the 3-dimensional matrix of fibers is stabilized by protein crosslinks to replicate the tensile strength of animal connective tissue. In some embodiments, the 3-dimensional matrix of fibers contains an isolated, purified cross-linking enzyme. The cross-linking enzyme can be, for example, a transglutaminase, a tyrosinase, a lipoxygenase, a protein disulfide reductase, a protein disulfide isomerase, a sulfhydryl oxidase, a peroxidase, a hexose oxidase, a lysyl oxidase, or an amine oxidase.

Some proteins (e.g., 8S globulin from Moong bean seeds, or the albumin or globulin fraction of pea seeds) have favorable properties for constructing meat replicas because of their ability to form gels with textures similar to animal muscle or adipose

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tissue. See also the proteins identified in Section III A and B. The proteins may be artificially designed to emulate physical properties of animal muscle tissue.

In some embodiments, one or more isolated and purified proteins accounts for about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component by weight of the meat replica. In some embodiments, one or more isolated and purified proteins accounts for about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein content of a consumable.

Skeletal muscles of animals such as beef cattle typically contain substantial quantities of glycogen, which can comprise on the order of 1% of the mass of the muscle tissue at the time of slaughter. After slaughter, a fraction of this glycogen continues to be metabolized, yielding products including lactic acid, which contributes to lowering the pH of the muscle tissue, a desirable quality in meat. Glycogen is a branched polymer of glucose linked together by alpha (1->4) glycosidic bonds in linear chains, with branch points comprising alpha (1->6) glycosidic bonds. Starches from plants, particularly amylopectins are also branched polymers of glucose linked together by alpha (1->4) glycosidic bonds in linear chains, with branch points comprising alpha (1->6) glycosidic bonds and can therefore be used as an analog of glycogen in constructing meat replicas. Thus, in some embodiments, the muscle or meat replica includes a starch or pectin.

Additional components of animal muscle tissue include sodium, potassium, calcium, magnesium, and other metal ions, lactic acid and other organic acids, free amino acids, peptides, nucleotides, and sulfur compounds. Thus, in some embodiments, a muscle replica can include sodium, potassium, calcium, magnesium, other metal ions such as iron, zinc, copper, nickel, lithium, or selenium, lactic acid, and other organic acids such as fatty acids, free amino acids, peptides, nucleotides and sulfur compounds glutathione, beta mercaptoethanol, or dithiothreitol. In some embodiments, the concentration of sodium, potassium, calcium, magnesium, other metal ions, lactic acid, other organic acids, free amino acids, peptides, nucleotides and/or sulfur compounds in

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the muscle replica or consumable are within 10% of the concentrations found in a muscle or meat being replicated.

The invention also provides methods for making a muscle replica. In some embodiments, the method includes forming the composition into asymmetric fibers prior to incorporation into the consumable. In some embodiments, these fibers replicate muscle fibers. In some embodiments the fibers are spun fibers. In other embodiments the fibers are extruded fibers. Accordingly, the present invention provides for methods for producing asymmetric or spun protein fibers. In some embodiments, the fibers are formed by extrusion of the protein component through an extruder. Methods of extrusion are well known in the art, and are described, for example, in U.S. Patent No. 6,379,738, U.S. Patent No. 3,693,533, and U.S. Patent Publication No. 20120093994, which are herein incorporated by reference. These methods can be applied to making the compositions provided herein.

Extrusion can be conducted using, for example, a Leistritz Nano-16 twin-screw co-rotating extruder (American Leistritz Extruder Corp. USA, Sommerville, NJ). Active cooling of the barrel section can be used to limit denaturation of proteins. Active cooling of the die section can be used to limit expansion of the extruded product and excessive moisture loss. Protein feed and liquid are added separately: protein is fed by a volumetric plunger feeder or a continuous auger-type feeder, and liquid can be added into the barrel through a high pressure liquid injection system. Die nozzles with various inner diameters and channel length can be used for precise control of extrudiate pressure, cooling rate and product expansion. In some examples, extrusion parameters were: screw speed 100-200 rpm, die diameter 3 mm, die length 15 cm, product temperature at the end of the die of 50°C, feed rate of 2 g/min, and water-flow rate of 3 g/min. Product temperature at the die during extrusion is measured by a thermocouple.

Spun fibers can be produced by preparing a high viscosity protein "dope" by adding sodium hydroxide to concentrated protein solutions or to precipitated proteins, and forcing the solution with a plunger-type device (in some examples, a syringe with a syringe pump) through a small steel capillary (in some examples, 27 gauge hypodermic needle) into a coagulating bath. In some examples, the bath is filled with a concentrated

acid solution (e.g. 3 M hydrochloric acid). In some examples, the bath is filled with a buffer solution at a pH approximately equal to the isoionic point of the protein. Coagulating protein solution jet forms a fiber that collects at the bottom of the bath.

Bundles of spun fibers can be produced by forcing protein "dope" through spinnerets with many small holes. In some examples, spinnerets are stainless steel plates with approximately 25,000 holes per cm^2, with diameter of each hole approximately 200 microns. In some embodiments muscle tissue replica is produced by immersing the 3-dimensional matrix of fibers (connective tissue replica) in solutions of proteins and creating protein gels incorporating 3-dimensional matrix of fibers.

H. FAT REPLICAS

Animal fat is important for the experience of eating cooked meat and are important for some of the nutritional value of meat. Accordingly, the present invention provides compositions derived from non-animal sources, which recapitulate key features of animal fat, including the texture and/or flavor, by using components that mimic the chemical composition and physical properties of, for example, ground beef. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources, which recapitulates animal fat. Such a composition will be labeled herein as an "adipose replica" or a "fat replica." In some embodiments, the adipose replica and/or meat substitute product comprising the adipose replica are partially derived from animal sources. The consumable can also include adipose replicas that recapitulate key features of non-animal fats, including texture, flavor, firmness, percent fat release, and/or temperature of fat release. The fat content of the consumable can be at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, 80%, 90%, or 95% fat.

Ground beef is typically prepared by mixing lean beef with adipose (fat) that is cut from steaks, with the adipose tissue added to 16-30% (Cox 1993). Without adipose, meat passed through a grinder is tough, crumbly, and dries out quickly. Fat is added to lean beef so that fat released during cooking provides a liquid surface to aid in cooking, and to generate key beef flavors, which largely are products of the fatty acids.

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Engineering an adipose tissue replica that plays the same key roles in texture and flavor of the plant-based ground beef is an important driver for texture and flavor.

The adipose tissue replicas described here have a great health benefit over the beef adipose tissue as the fatty acid composition can be controlled such that the amount of saturated fats can be decreased. Additionally, the plant-based adipose replicas are cholesterol free. The plant-based adipose replicas can contain a lower percent total fat, and still have the same amount of fat being released or retained for the desired cooking properties, flavor, and texture.

As described herein, adipose replicas comprising emulsions of plant-derived lipids and one or more isolated and purified proteins can be produced in which the composition (e.g., fatty acid composition), cooking characteristics (e.g., fat release temperature or percent fat release), and physical properties (e.g., firmness) can be controlled, allowing the plant based composition to mimic animal based adipose. The adipose tissue replica includes (1) a vegetable oil containing triacylglycerides of fatty acids; (2) one or more isolated and purified proteins from non-animal sources (e.g., a plant protein); and (3) a phospholipid such as lecithin. The proteins can be plant or microbial proteins as described above (e.g., RuBisCo, an oleosin, an albumin, a globulin, or other seed storage protein). See, also the proteins described in Sections III A and B. The vegetable oils can be any of the oils described herein. See, e.g., Section III C.

The fat replica can be a gelled emulsion. In some embodiments, the gel is a soft, elastic gel comprising proteins and optionally carbohydrates. The gelled emulsion can comprise a protein solution comprising multiple proteins, e.g., 1-5 or 1-3 isolated and purified proteins, wherein the protein solution accounts for 1-30% of the volume of the emulsion. The gelled emulsion can comprise a fat droplet, wherein the fat droplet accounts for 70-99% of the volume of the emulsion. The gelled emulsion can comprise an isolated, purified cross-linking enzyme, wherein the cross-linking enzyme accounts for 0.0005% to 0.5% of the emulsion weight by volume, 0.5-2.5% of the emulsion weight by volume, or 0.001% or lower of the emulsion weight by volume. The emulsion of fat droplets in the protein solution can be stabilized by forming the emulsion into a gel by the cross-linking enzyme, e.g., a transglutaminase, by gelling proteins via heating and

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cooling protein solutions, by forming a cold-set gel, by formation of a coacervate, or by combinations of these techniques as described for coacervates in section C and gel formation in section F.

In some embodiments, the fat replica comprises cross-linking enzymes that catalyze reactions leading to covalent crosslinks between proteins. Cross-linking enzymes can be used to create or stabilize the desired structure and texture of the adipose tissue replica, to mimic the desired texture of an equivalent desired animal fat. In some embodiments, the cross-linking enzymes are isolated and purified from a non-animal source, examples and embodiments of which are described herein. In some embodiments, the fat replica comprises at least 0.0001%, at least 0.001%, at least 0.01%, at least 0.1%, or at least1% (wt/vol) of a cross-linking enzyme. The cross-linking enzyme can be selected from, for example, transglutaminases, tyrosinases, lipoxygenases, protein disulfide reductases, protein disulfide isomerases, sulfhydryl oxidases, peroxidases, hexose oxidases, lysyl oxidases, and amine oxidases. In some embodiments, the cross-linking enzyme is transglutaminase, a lysyl oxidase (e.g., a *Pichia pastoris* lysyl oxidase), or other amine oxidase.

The fat replica can comprise a gel with droplets of fat suspended therein. The fat droplets used in some embodiments of the present invention can be from a variety of sources. In some embodiments, the sources are non-animal sources (e.g., plant sources). See, e.g., the examples provided in Section III C. In some embodiments, the fat droplets are derived from animal products (e.g., butter, cream, lard, and/or suet). In some embodiments, fat droplets are derived from pulp or seed oil. In other embodiments, the source may be algae, yeasts, oleaginous yeasts such as *Yarrowia lipolytica*, or mold. For instance, in one embodiment, triglycerides derived from *Mortierella isabellina* can be used. In some embodiments, the fat droplets contain synthetic or partially synthetic lipids

In some embodiments, the fat droplets are stabilized by addition of surfactants, including but not limited to phospholipids, lecithins, and lipid membranes. The lipid membranes may be derived from algae, fungi or plants. In some embodiments the surfactants comprise less than 5% of the fat replica. The fat droplets can in some examples range from 100 nm to 150 μm in diameter. The diameter of these stabilized

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droplets may be obtained by homogenization, high-pressure homogenization, extrusion or sonication.

In some embodiments, plant oils are modified to resemble animal fats. The plant oils can be modified with flavoring or other agents such as heme proteins, amino acids, organic acids, lipids, alcohols, aldehydes, ketones, lactones, furans, sugars, or other flavor precursor, to recapitulate the taste and smell of meat during and after cooking. Accordingly, some aspects of the invention involve methods for testing the qualitative similarity between the cooking properties of animal fat and the cooking properties of plant oils in the consumable.

In some embodiments, additional polysaccharides can be added to a fat replica, including flax seed polysaccharides and xanthan gum.

The creation of a plant-based adipose replica requires stabilization of the oil in water emulsions. Typically animal adipose tissue contains ~95% fat, and is stabilized by the phospholipid bilayer and associated proteins. Adipose replicas described herein can be created with up to 95% fat in some instances, with 80% fat under many conditions, or with lower amounts of fat (e.g., 50% or less) while mimicking the properties of animal fat. Achieving a high percent fat is controlled by the stabilization of the emulsion.

The composition (e.g., fatty acid composition), cooking characteristics (e.g., fat release temperature or percent fat release), and physical properties (e.g., firmness) can be manipulated by controlling the type and amount of fat, the amount of protein, the type and amount of lecithin, the presence of additives, and the method of gelling.

In some embodiments, the protein component comprises about 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, or 20%, 25%, or more of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises about 0.1-5% or about 0.5-10% or more of the fat replica by dry weight or total weight. In some embodiments, the protein component is 0.5 to 3.5% or 1 to 3% of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises a solution containing one or more isolated, purified proteins. The type of protein can affect the stability of the emulsion, RuBisCo and pea albumins allow for fat replicas to made a greater than 90%

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fat. Addition of polysaccharides including flax seed and xanthan gum aid in emulsifying the mixture, allowing for an increase in fat content.

The type and amount of fat can be controlled by choosing the source of the fat and its lipid composition. In general, oils with higher amounts of saturated fatty acids are better able to be emulsified at lower protein concentrations, while oils with more unsaturated fatty acids require higher protein concentrations to be emulsified. Protein is required to stabilize the emulsion, and an increase in protein content increases the stability. If the amount of protein added is too little to emulsify the amount of fat, the mixture will separate into layers.

Lecithin also is a modulator of emulsion, and can either stabilize or disrupt the emulation depending on the amount of protein present and type of oil used. For example, lecithin may disrupt the protein/fat matrix to make a less stable emulsion, but can be added at low levels to modulate other physical properties. Emulsions made from oils with higher amounts of unsaturated fats can be de-stabilized by a high amount of lecithin (1%), such that the emulsion does not solidify. Emulsions made from oils with higher amounts of saturated fats can solidify at high amounts of lecithin (1%), but are very soft.

As described herein adipose replicas can be prepared that can range from very soft to very firm. The composition and amount of the fat controls the firmness of the replica. Firmer oils, which contain more long-chained saturated fats, make firmer gels. Oils that produce softer gels typically contain more unsaturated fatty acids or short-chained saturated fatty acids. In general, the firmness of the gel increases as total percent fat increases, as long as the emulsion is held and does not separate. The amount of protein also contributes to the firmness of the replica. In general, an increase in protein concentration increases replica firmness. The amount of lecithin is a modulator of replica firmness. Higher amounts of lecithin (1%) are much softer than lower amounts of lecithin (0.05%) when gels are formed with high percent protein (3%). When protein is decreased (1.8%), all gels are softer, and there is little difference in firmness between low level of lecithin (0.05%) and high (1%) if emulsion is held.

Addition of polysaccharides to replicas including but not limited to xanthan gum and flax seed paste can increase the firmness of adipose-replica gels.

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When an adipose replica is cooked, fat leaks from the structured replica as it is cooked. Often there is fat that remains in the cooked product; it is important to achieve the balance between fat released to aid in cooking and fat retained for texture and taste. The percent fat released (per total fat) can be determined by measuring the amount of fat released upon cooking to completion. Percent fat released is reported as the weight of fat released per the total fat of the replica. For example, the percent fat release of an adipose tissue replica described herein can be 0 to 10%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, 50% to 60%, 60% to 70%, 70% to 80%, 80% to 90%, or 90% to 100% upon cooking. Adipose replicas typically release 0- 90% fat under standard cooking conditions. In comparison, beef adipose tissue typically releases 40-55% fat under equivalent conditions.

While vegetable oils have set melting temperatures, the range of temperatures over which adipose replicas can be made to release fat is wide. Fat release temperature is the temperature at which fat is visibly released from the replica at the cooking surface. As described herein, the fat release temperature of the adipose replica can be tailored based on the type and amount of fat, the amount of protein, the type and amount of lecithin, the presence of additives, the method of emulsification, and the method of gelling. The resulting adipose replicas can have a fat release temperature of between 23°C to 33°C, 34°C to 44°C, 45°C to 55°C, 56°C to 66°C, 67°C to 77°C, 78°C to 88°C, 89°C to 99°C, 100°C to 110°C, 111°C to 121°C, 122°C to 132°C, 133°C to 143°C, 144°C to 154°C, 155°C to 165°C, 166°C to 167°C, 168°C to 169°C, 170°C to 180°C, 181°C to 191°C, 192°C to 202°C, 203°C to 213°C, 214°C to 224°C, 225°C to 235°C, 236°C to 246°C, 247°C to 257°C, 258°C to 268°C, 269°C to 279°C, 280°C to 290°C, or 291°C to 301°C. Beef fat was measured to release fat at 100-150°C.

Emulsification also is a factor in controlling the temperature of fat release: Once fats are incorporated into a replica with protein, or protein and lecithin, the temperature at which the fat is released increases significantly above the temperature at which the fat alone melts.

Fatty acid composition also is a factor in the fat release temperature and percent fat release. Vegetable oils that contain a higher proportion of unsaturated fatty acids have

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low melting temperatures and many are liquid at room temperature. Vegetable oils that contain a higher proportion of saturated fatty acids have a higher melting temperature, and are solid at room temperature. Replicas with a greater amount of unsaturated fats have a higher temperature of fat leakage than the same replica made with more saturated fatty acids. Gels made from 75% oils with higher amounts of unsaturated fatty acids, a high protein content (3%), and minimal lecithin content (0.05%), where the mixture was emulsified by a hand-held homogenizer and gelled using the heat-cool method, can be heated to 200°C with little or no fat release. Replicas containing oils with more long-chained saturated fats typically have more fat release at high protein content, but release less percent total fat compared to replicas containing oils with more short chained fats and at low percent protein. Gels made from oils with a higher proportion of short-chained saturated fatty acids, high protein content (3%), and minimal lecithin content (0.05%), can be heated to 200°C with little fat release.

The percent of fat release as an adipose replica is cooked is also is a function of the amount of protein and the amount of lecithin. Typically, the adipose replica contains 1-3% protein by mass. Increasing protein content leads to increasing temperatures of fat release, and reduces the fraction of fat released. Increasing the lecithin content to 1% can decrease the fat release temperature to 60-115°C, and increase the fraction of fat released (e.g., 25-30%). The source or composition of the lecithin used can modulate the amount of fat release and the temperature threshold for fat release. Without being bound to a particular mechanism, it is thought that lecithin destabilizes the emulsion by disrupting the protein-protein interactions. In one embodiment, at a high protein concentration of 3%, increasing the lecithin content to 1% decreased the fat release temperature to 55-60°C, and increased the percent fat leaked to 60-65%.

The method of making the emulsion also is a factor in determining the amount of fat release. Emulsification forms a homogenous mixture of the fat held in a matrix of proteins and lecithin. Methods of emulsification can include high-pressure homogenization, sonication, or hand homogenization. The alternative methods result in characteristic differences in the size of the oil droplets in the emulsion, which influences the stability of the resulting emulsions and the maximum fat concentration at which

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stable emulsions can be formed.

The method of gelling the replica also is a factor in determining the amount of fat release. While adipose replicas can be formed without forming gels, gelation results in a firmer and more stable emulsion. Methods of gelling are described above, and can include, for example, the addition of a crosslinking enzyme such as a transglutaminase (TG), or subjecting the emulsion to a heat/cool cycle. For example, either treatment with TG or the heat/cool method can convert an emulsion, as described above, into a gel. Furthermore, gelled emulsions formed by crosslinking catalyzed by TG typically release fat at a temperature higher than that at which emulsions gelled by the heat/cool technique do so. Gels formed by crosslinking with a TG also typically release less fat than do gels formed by the heat-cool technique.

In some embodiments, a fat replica can be made with a protein content <1.5% and a minimal lecithin content (0.05%) and have a fat release temperature of 45-65°C, and a high amount of fat released (e.g., 70-90%). These gels are at the higher end of percent fat released.

In some embodiments, a fat replica can be made with a lower protein content (<1.5%) and a high lecithin (>1%), and have a lower fat release temperature (e.g., 30-50°C, e.g., 30 to 45°C), and with an intermediate percent fat leaked (45-65%). Thus, in gels formed from oils with short-chained fatty acids or long-chained fatty acids at low protein concentrations, lecithin may play a role in stabilizing the emulsion.

In some embodiments, >2% rubisco or pea albumin can be used to produce adipose replicas with greater than 70% fat. In some embodiments, gels formed with > 3% isolated and purified protein can result in adipose replicas with greater than 70% fat.

In some embodiments, adipose replicas made from oils with a higher proportion of long-chained saturated fatty acids, a protein content of 3%, and a minimal lecithin content (0.05%), can release fat at a temperature similar to that at which beef fat does so (50-100°C), and can release a low to intermediate level of fat (15-45%).

In some embodiments, an adipose replica with a higher protein concentration (> 3%), and a lecithin content > 1% can have a fat release temperature of 50-70°C, and a higher amount of fat release (50-80%). At high protein and low lecithin concentration,

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gels with higher saturated fatty acids typically leak about 10% more fat than do corresponding gels formed with unsaturated fats.

In some embodiments, protease treatment of the protein constituents before gel formation can lead to an increase in fat release.

In some embodiments, a adipose tissue replica matrix stabilized by crosslinking enzymes releases more fat than a adipose tissue replica matrix stabilized by heat/cool protein denaturation. In one embodiment, an adipose tissue matrix comprised of moong bean 8S protein and canola oil, or an equal mixture of coconut, cocoa, olive, and palm oils, retains more mass when formed upon heat/cool denaturation than when formed by cross-linking with an enzyme. In one embodiment, an adipose tissue matrix formed by heat/cool denaturation of a preformed protein-oil emulsion containing Rubisco and cocoa butter, has a higher melting temperature than an adipose replica of similar composition stabilized by a cross-linking enzyme.

In some embodiments, adipose tissue replicas constructed from 1.4% wt/v moong bean 8S protein with 90% v/v canola oil and 0.45% wt/v soybean lecithin, can be homogenized in the presence of variable concentrations of sunflower oleosins. Concentration of oleosins can be varied from 1:10 to $1:10^6$ molar ratio of oleosin:triglyceride. An increase in mass retention after cooking is observed as the concentration of oleosins in the adipose tissue replica increase.

Firmness of an adipose tissue replica constructed as stabilized protein-fat emulsion can be modified by varying the concentration of the protein within adiopose tissue replica matrix. For example, a series of adipose tissue replicas formed with varying concentrations of Rubisco with 70-80% v/v sunflower oil, varied in firmness. Adipose tissue replicas with 0% and 0.18% (wt/vol) Rubisco were very soft, whereas replicas formed with 1.6% (wt/vol) Rubisco were soft, and replicas formed with 1.9% (wt/vol) Rubisco were medium in firmness.

In one embodiment, the firmness of adipose replica formed by stabilizing protein oil emulsion can be modified by varying amount of protein in the adipose replica. In one embodiment, adipose tissue replicas made from Rubisco and 70% sunflower oil are softer

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at lower concentrations, such as 1%, of RuBisCo than at higher concentrations of Rubisco, such as 3%, in adipose tissue replicas.

In another aspect, the invention provides methods for making a fat replica. The fat can be isolated and homogenized. For example an organic solvent mixture can be used to help solubilize a lipid in a gel and then removed to provide the final gel. At this point, the lipid can be frozen, lyophilized, or stored. So in one aspect, the invention provides for a method for isolating and storing a lipid, which has been selected to have characteristics similar to animal fat. The lipid film or cake can then be hydrated. The hydration can utilize agitation or temperature changes. The hydration can occur in a precursor solution to a gel. After hydration the lipid suspension can be sonicated, homogenized, high-pressure homogenized or extruded to further alter the properties of the lipid in the solution.

In some embodiments, the fat replica is assembled to approximate the organization of adipose tissue in meat. In some embodiments some or all of the components of the fat replica are suspended in a gel (e.g., proteinaceous gel). In other embodiments, the gel can be a hydrogel, an organogel, or a xerogel. In some embodiments, the gel can be thickened to a desired consistency using an agent based on polysaccharides or proteins. For example fecula, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, alginin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, cellulose, methylcellulose, hydroxymethylcellulose, acadia gum, konjac, starch, pectin, amylopectin or proteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, of fungi can be used alone or in combination to thicken the gel, forming an architecture or structure for the consumable.

In some embodiments, the tensile strength of the fat replica mimics the tensile strength of adipose tissue. The tensile strength of the gelled emulsions can be increased by incorporation of fibers. The fibers may be derived from non-animal sources including, but not limited to, watermelon, jack fruit, squash, coconut, green hair algae, corn and/or cotton. In some embodiments, the fibers are derived from self-polymerization of proteins, e.g., oleosins and prolamins. In some embodiments the fibers

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are derived from electrospun or extruded proteins. The fibers may form a three dimensional mesh or strands wherein each fiber may be less than 1 mm in diameter.

The adipose replica can be an emulsion comprising a solution of one or more proteins and one or more fats suspended therein as droplets. Slowly adding the oil phase to the aqueous phase can provide a more robust emulsion and prevents occasional failures to emulsify. Adding lecithin can, in some circumstances, destabilize a protein-stabilized emulsion, allowing for increased fat leakage when the replica is cooked. In some embodiments, the emulsion is stabilized by one or more cross-linking enzymes into a gel. In some embodiments, the emulsion is stabilized by a matrix formed by proteins induced to gel by a heat-cool technique or a cold-set-gel technique. Heating a protein-stabilized emulsion can heat denature the proteins leading to an increase in the firmness of the adipose replica. Heating to a sufficient temperature also can reduce the viability of natural microflora by at least 100x. In some embodiments, the emulsion is stabilized by gelled protein matrix formed by a combination of one or more protein cross-linking enzymes and a heat/cool technique or a cold-set gel technique. After the emulsion has sufficiently cooled, but before gelling is complete, one or more optional ingredients can be added, such as a heme-containing protein (e.g., up to about 0.4% such as 0.15, 0.2., 0.25, 0.3, or 0.4%) to give the adipose a more natural looking pink color and/or one of more flavor compounds such as amino acids, sugars, thiamine, or phospholipids to provide an improved flavor to the final product.

The one or more proteins in solution can comprise isolated and purified proteins, e.g. a purified pea albumin enriched fraction, a purified pea globulin enriched fraction, a purified Moong bean 8S globulin enriched fraction, and/or a Rubisco enriched fraction. In other embodiments, the one or more fats are derived from plant-derived oils (a rice bran oil or canola oil). See, e.g., Section III C. In some cases the composition comprises a cross-linking enzyme such as a transglutaminase, lysyl oxidase, or other amine oxidase. Thus, in some embodiments, an adipose tissue replica can be made by isolating and purifying one or more proteins; preparing a solution comprising one or more proteins; emulsifying one or more fats in the solution; and stabilizing the solution into a gelled emulsification with one or more cross-linking reagents.

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In some embodiments, the fat replica is a high fat emulsion comprising a protein solution of purified pea albumin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel.

In some embodiments, the fat replica is a high fat emulsion comprising a protein solution of isolated moong bean 8S globulin emulsified with 40-80% rice bran oil or 40-80% canola oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel.

The fat can be isolated from plant tissues and emulsified. The emulsification can utilize high-speed blending, homogenization, high pressure homogenization, sonication, shearing, agitation or temperature changes. The lipid suspension can be sonicated or extruded to further alter the properties of the lipid in the solution. At this point, in some embodiments other components of the consumable are added to the solution followed by a gelling agent. In some embodiments crosslinking agents (e.g. transglutaminase or lysyl oxidase) are added to bind the components of the consumable. In other embodiments the gelling agent is added and the lipid/gel suspension is later combined with additional components of the consumable.

CONTROL OF MELTING POINT BY CONTROL OF FAT COMPOSITION

The process of cooking meat is integral to the experience of using and enjoying meat. One important property of meat is that as the meat is heated, fats are released from the meat, which lubricates the cooking surface and increases heat transfer and is a component of the visual, aural and olfactory experience of cooking meat. The amount of fat that is released rather than retained during cooking varies with cooking temperature and contributes to the visual, aural and olfactory experience of cooking meat.

The composition and ratio of fatty acids in triglycerides and phospholipids, along with the ratio of phospholipid headgroups, contribute to the generation of distinct flavor profiles of cooked meat. For example, increased levels of phosphatidylcholine and phosphatidylethanolamine in fat provide a more intense beef flavor. As discussed above, the flavor of meat replicas can be modified by varying the ratios and type of different oils, and phospholipids that comprise the meat replica. For example, the flavor of the cooked meat replica can be controlled by varying the amount of phospholipids, sterols

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and lipids (e.g., 0.2-1% wt/wt). In one embodiment, the flavor of the cooked meat replica can be controlled by varying the ratio of different phospholipid headgroups.

In some embodiments, the phospholipids comprise a plurality of amphipathic molecules comprising fatty acids, glycerol and polar groups. See, e.g., Section III C for examples of fatty acids, phospholipids, polar groups, and sterols associated with phospholipids. See also section III C for examples of useful plant oils.

In different cuts of meat, the fat has different properties, ranging from the structurally important nature of fat in bacon to the soft melting behavior of the marbling fat in Wagyu beef.

By controlling the melting point of adipose tissue replicas in the consumables, it is possible to replicate the cooking experience of different meat types. For example, adipose tissue replicas created from fats with a melting point of 23°C to 27°C can have melting points analogous to adipose tissue from wagyu beef; adipose tissue replicas created from fats with a melting point of 35°C to 40°C can have melting points analogous to adipose tissue from regular ground beef; and adipose tissue replicas created from fats with a melting point of 36°C to 45°C can have melting points analogous to adipose tissue from bacon. Adipose tissue replicas can be created and incorporated into consumables such that a ratio of fat which is released and the ratio of fat which is retained by adipose tissue replica during cooking is similar to the fat properties of meat, e.g. from ground beef.

In some embodiments, the fat release temperature of a fat replicas can be controlled by mixing different ratios of vegetable oils containing triacylglycerides and phospholipids (e.g., lecithin). The melting point of fats is governed by the chemical composition of fatty acids. In general, fats comprising saturated fatty acids (e.g., C10:0, C12:0, C14:0, C16:0, C18:0, C20:0, C22:0) are solid at refrigeration temperatures (e.g., about 1°C to about 5°C and at room temperature (e.g., about 20°C to 25°C). By controlling the fat release temperature upon cooking in a adipose tissue replica, the firmness of a adipose tissue replica during refrigeration (e.g., about 1.5°C to about 4°C) and at ambient temperature (e.g., about 20°C to 25°C) can be controlled. Fats comprising monounsaturated fatty acids (e.g., C16:1 or C18:1) are generally solid at refrigeration

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temperatures and liquid at room temperatures. Fats comprising polyunsaturated fatty acids (e.g., C18:2, C18:3, C20:5, or C22:6) are generally liquid at refrigeration temperatures and at room temperatures. For example, virgin coconut oil melts at about 24°C while hydrogenated coconut oil melts at 36-40°C.

For example, adipose tissue replicas containing triglycerides and phospholipids that are liquid at room temperature (about 20°C to 25°C) will be softer than adipose tissue replicas containing triglycerides and phospholipids that are solid at refrigeration temperatures.

Adipose tissue replicas can contain oils from a single or multiple sources that are liquid at both refrigeration and ambient room temperatures (e.g., canola oil, sunflower oil, and/or hazelnut oil). In one embodiment, a adipose tissue replica contains oils from single or multiple sources that are solid at refrigeration temperature, but liquid at room temperature (e.g., olive oil, palm oil, and/or rice brain oil). In one embodiment, a adipose tissue replica contains oils from single or multiple sources that are solid at room temperature but liquid at mouth-temperatures (about 37°C) (e.g., palm kernel oil, coconut oil, and/or cocoa butter. In one embodiment, a adipose tissue replica contains oils from single or multiple sources that are solid at mouth temperature (about 37°C) (e.g., oil from mango butter).

In one embodiment, a adipose tissue replica includes triglycerides and phospholipids with a high ratio of saturated fatty acids, and is firmer than a adipose tissue replica containing a higher ratio of monounsaturated and polyunsaturated triglycerides and lipids. For example, a adipose tissue replica containing sunflower oil is softer than a adipose tissue replica containing cocoa butter. Adipose tissue replicas can be formed with 0%, 0.18%, 1.6%, or 2.4% wt/v Rubisco with 70%, 80%, or 90% v/v sunflower or cocoa butter. Each adipose tissue replica that contained cocoa butter was firmer than the replicas that were formed with sunflower oil.

In one embodiment, a adipose tissue replica made as a stable emulsion of moong bean 8S protein with sunflower oil is softer than a adipose tissue replica made as a stable emulsion of moong bean 8S protein and cocoa butter. Adipose tissue replicas formed with 2%, 1%, or 0.5% wt/v moong bean 8S protein with 70%, 80%, or 90% v/v

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sunflower or cocoa butters. Each adipose tissue replica that contained cocoa butter was firmer than the replicas that were formed with sunflower oil.

In one embodiment, a adipose tissue replica made as a stable emulsion of moong bean 8S protein with canola oil is softer than a adipose tissue replica made as a stable emulsion of moong bean 8S protein with an equal mixture of coconut, cocoa, olive, and palm oils. Adipose tissue replicas can be formed with 1.4% wt/v moong bean 8S protein with 50%, 70%, or 90% v/v sunflower or a mixture of oils. Each adipose tissue replica that contained a mixture of oils was firmer than the replicas that were formed with sunflower oil.

In one embodiment, an adipose tissue replica made as a stable emulsion of soy proteins with sunflower oil is softer than a adipose tissue replica made as a stable emulsion of soy proteins and cocoa butter. Adipose tissue replicas were formed with 0.6%, 1.6%, or 2.6% wt/v Soy with 50%, 70%, 80%, or 90% v/v sunflower or mixture of oils. Each adipose tissue replica that contained a mixture of oils was firmer than the replicas that were formed with sunflower oil.

In some embodiments, the adipose tissue replicas comprising 0%, 0.18%, 1.6%, and 2.4% wt/v Rubisco with 70%, 80%, and 90% v/v cocoa butter are solid at room temperature but melt at about mouth temperature. In some embodiments, the adipose tissue replicas comprising 0.6%, 1.6%, and 2.6% wt/v soy with 50%, 70%, 80%, and 90% v/v cocoa butter are solid at room temperature but melt at about mouth temperature. In some embodiments, the adipose tissue replicas comprising 1.4% wt/v moong bean 8S protein with 50%, 70%, and 90% v/v of an equal mixture of coconut, cocoa, olive, and palm oil is solid at room temperature but melts at about mouth temperature. In one embodiment, the melting temperature of adipose tissue replicas will be similar to beef fat. In some embodiments the fat replicas comprise oils with a 1:1 ratio of saturated to unsaturated fatty acids. In some embodiments, the adipose tissue replica contains equal amounts of cocoa and mango butters. In some embodiments, the adipose tissue replica contains equal amounts of coconut oil, cocoa butter, olive oil and palm oil.

In one embodiment, a adipose tissue replica that comprises triglycerides and phospholipids will contain a ratio of fatty acids similar to that found in beef (C14:0 0-5%

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wt/wt, C16:0 0-25%, C18:0 0-20%, C18:1 0-60%, C18:2 0-25%, C18:3, 0-5%, C20:4 0-2%, and C20:6 0-2%). For example the adipose tissue replica may comprise equal proportions of olive oil, cocoa butter, coconut oil and mango butter. In another example, the adipose tissue replica may comprise equal proportions of olive oil and rice brain oil.

In one embodiment, the melting temperature of adipose tissue replicas will be similar to Wagyu beef fat. In some embodiments the fat replicas comprise oils with a 1:2 ratio of saturated to unsaturated fatty acids (e.g., for example 1 part coconut oil to 2 parts sunflower oil). In some embodiments, the adipose tissue replica contains equal amounts of olive oil, rice bran oil, cocoa butter and mango butter.

I. CONNECTIVE TISSUE REPLICA

Animal connective tissue provides key textural features that are an important component of the experience of eating meat. Accordingly, the present invention provides a composition derived from non-animal sources which recapitulates key features of animal connective tissue. The present invention additionally provides a meat substitute product that comprises a composition derived from non-animal sources, which recapitulates important textural and visual features of animal connective tissue. Such compositions will be labeled herein as "connective tissue replicas". In some embodiments, the connective tissue replica and/or meat substitute product comprising the connective tissue replica are partially derived from animal sources.

Animal connective tissue can generally be divided into fascia-type and cartilage-type tissue. Fascia-type tissue is highly fibrous, resistant against extension (has high elastic modulus), and has a high protein content, a moderate water content (ca. 50%), and low-to-no fat and polysaccharide content. Accordingly, the present invention provides a connective tissue replica that recapitulates key features of fascia type tissue. In some embodiments, the connective tissue replica comprises about 50% protein by total weight, about 50% by liquid weight, and has a low fat and polysaccharide component.

The fibrous nature of fascia type connective tissue is largely comprised of collagen fibers. Collagen fibers are observed to be cord or tape shaped species, 1-20 microns wide. These fibers consist of closely packed thin collagen fibrils 30 to 100

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nanometers thick. These fibrils also associate into elastic and reticular fibrous networks with individual fibers that may be 200 nanometers thick.

In one embodiment, the fascia-type connective tissue replica consists of a fibrous or fibrous-like structure which can consist of proteins. In some embodiments, the protein content is derived from non-animal source (e.g., a plant source, algae, bacteria, or fungi, see e.g., Sections IIIA and B). In some embodiments, the isolated proteins account for 50%, 60%, 70%, 80%, or 90% or more of the protein content by weight. In some embodiments, multiple isolated proteins are isolated and purified separately and account for the total protein content.

In fascia-type connective tissue, the prolamin family of proteins, individually or combinations thereof, demonstrates suitability for the protein component because they are highly abundant, similar in global amino acid composition to collagen (high fraction of proline and alanine), and amenable to processing into films. In some embodiments, the prolamin family proteins are selected from the group consisting of zein (found in corn), hordein from barley, gliadin from wheat, secalin, extensins from rye, kafirin from sorghum, or avenin from oats. In some embodiments, the one or more isolated and purified proteins is zein. In some embodiments, other proteins can be used to supplement prolamins in order to achieve targets specifications for physicochemical and nutritional properties. See, the list in Sections III A and B, including any major seed storage proteins, animal-derived or recombinant collagen, or extensins (hydroxyproline-rich glycoproteins abundant in cell walls e.g. *Arabidopsis thaliana,* monomers of which are "collagen-like" rod-like flexible molecules).

The proteins can be freeze-dried and milled and combined with one or more other ingredients (e.g., wheat gluten, fiber such as bamboo fiber, or soy protein isolate).

The fibrous or fibrous-like structures can be formed by extrusion. In some embodiments extrusion are conducted using Leistritz Nano-16 twin-screw co-rotating extruder (American Leistritz Extruder Corp. USA, Sommerville, NJ). Active heating and cooling of the barrel section is used to optimize the mechanical properties, extent of puffing and water content of fibers. For example, water content can be adjusted to about 50% to make a hard connective tissue replica. Protein feed and liquid are added

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separately: protein is feed by a volumetric plunger feeder, and liquid is added into the barrel through a high pressure liquid injection system. In some examples, extrusion parameters were: screw speed 200 rpm, product temperature at the die of 120°C, feed rate of 2.3 g/min, and water-flow rate of 0.7 g/min. Product temperature at the die during extrusion is measured by a thermocouple.

The fibrous or fibrous-like structures can be formed by extrusion through filament and multi-filament dies to produce fibrous structures. In some embodiments, dies incorporating multiple different orifice sizes in the range 10-300 microns can be used to create mixed fibrous tissue replicas with precise control over dimensions and compositions of fibers. Fibers of different sizes can be incorporated into the compositions to control the properties of the compositions.

Electrospinning can be used to create fibers in the <1-10 micron range. In some embodiments, electrospinning is used to create fibers in the <1-10 micron diameter range. For example, a concentrated solution of moong bean globulin (140 mg/ml) containing 400 mM sodium chloride can be mixed with solution of poly(vinyl alcohol) or poly(ethylene oxide) (9% w/v) to obtain mixed solutions with 22.5 mg/ml moong bean globulin and 6.75% w/v of the respective polymer. The resulting solution is slowly (for example, at 3 µl/min) pumped, using a syringe pump, from a 5 ml syringe through a Teflon tube and a blunted 21gauge needle. The needle is connected to a positive terminal of a high voltage supply (for example, Spellman CZE 30 kV) and fixed 20-30 cm from a collection electrode. Collection electrode is an aluminum drum (ca. 12 cm long, 5 cm in diameter) that is wrapped in aluminum foil. The drum is attached to a spindle that is rotated by an IKA RW20 motor at about 600 rpm. The spindle is connected to a ground terminal of the high voltage supply. Portein/polymer fiber accumulate on foil and, after electrospinning is completed, are removed from foil and added to tissue replicas.

The dimension and composition of the fibers produced by the methods of the invention has an effect upon the taste, texture, and mechanical properties of the tissue replicas. Tissues comprising between 1 and 50% of the fibers in the <1-10 micron range, and between 10 and 50% of fibers in the 10-300 micron range most closely approach animal connective tissues in terms of taste, mouthfeel and mechanical properties.

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Cartilage-type tissue is macroscopically homogenous, resistant against compression, has higher water content (up to 80%), lower protein (collagen) content, and higher polysaccharide (proteoglycans) contents (ca. 10% each). Compositionally, cartilage-type connective tissue replicas are similar to fascia-type tissue replicas with the relative ratios of each adjusted to more closely mimic 'meat' connective tissue. During extrusion, water content can be adjusted to about 60% to make a soft connective tissue replica.

Methods for forming cartilage-type connective tissue are similar to those for fascia-type connective tissue, but methods that produce isotropic non-fibrous gels are preferred.

A connective tissue replica can be made by isolating and purifying one or more proteins; and precipitating the one or more proteins, wherein the precipitating results in the one or more proteins forming physical structures approximating the physical organization of connective tissue. The precipitating can comprise solubilizing the one or more proteins in a first solution; and extruding the first solution into a second solution, wherein the one or more proteins is insoluble in the second solution, wherein the extruding induces precipitation of the one or more proteins.

In some embodiments some or all of the components of the consumable are suspended in a gel (e.g., a proteinacious gel). In various embodiments the gel can be a hydrogel, an organogel, or a xerogel. The gel can be thickened using an agent based on polysaccharides or proteins. For example fecula, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, alginin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, cellulose, methylcellulose, hydroxymethylcellulose, acadia gum, konjac, starch, pectin, amylopectin or proteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, of fungi can be used alone or in combination to thicken the gel, forming an architecture or structure for the consumable. Enzymes that catalyze reactions leading to covalent crosslinks between proteins can also be used alone or in combination to form an architecture or structure for the consumable. For example transglutaminase, tyrosinases, lysyl oxidases, or other

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amine oxidases (e.g. *Pichia pastoris* lysyl oxidase (PPLO)) can be used alone or in combination to form an architecture or structure for the consumable by crosslinking the component proteins. In some embodiments, multiple gels with different components are combined to form the consumable. For example a gel containing a plant-derived protein can be associated with a gel containing a plant-derived fat. In some embodiments fibers or strings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

The compositions of the invention can be puffed or expanded by heating, such as frying, baking, microwave heating, heating in a forced air system, heating in an air tunnel, and the like, according to methods well known in the art.

In some embodiments multiple gels with different components are combined to form the consumable. For example a gel containing a plant-derived protein can be associated with a gel containing a plant-derived fat. In some embodiments fibers or strings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

J. OMISSIONS FROM THE COMPOSITIONS

Because the consumable can be put together from defined ingredients, which may themselves be isolated and purified, it is possible to produce consumables that do not contain certain components. This, in some cases, allows for the production of consumables that are lacking ingredients that may be not desirable to consumers (e.g., proteins that some humans are allergic to can be omitted or additives). In some embodiments, the consumable contains no animal products. In some embodiments the consumable contains no or less than 1% wheat gluten. In some embodiments the consumable contains no methylcellulose. In some embodiments the consumable contains no carrageenan. In some embodiments the consumable contains no caramel color. In some embodiments the consumable contains no Konjac flour. In some embodiments the consumable contains no gum arabic (also known as acacia gum). In some embodiments the consumable contains no wheat gluten. In some embodiments the consumable contains no soy protein isolate. In some embodiments the consumable contains no tofu. In some

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embodiments the consumable contains less than 5% carbohydrates. In some embodiments the consumable contains less than 1% cellulose. In some embodiments the consumable contains less than 5% cellulose. In some embodiments the consumable contains less than 5% insoluble carbohydrates. In some embodiments the consumable contains less than 1% insoluble carbohydrates. In some embodiments the consumable contains no artificial color. In some embodiments the consumable contains no artificial flavorings.

In some embodiments the consumable contains one or more of the following characteristics: no animal products; no methylcellulose; no carrageenan; no Konjac flour; no gum arabic; less than 1% wheat gluten; no wheat gluten; no tofu; about 5% carbohydrates; less than 5% cellulose; less than 5% insoluble carbohydrates; less than 1% insoluble carbohydrates; no edible colorants such as caramel color, paprika, cinnamon, beet color, carrot oil, tomato lycopene extract, raspberry powder, carmine, cochineal extract, annatto, turmeric, saffron, F.D&C Red No.3, Yellow number 5, Yellow No 6., Green No.3, Blue No.2, Blue No. 1, Violet No. 1,FD&C Red No. 40 – Allura Red AC, and/or E129 (red shade); and/or no artificial flavorings. In some embodiments the consumable contains no soy protein isolate. In other embodiments, the consumable contains no soy protein or protein concentrate.

In some embodiments, the muscle tissue replica additionally contains less than 10%, less than 5%, less than 1%, or less than 0.1% wheat gluten. In some embodiments, the muscle tissue replica contains no wheat gluten.

IV. Combinations of the components

A. MEAT REPLICAS

A meat substitute product (alternatively a meat replica) can comprise compositions described herein. For example a meat replica can comprise a muscle replica; a fat tissue replica; and a connective tissue replica (or a sub-combination thereof). The muscle replica, adipose tissue replica, and/or connective tissue replica can be assembled in a manner that approximates the physical organization of meat. In some

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embodiments, a binding agent such as a coacervate is used to help binding the replicas to each other.

The percentage of different components may also be controlled. For example non-animal-based substitutes for muscle, adipose tissue, connective tissue, and blood components can be combined in different ratios and physical organizations to best approximate the look and feel of meat. The various components can be arranged to insure consistency between bites of the consumable. The components can be arranged to insure that no waste is generated from the consumable. For example, while a traditional cut of meat may have portions that are not typically eaten, a meat replicate can improve upon meat by not including these inedible portions (e.g, bone, cartilage, connective tissue, or other materials commonly referred to as gristle). Such an improvement allows for all of the product made or shipped to be consumed, which cuts down on waste and shipping costs. Alternatively, a meat replica may include inedible portions to mimic the experience of meat consumption. Such portions can include bone, cartilage, connective tissue, or other materials commonly referred to as gristle, or materials included simulating these components. In some embodiments the consumable may contain simulated inedible portions of meat products which are designed to serve secondary functions. For example a simulated bone can be designed to disperse heat during cooking, making the cooking of the consumable faster or more uniform than meat. In other embodiments a simulated bone may also serve to keep the consumable at a constant temperature during shipping. In other embodiments, the simulated inedible portions may be biodegradable (e.g., a biodegradable plastic).

In some embodiments, a meat substitute composition comprises between 10-30% protein, between 5-80% water, and between 5-70% fat, wherein the composition includes one or more isolated and purified proteins. Such a meat substitute can include no animal protein. In some embodiments, the meat substitute compositions comprise a transglutaminase.

In some embodiments, a meat substitute product includes a muscle replica, an adipose tissue replica, and connective tissue replica, where the muscle replica accounts for 40-90% of the product by weight, the adipose tissue replica accounts for 1-60% of the

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product by weight, and the connective tissue replica accounts for 1-30% of the product by weight.

In some embodiments, the meat substitute product comprises 60-90% water; 5-30% protein content; and 1-20% of a fat; wherein the protein content comprises one or more isolated and purified plant proteins.

In some embodiments the consumable contains components to replicate the components of meat. The main component of meat is typically skeletal muscle. Skeletal muscle typically consists of roughly 75 percent water, 19 percent protein, 2.5 percent intramuscular fat, 1.2 percent carbohydrates and 2.3 percent other soluble non-protein substances. These include organic acids, sulfur compounds, nitrogenous compounds, such as amino acids and nucleotides, and inorganic substances such as minerals. Accordingly, some embodiments of the present invention provide for replicating approximations of this composition for the consumable. For example, in some embodiments, the consumable is a plant-based meat replica comprising roughly 75% water, 19% protein, 2.5% fat, 1.2% carbohydrates; and 2.3 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 60-90% water, 10-30% protein, 1-20% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 60-90% water, 5-10% protein, 1-20% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 0-50% water, 5-30% protein, 20-80%% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances.

In some embodiments, a meat replica contains between 0.01% and 5% by weight of a heme containing protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin. Some meat also contains myoglobin, a heme containing protein, which accounts for most of the red color and iron content of some meat. It is understood that these percentages can vary in meat and the meat replicas can be produced to approximate the natural variation in meat. In embodiments that include a heme-containing protein and optional flavors, k-carrageenan can be used absorb some of

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the liquid contributed from the flavor and heme solution so the ground tissue is not excessively wet. During addition of flavor heme mix solution and the k-carrageenan powder are distributed evenly over the tissue mixture to ensure homogeneity in the final ground product.

It will be appreciated that when proteins are supplied as a solution, water removal techniques such as freeze-drying or spray drying can optionally be used to concentrate the protein. The proteins may then be reconstituted in an amount of liquid that prevents the ground tissue from being too moist.

Additionally, in some instances, the present invention provides for improved meat replicas, which comprise these components in unnatural percentages. The concentration of heme containing protein is an important determinant of meat flavor and aroma. Thus for example a meat replica could have a higher heme protein content than typical beef. For example a meat replica can be produced with a higher than typical average fat content. The percentages of these components may also be altered to increase other desirable properties.

In some instances a meat replica is designed so that, when cooked, the percentages of components are similar to cooked meat. So, in some embodiments, the uncooked consumable has different percentages of components than uncooked meat, but when cooked, the consumable is similar to cooked meat. For example, a meat replica may be made with a higher than typical water content for raw meat, but when cooked in a microwave, the resulting product has non-starch polysaccharides such as arabinoxylans, cellulose, and many other plant components such as resistant starch, resistant dextrins, inulin, lignin, waxes, chitins, pectins, beta-glucans, and oligosaccharide percentages of components similar to meat cooked over a fire.

In some embodiments, the consumable is a meat replica with a lower that typical water content for meat. In some embodiments the inventions provides for methods for hydrating a meat replica to cause the meat replica to have a water content similar to meat. For example a meat replica with a water content that would be low for meat, for example 1%, 10%, 20%, 30%, 40% or 50% water, can be hydrated to roughly 75% water. Once hydrated, in some embodiments, the meat replica is then cooked for human consumption.

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The consumable can have a protein component. In some embodiments the protein content of the consumable is 10%, 20%, 30%, or 40%. In some embodiments the protein content of the consumable is similar to meat. In some embodiments the protein content in the consumable is greater than that of meat. In some embodiments the consumable has less protein than meat.

The protein in the consumable can come from a variety or combination of sources. Non-animal sources can provide some or all of the protein in the consumable. Non-animal sources can include vegetables, non food biomass such as carrot tops and *Miscanthus*, seaweed, fruits, nuts, grains, algae, bacteria, or fungi. See, e.g, Sections III A and B. The protein can be isolated or concentrated from one or more of these sources. In some embodiments the consumable is a meat replica comprising protein only obtained from non-animal sources.

In some embodiments protein is formed into asymmetric fibers for incorporation into the consumable. In some embodiments these fibers replicate muscle fibers. In some embodiments the protein are spun fibers. Accordingly, the present invention provides for methods for producing asymmetric or spun protein fibers. In some embodiments the consumable contains a protein or proteins that have all of the amino acids found in proteins that are essential for human nutrition. In some embodiments the proteins added to the consumable are supplemented with amino acids.

The physical organization can be a determinant of the response of the meat substitute to cooking. For example, the flavor of meat is modified by the size of the particles. Ground meat that has been reduced to a paste provides different flavors than more crudely ground beef upon cooking. The ability to control the relative size and orientation of individual tissue replicas enables the flavor and aroma profile of consumables to be modified during cooking. For example, muscle tissue replicas and adipose tissue replicas provide different flavor profiles when cooked independently or when mixed. Further changes in the flavor profile are observed based on the method by which the different tissue replicas are mingled.

The physical organization of the meat substitute product can be manipulated by controlling the localization, organization, assembly, or orientation of the muscle, fat,

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and/or connective tissue replicas described herein. In some embodiments the product is designed in such a way that the replicas described herein are associated with one another as in meat. In some embodiments the consumable is designed so that after cooking the replicas described herein are associated with one another as in cooked meat.

Characteristic flavor and fragrance components of meat are mostly produced during the cooking process by chemical reactions, the substrates for which are amino acids, fats and sugars which are found in plants as well as meat. Therefore in some embodiments the consumable is tested for similarity to meat during or after cooking. In some embodiments human ratings, human evaluation, olfactometer readings, or GCMS measurements, or combinations thereof, are used to create an olfactory map of cooked meat. Similarly, an olfactory map of the consumable, for instance a meat replica, can be created. These maps can be compared to assess how similar the cooked consumable it so meat. In some embodiments the olfactory map of the consumable during or after cooking is similar to or indistinguishable from that of cooked or cooking meat. In some embodiments the differences are sufficiently small as to be below the detection threshold of human perception.

In some embodiments the individual tissue replicas are assembled in layers, sheets, blocks and strings in defined positions and orientations.

In some embodiments, the replicas are combined in the process of passing through the plates of a meat grinder with the holes set at less than 1/2 inch (e.g., at ¼ inch). The grinder provides multiple functions of reducing the particle size, providing additional mixing or working, and forming the material into cylindrical portions like what is typically done for ground beef. During the assembly, grinding, and forming, it is important to keep the replica tissues cold (e.g., 4 – 15°C) to control microbial growth, limit flavor reactions, and also to maintain the adipose in a solid state so discrete pieces of adipose will be maintained through the grinding process.

Prior to grinding, the replica tissues are usually broken down in some manner to a defined particle size. For example, in some embodiments, the individual tissue replicas can be formed into small pieces less than 1 cm in diameter or less than 5 mm in diameter before combination with the other tissue replicas. Adipose tissue can be crumbled into

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about 3 – 7 mm particles. This is important to both the appearance of the final material and the behavior of fat leakage during cooking. This size range allows for a natural appearance of adipose flecks in the final raw product. If the adipose tissues are too small (e.g., less than 2 mm), there will be an insufficient amount of fat leaked from the product when cooked.

Soft connective tissue replicas can be broken down into pieces of about 1 – 3 mm in length with ragged edges. If the pieces are too large (e.g., greater than about 4 mm), the texture of the final product can be too beady.

Sticky tissue or noodles tissue replicas, composed of amorphous or long noodle like tissue replica pieces, respectively, and raw tissue replicas can be manually broken down into pieces about 1 - 3 cm in diameter. Achieving particles in this size range allows for adequate mixing and suitable homogeneity in the final ground material.

In some embodiments, hard connective tissue replicas can be chopped to three levels, (e.g., coarse, intermediate, and fine). Chopping to three levels provides a greater amount of heterogeneity than a single step chopping process, and makes the mouthfeel of the final product more similar to ground beef.

In formulations containing gluten an additional function of the food grinder is to work the gluten and develop a gluten network of aligned gluten molecules. For gluten containing formulations it is important to minimize interaction of the adipose with the gluten network. This is done by prechilling the adipose replicas and ground tissue replicas prior to combining and also by minimizing the amount of manipulation after the adipose is added. Overworking of adipose replicas into the gluten will break down or "shorten" the gluten network.

Finally for gluten containing formulations the patties are allowed to rest at room temperature for 30 min or overnight at 4C prior to cooking. This allows time for the gluten network to relax, giving an overall better texture.

In some embodiments, the connective tissue replica is incorporated into the protein solution before formation of the muscle tissue replica.

In some embodiments, the connective tissue replica is directly incorporated into the emulsion before formation of the adipose tissue replica.

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In some embodiments, the adipose tissue replica is added to the muscle tissue replica in strands and sheets to replicate the effect of "marbling," or streaky bacon.

Mixed meat tissue replicas can increase the sense of flavors, such flavors including, but not limited to, multiple aromatic compounds associated with fruity/grean bean/metallic, nutty/green, peanut butter/musty, raw potato/roasted/earthy, vinegary, spicy/caramel/almond, creamy, sweet, fruity/stale beer, musty/nutty/coumarin/licorice/walnut/bread, coconut/woody/sweet, penetrating/sickening, minty, or toasty caramel aromas.

In some embodiments the mixed meat replicas increase the presence of volatile odorants, such as 2-pentyl-furan; 4-methylthiazole; ethyl pyrazine,; 2,3-dimethylpyrazine, acetic acid; 5-methyl-2-furancarboxaldehyde; butyrolactone; 2,5-dimethyl-3-(3-methyl butyl) pyrazine; 2-cyclopentene-1-one, 2-hydroxy-3-methyl; 3-acetyl-1h-pyrolline; pantolactone; 1-methyl 1(H)-pyrrole-2-2carboxaldehyde,; caprolactam; 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one. in some embodiments, the undesired flavors including but not limited to gasoline-like, petroleum, sour/putrid/fish-like, bland/woody/yogurt, fatty/honey/citrus, pungent/sweet/caramelic and nutty/burnt green aromas, form only in individual tissue replicas, but do not accumulate in mixed meat replicas. In some embodiments, the individual tissue replicas increase the presence of volatile odorants including but not limited to nonane, 2,6-dimethyl, 3-methyl 3-hexene,; pyridine; acetoin; octanal; 1-hydroxy-2-propanone; and/or ethenyl pyrazine. In some embodiments the levels to which all of the above compounds accumulate during cooking depend on the sizes of tissue replica units and how they are mixed (coarse, fine, or blended).

In some embodiments, the mixed meat tissue replicas increase the sense of flavors including but not limited to, multiple aromatic compounds associated with fruity/grean bean/metallic, nutty/green, peanut butter/musty, raw potato/roasted/earthy, vinegary, spicy/caramel/almond, creamy, sweet, fruity/stale beer, musty/nutty/coumarin/licorice/walnut/bread, coconut/woody/sweet, penetrating/sickening, minty, or toasty caramel aromas. In some embodiments, the mixed meat replicas increase the presence of volatile odorants including but not limited

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to phenylacetaldehyde, 1-octen-3-one, 2-n-heptylfuran, 2-thiophenecarboxaldehyde, 3-thiophenecarboxaldehyde, butyrolactone, 2-undecenal, methyl-pyrazine, furfural, 2-decanone, pyrrole, 1-octen-3-ol, 2-acetylthiazole, (E)-2-octenal, decanal, benzaldehyde, (E)-2-nonenal, pyrazine, 1-hexanol, 1-heptanol, dimethyl trisulfide, 2-nonanone, 2-pentanone, 2-heptanone, 2,3-butanedione, heptanal, nonanal, 2-octanone, 1-octanol, 3-ethylcyclopentanone, 3-octen-2-one, (E,E)-2,4-heptadienal, (Z)-2-heptenal, 2-heptanone, 6-methyl-, (Z)-4-heptenal, (E,Z)-2,6-nonadienal, 3-methyl-2-butenal, 2-pentyl-furan, thiazole, (E, E)-2,4-decadienal,, hexanoic acid, 1-ethyl-5-methylcyclopentene, (E,E)-2,4-nonadienal, (Z)-2-decenal, dihydro-5-pentyl-2(3H)-furanone, trans-3-nonen-2-one, (E,E)-3,5-octadien-2-one, (Z)-2-octen-1-ol, 5-ethyldihydro-2(3H)-furanone, 2-butenal, 1-penten-3-ol, (E)-2-hexenal, formic acid, heptyl ester, 2-pentyl-thiophene, (Z)-2-nonenal, 2-hexyl-thiophene, (E)-2-decenal,, 2-ethyl-5-methyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine, 2-ethyl-1-hexanol, thiophene, 2-methyl-furan, pyridine, butanal, 2-ethyl-furan, 3-methyl-butanal, trichloromethane, 2-methyl-butanal, methacrolein, 2-methyl-propanal, propanal, acetaldehyde, 2-propyl-furan, dihydro-5-propyl-2(3H)- furanone, 1,3-hexadiene, 4-decyne, pentanal, 1-propanol, heptanoic acid, trimethyl-ethanethiol, , 1-butanol, 1-penten-3-one, dimethyl sulfide, 2-ethyl furan, 2-pentyl-thiophene, 2-propenal, 2-tridecen-1-ol, 4-octene, 2-methyl thiazole,, methyl-pyrazine, 2-butanone, 2-pentyl-furan, 2-methyl-propanal, butyrolactone,, 3-methyl-butanal, methyl-thiirane, 2-hexyl-furan, butanal,, 2-methyl-butanal, 2-methyl-furan, furan, octanal, 2-heptenal, 1-octene, formic acid heptyl ester, 3-pentyl-furan, and 4-penten-2-one. In some embodiments the levels to which all of the above compounds accumulate during cooking depend on the sizes of tissue units and how they are mixed (coarse, fine, or blended).

The production of volatile odorants can be enhanced when the adipose, muscle and connective tissue replicas are contacting one another. In some embodiments, the production of volatile odorants is enhanced when the adipose, muscle and connective tissues are intimately mixed with a mean size of the individual tissue replicas of 5 mm. In some embodiments, the production of volatile odorants is enhanced when the fat, muscle and connective tissues are intimately mixed with mean size of the individual tissue replicas of 2 mm. In some embodiments, said production of volatile odorants is enhanced

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when the fat, muscle and connective tissue replicas are intimately mixed with mean size of the individual tissue replicas of 1 mm.

In some embodiments, the meat substitute is optimized for particular cooking methods (optimized for cooking in a microwave oven, or optimized for cooking in a stew).

In some embodiments, the meat substitute is optimized for dehydration.

In some embodiments, said meat substitute is optimized for fast rehydration upon exposure of the dehydrated meat replica to water.

In some embodiments, said meat substitute is optimized for use as emergency, camping or astronaut food.

The methods described herein can be used to provide a meat replica with defined cooking characteristics to allow the production of meat replicas that are optimized for particular cooking techniques. For example, stews require slow cooking to gelatinize the connective tissue in the meat, whereas meat replicas can be designed wherein the connective tissue replica is more easily gelatinized thus allowing stews to be prepared quickly.

B. INDICATORS OF COOKING MEAT

The consumable can include compositions which can indicate that the consumable is cooking or has cooked. The release of odorants upon cooking is an important aspect of meat consumption. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking beef. In some embodiments, the consumable when cooked generates an aroma recognizable by humans as typical of cooking pork, bacon, chicken, lamb, fish, or turkey. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with an odorant that is released upon cooking or that is produced by chemical reactions that take place upon cooking. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing mixtures of proteins, peptides, amino acids, nucleotides, sugars and polysaccharides and fats in

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combinations and spatial arrangements that enable these compounds to undergo chemical reactions during cooking to produce odorants and flavor-producing compounds.

In some embodiments, the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a volatile or labile odorant that is released upon cooking.

In some embodiments, the indicator is a visual indicator that accurately mimics the color transition of a meat product during the cooking progression. The color transition can be, for example, from red to brown, from pink to white or tan, or from a translucent to opaque color during the cooking progression.

In some embodiments, the indicator is an olfactory indicator that indicates cooking progression. In one embodiment, the olfactory indicator is one or more volatile odorants released during cooking.

In some embodiments, the indicator comprises one or more isolated, purified iron-containing proteins. In some embodiments, the one or more isolated, purified iron-containing proteins (e.g., a heme-containing protein, see section III B) is in a reduced state before cooking. In some embodiments, the one or more isolated and purified iron carrying proteins in a reduced or oxidized state has a similar UV-VIS profile to a myoglobin protein derived from an animal source when in an equivalent reduced or oxidized state. The *Aquifex aeolicus* hemoglobin has a peak absorbance wavelength at 413 nm; the *Methylacidiphilum infernorum* hemoglobin has a peak absorbance wavelength at 412 nm; the *Glycine max* leghemoglobin has a peak absorbance wavelength at 415 nm; the *Hordeum vulgare* and *Vigna radiata* non-symbiotic hemoglobins each have a peak absorbance wavelength at 412 nm. The *Bos taurus* myoglobin has a peak absorbance wavelength at 415 nm.

In some embodiments, the difference between the peak absorbance wavelength of the one or more isolated and purified iron-containing proteins and the peak absorbance wavelength of myoglobin derived from an animal source is less than 5%.

Odorants released during cooking of meat are generated by reactions that can involve as reactants fats, protein, amino acids, peptides, nucleotides, organic acids, sulfur compounds, sugars and other carbohydrates. In some embodiments the odorants that

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combine during the cooking of meat are identified and located near one another in the consumable, such that upon cooking of the consumable the odorants combine. So, in some embodiments, the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions involving amino acids, fats and sugars found in plants as well as meat. So, in some embodiments, the characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions involving one or more amino acids, fats, peptides, nucleotides, organic acids, sulfur compounds, sugars and other carbohydrates found in plants as well as meat.

Some reactions that generate odorants released during cooking of meat can be catalyzed by iron, in particular the heme iron of myoglobin. Thus in some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by iron. In some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by heme. In some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by the heme iron in leghemoglobin. In some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by the heme iron in a heme protein. For example, hemeproteins (e.g., from *Aquifex aeolicusm*, *Methylacidiphilum infernorum*, *Glycine max*, *Hordeum vulgare*, or *Vigna radiate*) provide a significantly different profile of volatile odorants when heated in the presence of cysteine and glucose than any subset of the three components when analysed by GC-MS. Volatile flavor components that are increased under these conditions include but are not limited to furan, acetone, thiazole, furfural, benzaldehyde, 2-pyridinecarboxaldehyde, 5-methyl-2-thiophenecarboxaldehyde, 3-methyl-2-thiophenecarboxaldehyde, 3-thiophenmethanol and decanol. Under these conditions, cysteine and glucose alone or in the presence of iron salts such as ferrous glucanate produced a sulfurous, odor but addition of heme proteins reduced the sulfurous odor and replaced it with flavors including but not limited to chicken broth, burnt mushroom, molasses, and bread.

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Additionally, a hemeprotein (e.g., from *Aquifex aeolicus*, *Methylacidiphilum infernorum*, *Glycine max*, *Hordeum vulgare*, or *Vigna radiata*) when heated in the presence of ground chicken increased specific volatile odorants that are elevated in beef compared to chicken when analyzed by GC-MS. Volatile flavor components that are increased under these conditions include but are not limited to propanal, butanal, 2-ethyl-furan, heptanal, octanal, trans-2-(2-pentenyl)furan, (Z)-2-heptenal (E)-2-octenal pyrrole, 2,4-dodecadienal, 1-octanal, or (Z)-2-decenal 2-undecenal.

C. COLOR INDICATORS

The color of meat is an important part the experience of cooking and eating meat. For instance, cuts of beef are of a characteristic red color in a raw state and gradually transition to a brown color during cooking. As another example, white meats such as chicken or pork have a characteristic pink color in their raw state and gradually transition to a white or brownish color during cooking. The amount of the color transition is used to indicate the cooking progression of beef and titrate the cooking time and temperature to produce the desired state of done-ness. In some aspects, the invention provides a non-meat based meat substitute product that provides a visual indicator of cooking progression. In some embodiments, the visual indicator is a color indicator that undergoes a color transition during cooking. In some embodiments, the color indicator recapitulates the color transition of a cut of meat as the meat progresses from a raw to a cooked state. In more embodiments, the color indicator colors the meat substitute product a red color before cooking to indicate a raw state and causes the meat substitute product to transition to a brown color during cooking progression. In other embodiments, the color indicator colors the meat substitute product a pink color before cooking to indicate a raw state and causes the meat substitute product to transition to a white or brown color during cooking progression.

The main determinant of the nutritional definition of the color of meat is the concentration of iron carrying proteins in the meat. In the skeletal muscle component of meat products, one of the main iron-carrying proteins is myoglobin. As described above, the myoglobin content of varies from under 0.05% in the white meat of chicken to 1.5-

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2.0% in old beef. So, in some embodiments, the consumable is a meat replica which comprises an iron-carrying protein (e.g., a heme-containing protein). In some embodiments, the meat replica comprises about 0.05%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, or more than about 2% of an iron-carrying protein (e.g., a heme-containing protein) by dry weight or total weight. In some cases, the iron carrying protein has been isolated and purified from a source. In other cases, the iron carrying protein has not been isolated and purified. In some cases, the source of the iron-carrying protein is an animal source, or a non-animal source such as a plant, fungus, or genetically modified organisms such as, e.g., plant, algae, bacteria or fungus. In some cases, the iron-carrying protein is myoglobin. In some embodiments the consumable is a plant based meat replica that has animal myoglobin added. So, for example a replica of young beef can have about 0.4-1% myoglobin. In some embodiments the consumable is a plant based meat replica that has a leghemoglobin or a cytochrome added. So, for example, a replica of young beef can have about 0.4-1% leghemoglobin or cytochrome.

Another example of iron-carrying proteins is hemoglobin, the iron-containing oxygen-binding protein in the red blood cells of vertebrates. Hemoglobin is similar in color to myoglobin. In some embodiments the invention provides methods of saving and recycling blood from animal farming to supplement the color of a consumable. For example, blood is saved from a slaughter house, and hemoglobin from the blood is used to enhance the color of a consumable. In some aspects the consumable is a plant-based meat replica containing hemoglobin.

Additional iron containing proteins exist in nature. In some embodiments the consumable comprises an iron containing protein that is not myoglobin. In some embodiments the consumable does not contain myoglobin. In some embodiments the consumable does not contain hemoglobin. In some embodiments the consumable is a meat replica that comprises an iron containing protein other than myoglobin or hemoglobin. See, for example, Section III B for examples of heme-containing proteins, as well as FIG. 3. For example, in some embodiments the consumable comprises a

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hemoprotein (e.g., a hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, a bacterial hemoglobin, a ciliate myoglobin, or a flavohemoglobin).

Leghemoglobin, similar in structure and physical properties to myoglobin, is readily available as an unused by-product of commodity legume crops (e.g., soybean or pea). The leghemoglobin in the roots of these crops in the US exceeds the myoglobin content of all the red meat consumed in the US.

In some embodiments, the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, and containing a heme protein (e.g., a leghemoglobin or member of the globin protein family). For example, a meat replica can be principally or entirely composed of ingredients derived from non-animal sources, including a muscle tissue replica, an adipose tissue replica, a connective tissue replica, and a heme protein. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a high iron content from a heme protein. In some embodiments the iron content is similar to meat. In some embodiments the consumable has the distinctive red color of meat, such color provided by leghemoglobin.

A heme protein (e.g., a heme-containing protein described in Section III B) can be used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin, which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.

In some embodiments, the increased shelf life is provided by an extension of the lifetime of the desired red color of food products (e.g., a non-meat based meat substitute).

In one embodiment, this invention provides hemoproteins that provide a desired color to non-meat meat substitutes. In some embodiments, the hemoproteins are derived from a non-animal source such as a plant, fungus, or genetically modified organisms such

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as, e.g., plant, algae, bacteria or fungus. See, e.g., section III B. In some embodiments the life time of the hemoproteins is extended by treatment with meat shelf life extenders.

In some embodiments, the meat shelf life extenders are selected from a groups consisting of carbon monoxide, nitrites, sodium metabisulfite, Bombal, rosemary extract, green tea extract, catechins and other anti-oxidants.

In one embodiment, this invention provides hemoproteins that provide a desired flavor profiles to food products (e.g., non-meat meat substitutes). In some embodiments, the ability of the hemoproteins to generate the desired flavor profile is similar to that of myoglobin

In some embodiments, the life time of the ability of the hemoproteins to generate the desired flavor profile is 10%, 20%, 30% 50%, or 100% or more greater than that of myoglobin.

D. Food products comprising isolated, purified heme proteins

In some embodiments, heme proteins described herein are added to meat or a consumable described herein to enhance the properties of the meat or consumable. For example, a heme protein containing solution can be injected into raw (e.g., raw white meat) or cooked meat to improve the organoleptic properties of the meat during cooking adding a "beefy" flavor (e.g., to white meats such as chicken).

In another example, a heme protein solution can be dripped over meat or a consumable of the invention to enhance appearance. In one embodiment, advertising, photography, or videography of food products such as meat or a meat substitute can be enhanced with a heme protein.

In another embodiment, a heme protein is added to the consumable as an iron supplement.

In one application of the invention, hemeproteins may be used as food dyes. In one embodiment, the heme proteins may be used as a safe, digestible replacement for FD&C Red No. 40 – Allura Red AC, E129 (red shade) in a variety of applications. A non-limiting list of such potential uses would include making pictures, especially in forms such as body-painting or as theatrical blood.

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In some embodiments, the present invention provides methods for obtaining hemeproteins (e.g., leghemoglobin) from a plant. Leghemoglobin can be obtained from a variety of plants. Various legumes species and their varieties (e.g., soybean, fava bean, lima bean, cowpeas, English peas, yellow peas, Lupine, kidney beans, garbanzo beans, peanuts, Alfalfa, Vetch hay, clover, Lespedeza, or pinto bean) contain nitrogen-fixing root nodules in which leghemoglobin has a key role in controlling oxygen concentrations (for example root nodules from a pea plant). In one embodiment leghemoglobin protein is purified from root nodules of legume plants (e.g., soybeans, favabeans, or peas) using ion-exchange chromatography. In an embodiment, leghemoglobin is purified from soybean, favabean or sweet pea root nodules.

Plants can be grown using standard agricultural methods, with the exception that, in some instances, fertilizer is not applied and soil is enriched in natural nitrogen-fixing bacteria from the *Rhizobium* genus. Either whole roots or root nodules can be harvested and lysed, for example in 20mM potassium phosphate pH 7.4, 100mM potassium chloride and 5mM EDTA using grinder-blender. During this process, leghemoglobin is released into the buffer. Root-nodule lysate containing leghemoglobin can be cleared from cell debris by filtration through 5μm filter. In some embodiments, filtration is followed by centrifugation (7000g, 20min). Clarified lysate containing leghemoglobin is then filtered through 200nm filter and applied onto anion-exchange chromatography column (High Prep Q; High Prep DEAE, GE Healthtcare) on fast protein liquid chromatography machine (GE Healthcare). Leghemoglobin is collected in the flowthrough fraction and concentrated over 3kDa filtration membrane to a desired concentration. Purity (partial abundance) of purified leghemoglobin is analyzed by SDS-PAGE gel: in lysate leghemoglobin is present at 20-40%, while after anion-exchange purification it is present at 70-80%. In another embodiment, soybean leghemoglobin flowthrough from anion-exchange chromatography is applied onto size-exclusion chromatography (Sephacryl S-100 HR, GE Healthcare). Soybean leghemoglobin is eluted as two fractions corresponding to dimer and monomer species. Purity (partial abundance) of leghemoglobin was analyzed by SDS-PAGE and determined to be ~ 90-100%.

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Proteins in legume root-nodule lysate can be transferred into 10 mM sodium carbonate pH 9.5, 50mM sodium chloride buffer, filtered through 200nm filter and applied onto anion-exchange chromatography column on fast protein liquid chromatography instrument (GE Healthcare). Leghemoglobin can be bound to anion-exchange chromatography matrix and eluted using sodium chloride gradient. Purity (partial abundance) of leghemoglobin can be analyzed by SDS-PAGE and determined to be ~ 60-80%.

Undesired small molecules from legume roots can be removed from purified leghemoglobin by passing leghemoglobin in solution over anion-exchange resin. These small molecules imbue varying shades of brown color to root-root nodule lysates, thus decreasing the color quality of leghemoglobin solution. In one embodiment, anion-exchange resin is FFQ, DEAE, Amberlite IRA900, Dowex 22, or Dowex 1x4. Leghemoglobin purified either by ammonium sulfate fractionation (60% wt/v and 90% wt/v ammonium sulfate) or by anion-exchange chromatography was buffer exchanged into 20mM potassium phosphate pH 7.4, 100mM sodium chloride and solution passed over one of the above mentioned anion-exchange resins. Flowthrough can be collected and its colored compared to the color of the solution before passage over anion-exchange resins. Color improvement to purified leghemoglobin solution as evaluated by visual inspection can be observed (from yellow/brown to more apparent red), however to different extent of removal of yellow-brown tinge.

Alternatively, the heme-containing protein can be recombinantly produced as described in section III B. For example, a non-symbiotic hemoglobin from moong bean can be recombinantly expressed in *E.coli* and purified using anion-exchange chromatography or cation-exchange chromatography. A cell lysate can be loaded over FF-Q resin on fast protein liquid chromatography instrument (GE Healthcare). Moong bean non-symbiotic hemoglobin eluted in the flowthrough fractions. Purity (partial abundance) of Moong bean non-symbiotic hemoglobin was analyzed by SDS-PAGE and determined to be as a fraction of total protein: 12 % in *E.coli* lysate, and 31% after purification on FFQ. UV-Vis analysis of purified protein showed spectra characteristic of heme bound protein.

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Alternatively, the cell lysate can be loaded over a FF-S resin on a fast protein liquid chromatography instrument (GE Healthcare). Moong bean non-symbiotic hemoglobin can be bound to FF-S column and eluted using sodium chloride gradient (50mM- 1000mM). Purity (partial abundance) of Moong bean non-symbiotic hemoglobin can be analyzed by SDS-PAGE and determined to be: *E.coli* lysate 13 %, after purification on FFQ 35%. UV-Vis analysis of purified protein can show spectra characteristic of heme bound protein.

In some embodiments, the heme proteins are utilized as ingredients in food products where the flavor of blood is desired. Heme-containing proteins of the invention were tasted by a panel of volunteers and in each case described as tasting like blood.

Heme proteins, for example leghemoglobin, can be combined with other plant based meat replica components. In some embodiments the heme proteins are captured in a gel which contains other components, for example lipids and or other proteins. In some aspects, multiple gels are combined with non-gel based heme proteins. In some embodiments, the combination of the heme proteins and the other compounds of the consumable are done to insure that the heme proteins are able to diffuse through the consumable. In some embodiments the consumable is soaked in a heme-protein containing solution, for instance a leghemoglobin solution, e.g., for 1, 5, 10, 15, 30, or 45 minutes or for 1, 5, 10, 15, 20 or 30 hours.

Given the usefulness of heme proteins for coloring consumables, it is useful to detect whether a product contains a particular heme protein. Accordingly the present invention includes in some embodiments methods to determine whether a product contains a heme protein. For example, an ELISA, a proximity-ligation assay, a luminex assay, or western blot analysis can be performed to determine whether leghemoglobin or other heme-containing protein is present in a food product such as meat or a meat replica. In one embodiment the detection methods are performed to determine whether meat has been altered with leghemoglobin or other heme-containing protein.

E. MAYONNAISE SPREAD REPLICA.

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Mayonnaise is thick, creamy sauce. Traditional mayonnaise is a stable emulsion of oil and egg yolk. It is thought that lecithin and proteins from egg yolk stabilize the emulsion. Traditional commercial mayonnaise typically contains 70-80% (wt/wt) fat and 5% (wt/wt) of egg yolk. Lower fat commercial products can contain ~ 20% wt/wt fat. The consumable can comprise a composition that has comparable properties to mayonnaise.

In one embodiment, purified plant proteins can be used as substitute for egg proteins to make stable, creamy protein-fat emulsions whose visual and mouth-feel appearance resemble traditional mayonnaise. Fat (~ 20- 80% wt/wt) can be from a single source or from multiple sources as described herein. Non-traditional mayonnaise products can be used for all the culinary applications that traditional mayonnaise is used for. In one embodiment, vinegar and/or lemon and/or lime juice are added as flavor additives. In one embodiment, the purified plant proteins are not soy proteins. In one embodiment, the flavor can be modified by addition of mustard, spices, herbs, and/or pickles.

A mayonnaise replica can comprise a mixture of non-animal proteins. In one embodiment, a mayonnaise replica is a mixture of 50% (wt/v) rice bran oil and 7% (wt/v) Moong bean 8S protein. In one embodiment, a mayonnaise replica is a mixture of 70% (wt/v) sunflower oil or cocoa butter, 2.4% (wt/v) RuBisCo,0.29% (wt/wt) soybean lecithin, and optionally 8 μM oleosin.

The mixture can be emulsified, and the stability of the emulsion can be controlled by modifying the size of the oil-water-protein particles by high pressure homogenization or sonication. Oil can be added as liquid. Protein can be added as solution in buffer. Soybean lecithin can be resuspended in water and sonicated prior to mixing with an oil and protein solution. A resulting oil, protein and lecithin solution can be homogenized, for example, first at 5000psi and then at 8000psi, or can be sonicated at 40% duty cycle for 2 minutes at maximum setting. Thickness, texture, creaminess and visual appearance of resulting products are similar to one of traditional mayonnaise. In some instances (e.g. using Moong bean 8S protein and rice bran oil), a product is a light off-white in color.

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F. CREAM LIQUOR REPLICA

Traditionally, cream liquors contain dairy cream and liquor as their base. Examples of liquor include whiskey, Irish whiskey, Scotch whiskey, rum, vodka, grappa, or fermented fruits (e.g., cherry liquor), plum brandy, tequila, or herbal bitters. A cream liquour replica can be produced by substituting the dairy cream in cream liquor with a non-dairy cream fraction from plant sources. In one embodiment, dairy cream in a cream liquor can be substituted by a stable emulsion of plant fats and isolated or purified proteins of a consistency similar to dairy cream. In one embodiment, purified plant proteins and/or plant fats can be from single or multiple sources as described herein. For example, a cream liquor can include a sunflower cream fraction, RuBsiCo and whiskey, and one or more optional flavorings (e.g., vanilla, chocolate, and or coffee).

G. PROTEIN ENRICHED ALCOHOLIC BEVERAGE

Traditionally, alcoholic beverages contain negligible to low amounts of protein. Addition of plant proteins to various alcoholic beverages would positively modify their flavor, mouthfeel, physical state and increase their nutritional protein content. In addition, the presence of protein in various alcoholic beverages used in cocktails would positively modify the cocktail's flavor, mouthfeel, physical state and increase their nutritional protein content. Different classes of alcoholic beverages contain different amounts of alcohols. For example, wine coolers contain about 4-7% alcohol, beer contains about ~ 3-10% alcohol, wine contains about 8-14% v/v alcohol, dessert wines contain about 17-20% alcohol, whiskey contains about ~ 40% alcohol, and vodka contains about 35-50% alcohol. In addition, some traditional alcoholic beverages include sugars (for example, Bacardi Razz at 10% wt/v).

Accordingly beverages containing alcohol can be supplemented by addition of purified plant proteins at for example 0.1-5% wt/v and optionally sugar (1-15% wt/v). The sugar can be, for example, cane sugar, brown sugar, sucrose, or glucose. For example, purified Rubisco at 180mg/ml in 20mM K-phosphate pH 7.0, 150mM NaCl can be added to a whiskey. Jameson whiskey supplemented with 5% wt/v Rubsico formed a soft gel, with a consistency similar to traditional Jello shots.

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For example, Rubsico, moong bean 8S, and pa Globulin enriched alcoholic beverages were made by adding purified Rubsico, Moong bean 8S and pea globulin proteins at final protein concentrations of 0.5%, 1% and 5% wt/v, respectively, to Corona beer, Pinot grigio wine and Jameson whiskey. Zein was added at 0.5%, 1% and 5% wt/v to a 60% ethanol, 5% sucrose solution in water.

Pea proteins were extracted from pea flour by resuspending the flour in 5%, 20% or40% ethanol, 5% sucrose solutions in water, followed by incubation for 1hr at room temperature. Any undissolved solids were removed by centrifugation at 5000g for 10min. The resultant supernatant solution was clear in appearance. The 5% ethanol solution was particularly useful.

A sensory panel evaluated all protein enriched alcoholic beverages as having aromas and flavors different from beverages not-enriched in protein. In some cases, generated aromas and flavors were judged as neutral, in some cases as more appealing and in some cases as less appealing than controls. In particular example, addition of both 0.5% wt/v and 1% wt/v of Moong bean 8S protein to Jameson whiskey softened the aroma and mouthfeel of Jameson. Addition of 0.5% wt/v of moong bean to Jameson whisky added a slightly creamy flavor to Jameson, with an aroma similar to a traditional White Russian cocktail. Addition of 5% wt/v zein to Jameson whiskey generated aromas and flavor characterized as moldy beans and raw potato.

In another example where Corona beer was enriched with 0.5% wt/v pea Globulin, the aroma changed to hoppy and resembled one of Indian Pale Ale, and the flavor changed to carrying pea notes. Addition of 0.5% wt/v and 5% wt/v of moong bean 8S protein changed the Corona aroma towards sweet peony flower with an intensified hop aroma. The flavor was neutral in the case of 0.5% wt/v moong bean 8S and carried planty-nutty notes in the case of 5% wt/v moong bean 8S.

In another example where Pinot Grigio wine was enriched with 1% wt/v moong bean 8S protein, additional aroma notes of sweet and citrus were detected, and the flavor changed to that of carrying notes of peanut-butter. Addition of 1% wt/v of Pea Globulins modified the aroma to that of strong moldy oak and wet leaves. Flavor was modified to

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carry notes of mud. Addition of 5% wt/v of Rubisco generated aroma and flavor of wet hay.

A zein enriched 60% ethanol, 5% sucrose solution carrried burnt tortilla chip aroma notes compared to a corresponding solution without zein. There was no difference in flavor.

Pea proteins enriched 5%, 20% and 40% ethanol, 5% sucrose solutions all developed earthy aroma and flavor compared to protein free controls. In addition, flavor of peas was detected and bitter flavor increased at higher alcohol content.

H. CHOCOLATE SPREAD

Chocolate spread is a chocolate flavored spread whose traditional main ingredients are cocoa powder, dairy milk, plant oil, and sugar. Traditional chocolate spreads are either firm or soft solids at ambient room temperature and melt at temperatures below that of cocoa butter. The product can be used as a spread on bread, crepes, pancakes, icing for cakes and cookies, filling for chocolate confectionary, or non-dairy chocolate cake filling.

In one embodiment, dairy milk and milk products such as ice cream, whey, cream, yoghurt, sour cream or butter fat are substituted by a non-dairy cream fraction made as described herein. In one embodiment, a non-dairy cream fraction comes from a single source or multiple sources described herein. In one embodiment, dairy milk and milk products are substituted by any non-dairy milk described herein. In one embodiment, dairy milk and milk products are substituted by purified plant proteins described herein. In one embodiment, dairy milk and milk products are substituted by soft solid stable emulsions made from single or multiple plant oil and single or multiple purified plant proteins.

I. OTHER APPLICATIONS:

In one embodiment, non-dairy plant cream fraction can be used as a substitute for dairy milk and dairy milk products to make non-dairy milk chocolate bars or non-dairy milk chocolate confectionary.

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In one embodiment, non-dairy plant cream fraction and purified plant proteins can be used as substitute for dairy milk and dairy milk products to make non-dairy milk chocolate bars or a non-dairy milk chocolate confectionary.

In one embodiment, non-dairy plant cream fraction and plant proteins can be used to make chocolate mousse. Traditional main chocolate mousse ingredients are bittersweet or semisweet chocolate, dairy butter and eggs. In one embodiment, dairy butter can be substituted by a non-dairy plant cream fraction. In one embodiment, dairy butter and eggs can be substituted by a non-dairy plant cream fraction and a foam stabilizing plant seed storage proteins such as pea albumin.

In one embodiment, a vegan consumable such as pate analog can be made. Vegan pate analog can be made by finely chopping 10 g of fat replica and heating it on a frying pan with finely chopped shallots for 2-3 min. Muscle replica (20 g) made without connective tissue replica fibers can be chopped into ½-inch cubes and browned in the fat and shallots mix for another 3-5 min. The mixture can be forced through a sieve until homogeneous. The pan, while still warm, can be rinsed with a tablespoon of madeira without allowing it to evaporate fully. The liquid from the pan is added to the homogenized mix, spices (salt, pepper) are added to taste, and the mix is forced through a sieve again. After chilling in a refrigerator (e.g., for 15 minutes), the pate is ready to be served.

In some embodiments, other fat-to-muscle replica ratios are used to create leaner or richer pates. For example, pate can contain 0.5-10%, about 5%-40%, about 10%-60%, or about 30-70%, or >70% of an adipose tissue replica.

In some embodiments, a muscle tissue replica with a higher iron content can be used for pate to make it a closer imitation of pig or bird liver pate. For example, muscle tissue replica can contain about 1%, about 1.5%, about 2%, or >2% of a heme protein.

In some embodiments, muscle tissue replica with lower iron content can be used for pate to make it closer imitation of bird meat or fish pate. For example, muscle tissue replica can contain about 1%, about 0.5%, about 0.2%, or <0.2% of a heme protein.

In one embodiment, a vegan consumable such as a blood sausage analog can be made. Vegan blood sausage is made from a blood analog created by mixing solutions of

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heme protein and purified plant protein. For example, 35 ml of a mixed solution of leghemoglobin (120 mg/ml) and pea albumin (100 mg/ml), which approximates the composition of blood, can be carefully mixed with a slurry of corn flour in salt water (6:5 w/v flour to water ratio). A tablespoon of chopped onion can be fried with 10 g of chopped adipose tissue replica, mixed with a few raisins and cooled to room temperature before mixing with blood/flour mix. The mixture can be seasoned to taste (for example, using salt, pepper, parsley and/or cinnamon), loaded into vegetarian sausage casings, and poached in near-boiling water for about 45 min. After cooling, the sausage can be consumed as is or further cooked, for example smoked, crisped in an oven or roasted.

In some embodiments, muscle replica can be included in the recipe to imitate meat/blood sausages. In some embodiments, barley, buckwheat, oat, rice, rye, sorghum, wheat or other grains can be used in the blood sausage. In some embodiments, bread, chestnuts, potato, sweet potato, starch or other fillers can be added to, or substitute for, grains in blood sausage.

EXAMPLES

EXAMPLE 1: PROTEIN ISOLATION.

All steps were carried out at 4˚C or room temperature. Centrifugation steps were at 8000 g for 20 mins, 4˚C or room temperature. The flour is suspended in a specific buffer, the suspension is centrifuged and the supernatant is microfiltered through a 0.2 micron PES membrane and then concentrated by ultrafiltration on a 3 kDa, 5 kDa, or 10 kDa molecular weight cutoff PES membrane on a Spectrum Labs KrosFlo hollow fiber tangential flow filtration system.

Once fractionated, all ammonium sulfate precipitate fractions of interest were stored at -20˚C until further use. Prior to their use in experiments, the precipitates were resuspended in 10 volumes of 50 mM K Phosphate buffer, pH 7.4, + 0.5 M NaCl. The suspensions were centrifuged and the supernatants microfiltered through a 0.2 micron PES membrane and then concentrated by ultrafiltration on a 3 kDa, 5 kDa, or 10 kDa molecular weight cutoff PES membrane on a Spectrum Labs KrosFlo hollow fiber tangential flow filtration system. Protein composition at individual fractionation steps

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was monitored by SDS-PAGE and protein concentrations were measured by standard UV-Vis methods.

(i) Pea-albumins: Dry green or yellow pea flour was used as a source of pea albumins. The flour was suspended in 10 volumes of 50 mM sodium acetate buffer pH 5 and stirred for 1 hr. Soluble protein was separated from un-extracted protein and pea seed debris by either centrifugation (8000 g, 20 minutes) or filtration through a 5 micron filter. Supernatant or filtrate, respectively, was collected. To this crude protein extract, solid ammonium sulfate was added to 50% wt/v saturation. The solution was stirred for 1 hour and then centrifuged. To the supernatant from this step, ammonium sulfate was added to bring to 90% wt/v saturation. The solution was stirred for 1 hour, and then centrifuged to collect the pea albumin proteins in the pellet. The pellet was stored at -20˚C until further use. Protein was recovered from the pellet and prepared for use as described above, with the exception that final buffer can contain 0-500 mM sodium chloride.

In some embodiments, the flour was suspended in 10 volumes of 50 mM NaCl, pH 3.8 and stirred for 1 hour. Soluble protein was separated from un-extracted protein and pea seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 Kda cutoff PES membrane.

(ii) Pea-globulins: Dry green pea flour was used to extract pea globulin proteins. The flour was suspended in 10 volumes of 50 mM potassium phosphate buffer pH 8 and 0.4M sodium chloride and stirred for 1hr. Soluble protein was separated from pea seed debris by centrifugation. The supernatant was subjected to ammonium sulfate fractionation in two steps at 50% and 80% saturation. The 80% pellet containing globulins of interest was stored at -20˚C until further use. Protein was recovered from the pellet and prepared for use as described above.

iii) Soybean 7S and 11S globulins: Globulins from soybean flour were isolated by first suspending lowfat/defatted soy flour in 4-15 volumes of 10 (or 20) mM potassium phosphate pH 7.4. The slurry was centrifuged at 8000 rcf for 20 mins or clarified by 5 micron filtration and the supernatant was collected. The crude protein extract contained both the 7S and 11S globulins. The solution then was 0.2 micron filtered and

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concentrated using a 10 kDa molecular weight cutoff PES membrane on a Spectrum Labs KrosFlo hollow fiber tangential flow filtration system or by passing over anion-exchange resin prior to use in experiments. The 11S globulins were separated from the 7S proteins by isoelectric precipitation. The pH of the crude protein extract was adjusted to 6.4 with dilute HCl, stirred for 30 min-1 hr and then centrifuged to collect the 11S precipitate and 7S proteins in the supernatant. The 11S fraction was resuspended with 10mM Potassium phosphate pH 7.4 and the protein fractions were microfiltered and concentrated prior to use.

Soybean proteins also can be extracted by suspending the defatted soy flour in 4-15 volumes (e.g., 5 volumes) of 20 mM sodium carbonate, pH 9 (or water, pH adjusted to 9 after addition of the flour) or 20 mM potassium phosphate buffer pH 7.4 and 100 mM sodium chloride to decrease off-flavors in the purified protein. The slurry is stirred for one hour and centrifuged at 8000 xg for 20 minutes. The extracted proteins are ultrafiltered and then processed as above or alternatively, the supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(iv) Moong bean 8S globulins: Moong bean flour was used to extract 8S globulins by first suspending the flour in 4 volumes of 50 mM KPhosphate buffer pH 7 (+ 0.5M NaCl for lab scale purifications). After centrifugation, proteins in the supernatant were fractionated by addition of ammonium sulfate in 2 steps at 50% and 90% saturation respectively. The precipitate from the 90% fraction contained the 8S globulins and was saved at -20˚C until further use. Protein was recovered from the pellet and prepared for use as described above.

Moong bean globulins also can be extracted by suspending the flour in 4 volumes of 20 mM sodium carbonate buffer, pH 9 (or water adjusted to pH 9 after addition of the moong flour) to reduce off-flavors in the purified protein fractions. The slurry is centrifuged (or filtered) to remove solids, ultrafiltered and then processed as described above.

(v) Late embryogenesis abundant proteins: Flour (including but not limited to moong bean and soy flour) was suspended in 20 mM Tris–HCl, pH 8.0, 10 mM NaCl,

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and stirred at room temperature for 1 hour then centrifuged. Acid (HCl or acetic acid) was added to the supernatant to a 5% concentration (v/v), stirred at room temperature then centrifuged. The supernatant was heated to 95°C for 15 minutes, and then centrifuged. The supernatant was precipitated by adding Trichoroacetic acid to 25%, centrifuged, then washed with acetone. Heating and acid wash steps can be carried out in the reverse direction as well.

(vi) Pea-Prolamins: Dry green pea flour was suspended in 5x (w/v) 60% ethanol, stirred at room temperature for one hour, then centrifuged (7000g, 20min) and the supernatant collected. The ethanol in the supernatant was evaporated by heating the solution to 85°C and then cooling to room temperature. Ice-cold acetone was added (1: 4 v/v) to precipitate the proteins. The solution then was centrifuged (4000g, 20min), and protein recovered as the light-beige colored pellet.

(vii) Zein-Prolamins: Corn protein concentration or flour was suspended in 5x (w/v) 60% ethanol, stirred at room temperature for one hour, then centrifuged. Ethanol in supernatant was evaporated with heat, and then the solution is centrifuged, and the protein recovered as the pellet.

(viii) RuBisCO was fractionated from alfalfa greens by first grinding leaves with 4 volumes of cold 50 mM potassium phosphate buffer pH 7.4 buffer (0.5M NaCl + 2mM DTT + 1mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (crude lysate) was used in further purification steps. Proteins in the crude lysate were fractionated by addition of ammonium sulfate to 30% (wt/v) saturation. The solution was stirred for 1hr and then centrifuged. The pellet from this step was discarded and additional ammonium sulfate was added to the supernatant to 50 % (wt/v) ammonium sulfate saturation. The solution was centrifuged again after stirring for 1hr. The pellet from this step contains RuBisCO, and was kept at -20°°C until used. Protein was recovered from the pellet and prepared for use as described above.

RuBisCO also can be purified by adjusting the crude lysate to 0.1M NaCl and applying to an anion exchange resin. The weakly bound protein contaminants are washed with 50 mM KPhosphate buffer pH 7.4 buffer + 0.1M NaCl. RuBisCO was then eluted with high ionic strength buffer (0.5M NaCl).

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RuBisCO solutions were decolorized (pH 7-9) by passing over columns packed with activated carbon. The colorants bound to the column while Rubisco was isolated in the filtrate.

RuBisCO solutions were also alternatively decolorized by incubating the solution with FPX66 (Dow Chemicals) resin packed in a column (or batch mode). The slurry is incubated for 30mins and then the liquid is separated from the resin. The colorants bind to the resin and RuBisCO was collected in the column flow-through.

In some embodiments, RuBisCO was isolated from spinach leaves by first grinding the leaves with 4 volumes of 20mM potassium Phosphate buffer pH 7.4 buffer + 150 mM NaCl + 0.5 mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (crude lysate) was filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

In some embodiments, RuBisCO was extracted from alfalfa or wheatgrass juice powder by mixing the powder with 4 volumes of 20mM potassium Phosphate buffer pH 7.4 buffer + 150 mM NaCl + 0.5 mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (crude lysate) was filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(ix) Leghemoglobin. Soy root nodules were suspended and lysed in 20 mM potassium phosphate pH 7.4, 100mM potassium chloride and 5mM EDTA using grinder-blender. During this process leghemoglobin is released into the buffer. Root-nodule lysate containing leghemoglobin was cleared from cell debris by filtration through 5 micron filter. In some embodiments, filtration was followed by centrifugation (7000g, 20min). Clarified lysate containing leghemoglobin was then filtered through 0.2 micron filter and applied onto anion-exchange chromatography column (High Prep Q; High Prep DEAE, GE Healthtcare) on fast protein liquid chromatography instrument (GE Healthcare). Leghemoglobin was collected in flowthrough fraction and concentrated over 3kDa molecular weight cutoff PES membrane on a Spectrum Labs KrosFlo hollow fiber tangential flow filtration system to a desired concentration. Purity (partial abundance) of purified leghemoglobin was analyzed by SDS-PAGE gel: in lysate leghemoglobin is present at 20-40%, while after anion-exchange purification it is present at 70-80%. In

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another embodiment, soybean leghemoglobin flow through from anion-exchange chromatography was applied onto size-exclusion chromatography (Sephacryl S-100 HR, GE Healthcare). Soybean leghemoglobin eluted as two fractions corresponding to dimer and monomer species. Purity (partial abundance) of leghemoglobin was analyzed by SDS-PAGE and determined to be ~ 90-100%. Analysis of UV-VIS spectra (250-700nm) revealed spectral signature consistent with heme loaded leghemoglobin.

(x) Non-symbiotic hemoglobin from moong bean was cloned into pJexpress401 vector (DNA2.0) and transformed into *E. coli* BL21. Cells were grown in LB media containing soytone instead of tryptone, kanamycin, 0.1mM ferric chloride and 10 μg/ml 5-aminolevulinic acid. Expression was induced by 0.2mM IPTG and cells grown at 30°C for 20hr. *E.coli* cells expressing moong bean non-symbiotic hemoglobin were collected and resuspended in 20mM MES buffer pH 6.5, 50mM NaCL, 1mmM $MgCl_2$, 1mM $CaCl_2$. Add a bit of DNAaseI, and protease inhibitors. Cells were lysed by sonication. Lysate was cleared from cell debris by centrifugation at 16 000g for 20 min, followed by filtration over 200nm filter. Cell lysate was then loaded over FF-S resin on fast protein liquid chromatography instrument (GE Healthcare). Moong bean non-symbiotic hemoglobin bound to FF-S column and was eluted using sodium chloride gradient (50mM- 1000mM). Purity (partial abundance) of moong bean non-symbiotic hemoglobin was analyzed by SDS-PAGE and determined to be: *E.coli* lysate 13 %, after purification on FFQ 35%. UV-Vis analysis of purified protein showed spectra characteristic of heme bound protein.

(xi) Hemeproteins were synthesized with an N-terminal His6 epitope tag and a TEV cleavage site, cloned into pJexpress401 vector (DNA2.0), and transformed into *E. coli* BL21. Transformed cells were grown in LB media containing soytone instead of tryptone, kanamycin, 0.1 mM ferric chloride and 10 μg/ml 5-aminolevulinic acid. Expression was induced by 0.2mM IPTG and cells grown at 30°C for 20hr. *E.coli* cells expressing heme proteins were collected and resuspended in 50 mM potassium phosphate pH 8, 150 mM NaCl, 10 mM imidazole, 1 mM $MgCl_2$, 1 mM $CaCl_2$, DNAaseI, and protease inhibitors. Cells were lysed by sonication and clarified by centrifugation at 9000 x g. Lysate was incubated with NiNTA resin (MCLAB), washed with 5 column volumes

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(CV) of 50 mM potassium phosphate pH 8, 150 mM NaCl, 10 mM imidazole, and eluted with 50 mM potassium phosphate pH 8, 150 mM NaCl, 500 mM imidazole. SDS-PAGE and UV-vis spectra confirmed expected molecular weights and complete heme-loading, respectively.

In some embodiments, transformed cells were grown in seed media comprised of 10g/L glucose monohydrate, 8g/L Monopotassium Phosphate, 2.5g/L Sensient Amberferm 6400, 2.5g/L Sensient Tastone 154, 2g/L Diammonium Phosphate, 1mL/L Trace Metals Mixture (Teknova 1000x Trace Metals Mixture Cat. No. T1001), 1g/L Magnesium Sulfate, 0.25mL 0.1M solution Ferric Chloride, 0.5mL/L Sigma Anti-foam 204, 1mL/L Kanamycin Sulfate 1000x solution. 250mL of media was used in a (4)-1L baffeled shakeflasks, innoculated with 0.25mL each from a single vial of glycerol stock culture. Shakeflasks were grown for 5.5 hours, with 250RPM agitation at 37°C. 40L of seed media was steam-sterlized in a 100L bioreactor, cooled to 37°C, pH-adjusted to 7.0 and innoculated with 800mL of shakeflask culture once a shakeflask OD of 2.5 was achieved. Aeration to the bioreactor was supplied at 40L/m and agitation was 250RPM. After 2.2 hours of growth, an OD of 2.20 was reached and 22L of culture was transferred to the final $4m^3$ bioreactor. The starting media for the final bioreactor comprised of the following components steamed-in-place: 1775L deionised water, 21.75kg Monopotassium Phosphate, 2.175kg Diammonium Phosphate, 4.35kg Ammonium Ferric Citrate, 8.7kg Ammonium Sulfate, 10.875kg Sensient Amberferm 6400, 10.875kg Sensient Tastone 154. After 30 minutes of steaming, the media components were cooled to 37°C and post-sterilzation additions were made: 2.145L of 0.1M Ferric Chloride solution, 59.32kg 55%w/w Glucose Monohydrate, 3.9L of Trace Metals Mixture (Teknova 1000x Trace Metals Mixture Cat. No. T1001), 10.88L of 200g/L Diammonium Phosphate, 36.14L 1M Magnesium Sulfate, and 2.175L Sigma Anti-foam 204, 2.175L Kanamycin Sulfate 1000x solution. pH was controlled at 7.0 via the addition of 30% Ammonium Hydroxide. Aeration was supplied at $2.175m^3$/min, dissolved oxygen was controlled at 25% by varying agitation between 60-150RPM. At two timepoints (EFT=4 and EFT8), bolus additions of additional nutrients were supplied. Each addition added 5.5kg of Sensient Amberferm 6400, 5.5kg of Sensient Tastone 154 and 4.4kg of

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Diammonium Phosphate, in autoclaved solutions (100g/L solution for Amberferm and Tastetone, 200g/L for Diammonium Phosphate). A sterile glucose solution of 55%w/w Glucose Monohydrate was fed into the bioreactor to maintain a level of residudual glucose of 2-5g/L. Once an OD of 25 was reached, the temperature was reduced to 25°C and the culture was induced with 0.648L of 1M Isopropyl β-D-1-thiogalactopyranoside. The culture was allowed to grow for a total time of 25 hours, at which point the culture was diluted 1:1 with deionized water, then centrifuged, concentrating the centrate to 50%v/v solids content. Cell centrate was frozen at -20°C. Centrate was thawed to 4°C and diluted in 20 mM potassium phosphate pH 7.8, 100 mM NaCl, 10 mM imidazole, and homogenized at 15,000 PSI. Homogenized cells were 0.2 um filtered by tangential flow filtration (TFF) and filtered lysate was loaded directly onto a zinc-charged IMAC column (GE). Bound proteins were washed with 10 column volumes (CV) 20 mM potassium phosphate pH 7.4, 100 mM NaCl, 5 mM histidine and eluted with 10 CV 500 mM potassium phosphate monobasic, 100 mM NaCl. Eluted leghemoglobin was concentrated and diafiltered using a 3kDa molecular weight cutoff PES membrane and TFF. The concentrated sample was reduced with 20 mM sodium dithionite and desalted using G-20 resin (GE). Desalted leghemoglobin samples were frozen in liquid nitrogen and stored at -20 C. Leghemoglobin concentration and purity were determined by SDS-PAGE and UV-vis analysis.

(xi) Oleosin. Sunflower oil bodies were purified from sunflower seeds. Sunflower seeds were blended in 100 mM sodium phosphate buffer pH 7.4, 50mM sodium chloride, 1 mM EDTA at 1:3 wt/v. Oil-bodies were collected by centrifugation (5000g, 20min), and resuspended at 1:5 (wt/v) in 50 mM sodium chloride, 2M urea and stir for 30min, 4°C. 2M urea wash and centrifugation steps were repeated. Oil-bodies collected by centrifugation were resuspended in 100 mM sodium phosphate buffer pH 7.4, 50mM sodium chloride. Centrifugation and washing steps were repeated once more, and the final washed oil-bodies fraction was obtained from a last centrifugation step. Oil-bodies were resuspended at 10% wt/w in 100 mM sodium phosphate buffer pH 7.4, 50mM sodium chloride, 2% wt/v vegetable oil fatty acid salts, homogenized at 5000 psi and incubated at 4°C for 12 hr. Solution was centrifuged (8000g, 30min), top layer removed

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and soluble fraction collected. SDS-PAGE analysis suggested that oleosins are a major protein present in the soluble fraction. Oleosin concentration was 2.8 mg/ml.

(xii) Pea total proteins: Dry green or yellow pea flour was used to extract total pea proteins. The flour was suspended in 10 volumes of 20mM potassium phosphate buffer pH 8 and 100 mM sodium chloride and stirred for 1hr. Soluble protein was separated from pea seed debris by centrifugation. The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 Kda cutoff PES membrane.

(xiii) Pea vicilin and Pea legumin: Dry green or yellow pea flour was used to extract total pea proteins as described above. The crude pea mixture obtained thereof was fractionated into pea vicilin and pea legumin using ion-exchange chromatography. Material was loaded on Q Sepharose FastFlow resin and fractions were collected as salt concentration was varied from 100 mM to 500 mM NaCl. Pea vicilin was collected at 350 mM sodium chloride while pea legumin was collected at 460 mM sodium chloride. The collected fractions were concentrated using a 10 KDa cutoff PES membrane.

(xv) Lentil total proteins: Air classified lentil flour was used to extract crude mixture of lentil proteins. Flour was suspended in 5 volumes of 20 mM potassium phosphate buffer pH 7.4 and 0.5 M sodium chloride and stirred for 1 hr. Soluble protein was separated from un-extracted protein and lentil seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(xvi) Lentil albumins: Air classified lentil flour was suspended in 5 volumes of 50 mM sodium chloride, pH 3.8 and stirred for 1 hr. Soluble protein was separated from un-extracted protein and lentil seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(xvii) Chickpea / Garbanzo bean total proteins: Garbanzo bean flour was suspended in 5 volumes of 20 mM potassium phosphate buffer pH 7.4 and 0.5 M sodium chloride and stirred for 1 hr. Soluble protein was separated from un-extracted protein and chickpea seed debris by centrifugation (8000 g, 20 minutes). The supernatant was

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collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(xviii) Chickpea/ Garbanzo bean albumins: Garbanzo bean flour was suspended in 5 volumes of 50 mM sodium chloride, pH 3.8 and stirred for 1 hr. Soluble protein was separated from un-extracted protein and lentil seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(xix) Amaranth flour dehydrins: Amaranth flour was suspended in 5 volumes of 0.5 M sodium chloride, pH 4.0 and stirred for 1 hr. Soluble protein was separated from un-extracted protein and lentil seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 3 KDa cutoff PES membrane. Further enrichment of dehydrins from this fraction was obtained by boiling the concentrated protein material, spinning at 8000 g for 10 minutes and collecting the supernatant.

EXAMPLE 2: CONSTRUCTING A MUSCLE TISSUE ANALOG

To prepare a muscle tissue replica, 8 ml of moong bean protein solution (114 mg/ml in 20 mM phosphate buffer (pH 7.4) and 400 mM sodium chloride) were mixed with 16 ml of leghemoglobin solution (6 mg/ml leghemoglobin in 20 mM potassium phosphate, 400 mM NaCl, pH 7.3). The resulting mixture was concentrated using Amicon spin concentrators (10 kDa cut-off) to a final concentration of moong bean 8S globulin 61 mg/ml, and of leghemoglobin 6.5 mg/ml. Approximately 400 mg of transglutaminase powder were added to the solution, which were thoroughly mixed, and divided into two 50 ml Falcon tubes and incubated overnight at room temperature. Final total protein concentrations was 67.5 mg/ml total protein. The muscle tissue replica formed an opaque gel of reddish-brown color, with small amounts (< 1 ml) of inclusions of dark red, venous blood colored liquid.

EXAMPLE 3: INCREASED TENSILE STRENGTH ADIPOSE TISSUE REPLICA

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A 40 ml aliquot of rice bran oil and a 40 ml aliquot of moong bean protein (114 mg/ml) were combined in a 250ml Pyrex beaker. The beaker was placed in a water bath and emulsified using a Branson Sonifer 450 sonicator with a 12mm tip for a 6 minute, 60% duty cycle at power level 5.

In an 18cm x 18cm x 2.5cm synthetic rubber Ikea plastic ice cube tray, 48 mg of electrospun fibers (from connective tissue Example 14) were laid longitudinally and as homogenously as possible across the bottom of one triangular 13.97cm x 1.27cm x 1.5875cm mold. Approximately 20 ml of the rice bran oil/moong bean protein emulsions then were poured on top of the fibers. An additional 20 ml of the emulsions were then poured into a similarly sized blank mold on the same tray to be used as a control.

The ice cube tray was floated in boiling water for 15 minutes, removed, and cooled to room temperature.

Using a razor blade, each of the resulting gels was cut into 3 segments, each 4.66cm long with a cross-sectional area of $1cm^2$. A Stable Micro Systems TA XTExpress Enhanced texture analyser with attached TA-96B probe was used to assess the tensile strength. The fiber containing the fat replica had a tensile strength of 23 kPa, whereas the fat replica with no fibers had a tensile strength of 20 kPa.

EXAMPLE 4 HIGH PERCENTAGE FAT ADIPOSE-REPLICA

An adipose tissue replica comprising a protein-oil emulsification formed with 3.3% wt/v pea globulin, 70% v/v oil that consisted of an equal mixture of coconut, cocoa, olive, and palm oils, and 0.5% wt/v lecitin, was cross-linked with a 2% transglutaminase (Ajinomoto Activa® TI). After draining and dehydrating, the resulting gel was medium soft and the fat content was confirmed to be 75% (wt/wt).

An adiose tissue matrix comprising a protein-oil emulsification was formed with 1.6 % wt/v Rubisco and 80% v/v cocoa butter. The resulting gel was soft.

EXAMPLE 5: METHOD OF PREPARING ADIPOSE TISSUE REPLICAS

Oils are melted if necessary by warming to room temperature or gently heated. If the oils are solid at room temperature, they are kept near the melting point during the rest

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of the procedure. Proteins are obtained per specified protocols (see Example 1). Lecithin is weighed and resuspended in water, then sonicated to create a homogenous solution. Components are combined at specified ratios and brought to volume with buffer if necessary (20 mM sodium phosphate pH 7.4 with 50 mM sodium chloride) then are subjected to homogenization or sonication to control particle size within an emulsion. Afterwards, emulsions are gelled by either: (a) heating/cooling, (b) cross-linking with a transglutaminase enzyme or (c) heating/cooling followed by addition of a transglutaminase enzyme. Control samples (no heating/cooling nor transglutaminase cross-linking treatments) were prepared for comparisons. Emulsions stabilized by heating/cooling treatment are prepared by placing emulsion in 90-100°C water bath for five minutes, then letting samples slowly cool to room temperature. Emulsions stabilized by transglutaminase cross-linking are prepared by adding transglutaminase to 2% wt/v and incubating at 37°C for 12-18hr. Emulsions stabilized by heating/cooling followed by addition of transglutaminase enzyme were prepared by first undergoing the heat/cool protocol, then adding the enzyme once the samples cooled to room temperature. All emulsifications are incubated for 8-12 hours at 37°C.

EXAMPLE 6: METHOD TO ANALYZE ADIPOSE TISSUE REPLICAS

After different gelling treatments, gelled emulsions are moved to room temperature for evaluation. The total volume of gelled emulsions, and volumes of phase separated water and/or oil volumes (if gelled emulsions are not in a single phase) are recorded. Firmness of the adipose tissue replica is evaluated by gentle poking of gelled emulsions. Cooking experiments are performed by transferring the mass to a heated surface and measuring the temperature of the liquid immediately after cooking.

EXAMPLE 7: ADIPOSE REPLICA OF BEEF FAT

An adipose tissue replica was made by gelling a solution of purified moong bean 8S protein emulsified with equal amounts of cocoa butter, coconut butter, olive oil and palm oil. Moong bean 8S protein was purified as described in Example 1, and had a concentration of 140 mg/ml in 20mM K-phosphate pH 7.4, 400mM NaCl. A fat mixture

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was prepared by melting individual fats from solid to liquid state at 45°C for 30 min. Individual fats (cocoa butter, coconut butter, olive oil and palm oil) in liquid states were then mixed at a 1:1:1:1 (v/v) ratio. A protein-fat emulsion was formed by mixing a 70% v/v liquid fat mixture with 4.2% wt/v moong bean 8S protein, 0.4% wt/v soybean lecithin and emulsified by vortexing for 30 sec followed by sonication for 1 min. After homogenization, the fat-protein emulsion was in a single liquid phase as judged by visual observation.

One adipose tissue replica emulsion was stabilized by cross-linking with 0.2% wt/v transglutaminase enzyme at 37°C for 12hr. Another fat tissue replica was stabilized by gelling of proteins by heating to 100°C in a water bath followed by cooling to ambient room temperature. The resulting adipose tissue replicas were in a single phase. The adipose tissue replica matrix formed by the transglutaminase was a softer solid than the adipose tissue replica matrix formed by heat/cool induced gelling.

EXAMPLE 8: AN ADIPOSE REPLICA OF WAGYU BEEF FAT

An adipose tissue replica was made by gelling an emulsion of purified pea globulin proteins and equal amounts of cocoa butter, coconut butter, olive oil and palm oil. Pea globulin proteins were purified as described in Example 1 and had a concentration of 100 mg/ml, in 20mM K-phosphate pH 8, 400mM NaCl. A fat mixture was prepared by melting individual fats from solid to a liquid state at 45°C for 30 min. Individual fats (cocoa butter, mango butter, olive oil) in liquid state where then mixed at 2:1:1 (olive oil: cocoa butter: mango butter) v/v ratio. A protein-fat emulsion was formed by mixing a liquid fat mixture with a 5% wt/v solution of pea globulins in a 1:1 ration, and emulsifying using a hand-held homogenizer at the maximum setting for 30sec. After homogenization, the fat-protein emulsion was in a single liquid phase as judged by visual observation. Emulsion was stabilized by cross-linking with 0.2% wt/v transglutaminase enzyme at 37°C for 12hr. The resulting adipose tissue replica was in a single phase, was a soft solid and was salty in flavor.

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EXAMPLE 9: ADIPOSE TISSUE REPLICA WITH FATTY ACID DISTRIBUTION OF BEEF:

An adipose tissue replica was made by gelling an emulsion of purified pea globulin proteins and equal amounts of cocoa butter, mango butter, olive oil and rice bran oil. Pea globulin proteins were purified as described in Example 1, and had a concentration of 100mg/ml, in 20mM K-phosphate pH 8, 400mM NaCl. A fat mixture was prepared by melting individual fats from solid to liquid state at 45°C for 30min. Individual fats (cocoa butter, mango butter, olive oil and rice bran oil) in a liquid state where then mixed at 1:1:1:1 v/v ratio. A protein-fat emulsion was formed by mixing 50% v/v liquid fat mixture with 5% wt/v Pea globulins, and emulsifying using a hand-held homogenizer at the maximum setting for 30sec. After homogenization, the fat-protein emulsion was in a single liquid phase as judged by visual observation. The emulsion was stabilized by cross-linking with 0.2% wt/v transglutaminase enzyme at 37°C for 12hr. The resulting adipose tissue replica was in a single phase, was a soft solid and was salty in flavor.

EXAMPLE 10: ADIPOSE TISSUE REPLICA WHERE FIRMNESS OF FAT TISSUE AT REFRIGERATION AND AMBIENT TEMPERATURES IS CONTROLLED BY MELTING TEMPERATURE OF FAT IN ADIPOSE TISSUE REPLICA.

A adipose tissue replica made as a stable emulsion of RuBisCo with sunflower oil is softer than a adipose tissue replica made as a stable emulsion of RuBisCo and cocoa butter. Adipose tissue replicas were formed with 0.18%, 1.6%, and 2.4% wt/v Rubisco with 70%, 80%, and 90% v/v sunflower or cocoa butters. Each adipose tissue replica that contained cocoa butter was firmer than the corresponding replicas that were formed with sunflower oil. Adipose tissue replicas comprising 0.18%, 1.6%, and 2.4% wt/v Rubisco with 70%, 80%, and 90% v/v cocoa butter were solid at room temperature but melted at close to mouth temperature. In the adipose tissue replicas formed with varying concentrations of Rubisco (0.18, 1.6 1.9 % wt/v) and 70-80% v/v sunflower oil, the replicas were firmer as the amount of protein in the adipose tissue replica matrix

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increased. Adipose tissue replicas with 0.18% wt/v Rubisco were very soft; adipose tissue replicas with 1.6% wt/v Rubisco were soft; and adipose tissue replicas with 1.9% wt/v RuBisco were of medium firmness.

Adipose tissue replicas made as a stable emulsion of Moong Bean 8S protein with sunflower oil were softer than adipose tissue replicas made as a stable emulsion of Moong Bean 8S protein and cocoa butter. Adipose tissue replicas were formed with 2%, 1%, and 0.5% wt/v Moong Bean 8S protein with 70%, 80%, and 90% v/v sunflower or cocoa butters. Each adipose tissue replica that contained cocoa butter was firmer than the corresponding replica that was formed with sunflower oil.

Adipose tissue replicas made as stable emulsions of Moong Bean 8S protein with canola oil were softer than the corresponding adipose tissue replicas made as stable emulsions of Moong Bean 8S protein with an equal mixture of coconut, cocoa, olive, and palm oils. Adipose tissue replicas were formed with 1.4% wt/v Moong Bean 8S protein with 50%, 70%, and 90% v/v sunflower or mixture of oils. Each adipose tissue replica that contained a mixture of oils was firmer than the corresponding replica that was formed with sunflower oil. A adipose tissue replica comprising1.4% wt/v Moong Bean 8S protein with 50%, 70%, and 90% v/v of an equal mixture of coconut, cocoa, olive, and palm oil was solid at room temperature but melted at close to mouth temperature.

Adipose tissue replica made as a stable emulsion of soy proteins with sunflower oil was softer than the adipose tissue replica made as stable emulsion of soy proteins and cocoa butter. Adipose tissue replicas were formed with 0.6%, 1.6%, and 2.6% wt/v Soy with 50%, 70%, 80%, and 90% v/v sunflower or mixture of oils. Each adipose tissue replica that contained mixture of oils was firmer than the corresponding replica that was formed with sunflower oil. Adipose tissue replicas comprising 0.6%, 1.6%, and 2.6% wt/v soy proteins with 50%, 70%, 80%, and 90% v/v cocoa butter were solid at room temperature but melted at close to mouth temperature.

EXAMPLE 11: ADIPOSE TISSUE REPLICA COOKING: STRUCTURE OF FAT TISSUE MATRIX CONTROLS MELTING POINT DURING COOKING.

An adipose tissue comprising a stabilized protein-oil emulsion constructed as described

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in Example 5 and Example 6 above, formed with 2% w/v Rubisco and 50%, 70%, and 90% v/v cocoa butter, melted at a higher temperature when formed upon heat/cool denaturation and at a lower temperature than when formed by cross-linking with a transglutaminase.

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EXAMPLE 12: COOKING ADIPOSE TISSUE: ARRANGEMENT AND STRUCTURAL ORGANIZATION OF PROTEINS AND FAT WITHIN A FAT TISSUE MATRIX CONTROLS AMOUNT OF FAT RELEASED AND FAT RETAINED BY THE ADIPOSE TISSUE REPLICA DURING COOKING.

10 During cooking of a adipose tissue replica matrix comprising a protein-oil emulsion formed with 2% w/v Rubisco and 50%, 70%, or 90% v/v cocoa butter, more adipose tissue replica mass was retained after cooking when the adipose tissue replica was formed upon heat/cool denaturation than when formed by cross-linking with a transglutaminase. Released mass was liquid and appeared oily.

15 During cooking of adipose tissue replica matrix comprising a protein-oil emulsion formed with 2.6 and 0.6 % w/v Soybean protein and 50%, 70%, or 90% v/v cocoa butter, more adipose tissue replica mass was retained when formed upon heat/cool denaturation than when formed by cross-linking with transglutaminase. Released mass was liquid and appeared oily.

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EXAMPLE 13: COOKING AN ADIPOSE TISSUE REPLICA: CONCENTRATION OF PARTICULAR PROTEINS WITHIN FAT TISSUE MATRIX CONTROLS MASS OF ADIPOSE TISSUE REPLICA THAT REMAINS AFTER COOKING

25 A series of adipose tissue replicas constructed from 1.4% wt/v moong bean 8S protein with 90% v/v canola oil and 0.45% wt/v soybean lecithin, were homogenized and increasing concentration of sunflower oleosins were added at varying concentrations to the emulsion. The concentration of oleosin varied from 1:10 to $1:10^6$ oleosin to triglyceride molar ratio. An increase in mass retention after cooking was observed when
30 the ratio of oleosins to oil in adipose tissue replica was greater.

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A series of adipose tissue replicas formed with varying concentrations of Rubisco with 70% v/v sunflower oil, retained more mass upon cooking as the concentration of Rubisco increased. Adipose tissue replicas containing RuBisCo at 0% wt/v completely melted, while 1.9% wt/v Rubisco retained 10% mass, and adipose tissue replica containing 2.4% wt/v Rubisco retained 20% mass upon cooking

EXAMPLE 14: CONNECTIVE TISSUE ANALOG

Connective tissue fiber replicas were manufactured by electrospinning a solution of moong bean globulin (22.5 mg/ml) containing 400 mM sodium chloride, 6.75% w/v of poly(vinyl alcohol) and trace amounts of sodium azide (0.007% w/v). The resulting solution was pumped at 3 µl/min using a syringe pump, from a 5 ml syringe through a Teflon tube and a blunted 21gauge needle. The needle was connected to a positive terminal of a Spellman CZE 30 kV high voltage supply set at 17kV and fixed 12 cm from a an aluminum drum (ca. 12 cm long, 5 cm in diameter) that was wrapped in aluminum foil. The drum was attached to a spindle that is rotated by an IKA RW20 motor at about 220 rpm. The spindle was connected to a ground terminal of the high voltage supply. The protein/polymer fibers that accumulated on the foil were scraped off and used as the connective tissue replicas.

EXAMPLE 15: EXTENDING THE LIFETIME OF REDUCED (HEME-FE2+) LEGHEMOGLOBIN

Equine myoglobin was purchased from Sigma. Myoglobin was resuspended at 10 mg/ml in 20 mM potassium phosphate, pH 8.0, 100 mM NaCl. SDS-PAGE analysis suggested that protein purity was ~ 90%.

Soy leghemoglobin was purified from *Glycine max* root nodules via ammonium sulfate precipitation (60%/90% fractionation) as detailed in Example 1. Resuspended 90% ammonium sulfate leghemoglobin was further purified by anion-exchange chromatography (HiTrap Q FF 5mL FPLC column) in 20 mM potassium phosphate, pH 8.0, 100 mM NaCl. The leghemoglobin eluted in flow through fractions. SDS-PAGE analysis suggested that protein purity was ~ 70%. Leghemoglobin was buffer exchanged

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into 20 mM potassium phosphate, pH 7.4, 100 mM NaCl and concentrated to 10 mg/ml on 3.5kDa membrane concentrators.

Carbon monoxide treatment: Myoglobin at 10 mg/ml in 20 mM potassium phosphate, pH 8.0, 100 mM NaCl and leghemoglobin at 10 mg/ml in 20 mM potassium phosphate, pH 7.4, 100 mM NaCl were first degassed under vacuum for 1 hour at 4°C then perfused with carbon monoxide gas for 2 minutes. Globins were then reduced from heme-Fe^{3+} to heme-Fe^{2+} state by adding 10 mM sodium dithionite, 0.1mM sodium hydroxide for 2 minutes. Sodium dithionite and sodium hydroxide were removed from the protein solution by using size-exclusion chromatography (PD-10 desalting column) in 20 mM potassium phosphate, pH 8.0, 100 mM NaCl and 20 mM potassium phosphate, pH 7.4, 100 mM NaCl respectively. Globins fractions were collected as a peak red colored fractions as evaluated by visual estimation. UV-VIS spectra confirmed the presence of heme-Fe^{2+} state for both proteins. After desalting, the solution was again perfused with gas for another 2 minutes. The color of the solutions was evaluated by taking UV-Vis spectra (250nm- 700nm) every 20 minutes using the nanodrop spectrophotometer. Control samples were not treated with carbon monoxide.

Sodium nitrite treatment: Myoglobin at 10 mg/ml in 20 mM potassium phosphate, pH 8.0, 100 mM NaCl and leghemoglobin at 10 mg/ml in 20 mM potassium phosphate, pH 7.4, 100mM NaCl were reduced from heme-Fe^{3+} to heme –Fe^{2+} by adding 10 mM sodium dithionite, 0.1mM sodium hydroxide for 2 minutes. Sodium dithionite and sodium hydroxide were removed from the protein solution by using size-exclusion chromatography (PD-10 desalting column) in 20 mM potassium phosphate, pH 8.0, 100 mM NaCl and 20 mM potassium phosphate, pH 7.4, 100 mM NaCl, respectively. Globins fractions were collected as peak red colored fractions as evaluated by visual estimation. UV-VIS spectra confirmed the presence of heme-Fe^{2+} state for both proteins. Sodium nitrite was then added to a final concentration of 1 mM from 100 mM nitrite in phosphate buffer pH 7.4. The lifetime of heme-Fe^{2+} state was followed by recording UV-VIS spectra (250-700nm) using spectrophotometer as a function of time. Control samples were not treated with sodium nitrite.

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Data analysis of heme-Fe^{2+} life-times for myoglobin and leghemoglobin samples treated with carbon monoxide and sodium nitrite was performed in Microsoft Excel by plotting the amplitude of the absorbance peak at the 540nm wavelength. The "baseline" of the 540 nm absorbance was determined by the state of the UV-vis spectrum of the globin solutions prior to the addition of any additives, dithionite reduction, or desalting. The built-in curve fit function was used to produce an exponential line-of-best-fit, the exponent of which directly relates to the half life of the peak amplitude.

The life-time of the heme-Fe^{2+} state and accompanying red color of myoglobin and leghemoglobin solutions in the absence of carbon monoxide and sodium nitrite were ~ 6hr and ~ 4hr respectively. Addition of sodium nitrite extended the life-time of the heme-Fe^{2+} state and accompanying red color to more than seven days. Addition of carbon monoxide extended the life-time of heme-$Fe2^{+}$ state and accompanying red color to more than two weeks.

EXAMPLE 16: PREPARATION OF MEAT REPLICAS IN WHICH THE PARTICLE SIZE OF INDIVIDUAL TISSUE REPLICA UNITS IS VARIED TO CONTROL AROMA GENERATION DURING COOKING.

Muscle tissue replica and adipose tissue replica were prepared separately and then combined into a meat tissue replica such that the size of individual tissue replica units was varied to control aroma generation during cooking. Individual fat, muscle and connective tissue replicas were constructed in the following manner.

A muscle tissue replica was prepared as in Example 2. The muscle tissue replica formed an opaque gel of reddish-brown color, with small amounts (< 1 ml) of inclusions of dark red, venous blood colored liquid. A connective tissue replica was prepared as in Example 14. An adipose tissue replica was prepared as in Example 7.

Meat replicas with a lean-to-fat ratio 85/15 were prepared by combining individual muscle, connective and adipose tissues such that particle size of individual tissues replicas varied. (a) 2.1 g of muscle replica with 0.9 g of chunks of fat replica 5-10 mm in size ("coarse mix"); (b) 2.1 g of muscle replica with 0.9 g of fat replica chopped to 2-3 mm in size ("fine mix"); and (c) 2.1 g of muscle replica with 0.9 g of fat replica

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thoroughly blended to <1 mm in size ("blend"). "Muscle only" control sample contained 3 g of muscle replica alone. "Fat only" control sample contained 3 g of fat replica alone as 5-10 mm sized particle. Meat, muscle and fat tissue samples were cooked in sealed glass vials at 150°C for 10 min. Aroma profiles of the samples were analyzed by a panel of testers, and by GC-MS.

Sensory olfactory analysis of meat replica samples performed by a panel of testers, suggested that the size of individual tissue units and the extent of their mixing within meat tissue replicas correlated with generation of different aromas. Muscle tissue replica cooked by itself generated aromas associated with store-bought gravy, faint citrus and star anise. Adipose tissue replica cooked by itself generated aromas associated with musty, rancid and sweet aromas. Cooked meat tissue replica (coarse particle size) generated aromas of store-bought gravy, sweet, slightly musty and star-anise. Cooked meat tissue replica (fine particle size) generated aromas associated with soy sauce, musty, slightly rancid and beef bouillon. Cooked meat tissue replica (very fine particle size) generated aromas associated with sweet, musty and soy-sauce. All samples with the exception of adipose tissue replica generated aromas associated with burnt meat smell, however to varying intensities.

Analysis of GCMS data indicated that the size of individual tissue units and the extent of their mixing within meat tissue replicas had profound effects on the generation of aromatic compounds upon cooking. In particular, multiple aromatic compounds associated with fruity/grean bean/metallic (2-pentyl-furan); nutty/green (4-methylthiazole); peanut butter/musty (pyrazine, ethyl); raw potato/roasted/earthy (Pyrazine, 2,3-dimethyl); vinegary (acetic acid); spicy/caramel/almond (5-methyl-2-furancarboxaldehyde); creamy (butyrolactone); sweet (2,5-dimethyl-3-(3-methyl butyl) pyrazine); fruity/stale beer (2-cyclopentene-1-one, 2-hydroxy-3-methyl); musty/nutty/coumarin/licorice/walnut/bread (3-acetyl-1H-pyrolline); coconut/woody/sweet (pantolactone); penetrating (1-H-pyrrole-2-2carboxaldehyde, 1-methyl); minty (caprolactam); toasty caramel (4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl) aromas appeared only in mixed meat replicas, but not in individual tissue replicas. Some other aromatic compounds, for example associated with gasoline-

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like (nonane, 2,6-dimethyl), petroleum-like (3-hexene, 3-methyl); sour/putrid/fish-like (pyridine); bland/woody/yogurt (acetoin); fatty/honey/citrus (octanal); pungent/sweet/caramelic (2-propanone, 1-hydroxy) and nutty/burnt green (ethenyl pyrazine) aromas appeared only in individual tissue replicas, but did not accumulate in mixed meat replicas. Furthermore, the levels to which all of the above compounds accumulated during cooking depended on the sizes of tissue units and how they are mixed (coarse particle size, fine particle size, or very fine particle size (blended)).

Similar to meat tissue replica, it was found that structural organization and particle size of beef tissues modify response of beef tissues to cooking. For example, the flavor of meat is modified by the size of the particles. Beef samples were prepared as following: samples of beef muscle and beef fat were cut separately with a knife and: (a) "ground", where knife-cut tissue cubes were passed through standard meat grinder. 80/20 (wt/wt) lean/fat ground beef sample was prepared by mixing muscle and fat tissue cubes at appropriate ratio prior to grinding. This sample preparation is referred to as a "fine size particle mix". (b) Ground tissue particle size was further reduced by freezing ground tissue in liquid nitrogen and crushing it using mortar and pestle to a very fine powder (particle size <1mm). This sample preparation is referred to as a "very fine size particle mix". All samples were cooked in sealed glass vials at 150 °C for 10 min. Aroma profiles of the samples were analyzed by a panel of testers, and by GC-MS, as described in Example 1. "Muscle only" control sample contained 3 g of muscle tissue alone. "Fat only" control sample contained 3 g of fat tissue alone. Ground beef sample contained 3 g of a 80/20 (wt/wt) muscle/fat mixture.

Sensory olfactory analysis of beef samples performed by a panel of testers, suggested that the size of individual tissue units and the extent of their mixing within the samples correlated with generation of different aromas. Beef muscle cooked by itself generated typical aromas associated with cooked ground beef. Fat tissue replica cooked by itself generated slightly sweet aromas, and aromas associated with burnt mushrooms. Cooked ground beef with "fine size particle mix" generated typical aromas associated with cooked ground beef, with presence of slightly sweet aromas characteristic of cooked fat. Cooked ground beef with "very fine size particle mix" generated aromas associated

with cooked ground beef, but no slightly sweet aroma characteristic of cooked fat was detected.

Analysis of GCMS data indicated that the particle size of the individual tissue units has effect on generation of aromatic compounds upon cooking. In particular generation and/or amount of multiple aromatic compounds by individual tissue samples or ground beef sample varied in correlation with particle size of the tissue. Some of the aromatic compounds that differed between fine and very fine particle size of muscle tissue: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 3-Acetyl-1H-pyrolline, 1-(6-methyl-2-pyrazinyl)-1-ethanone, 2,5-dimethyl-3-(3-methyl butyl) pyrazine, 2-furancarboxyaldehyde, 5-methyl, Acetic acid, Ethenyl pyrazine, Pyrazine, 2,3-dimethyl , 2-Propanone, 1-hydroxy, Octanal, Acetoin, 4-Methylthiazole, Pseudo-2-pentyl-furan, 2-pentyl-furan. Some of the aromatic compounds that differed between fine and very fine particle size of fat tissue: triethylene glycol: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, Caprolactam, 1-(6-methyl-2-pyrazinyl)-1-ethanone, 2-Cyclopentene-1-one, 2-hydroxy-3-methyl, Butyrolactone, 2-furancarboxyaldehyde, 5-methyl, Ethanone, 1(2 furanyl), Acetic acid, 2-ethyl-5-methyl pyrazine, Pyrazine, 2,3-dimethyl, Pyrazine, ethyl, Octanal, Acetoin, 4-Methylthiazole, Pseudo-2-pentyl-furan, Pyridine, Nonane, 2,6-dimethyl. Some of the aromatic compounds that differed between fine and very fine particle size of 80/20 muscle/fat sample: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, Caprolactam, 1H-1Pyridine, 3-carbonitrile, 4-ethyl-2-oxo-2,5, 1-H-Pyrrole-2-2carboxaldehyde, 1-methyl, 2-Cyclopentene-1-one, 2-hydroxy-3-methyl , 2,5-dimethyl-3-(3-methyl butyl) pyrazine, Butyrolactone, 2-furancarboxyaldehyde, 5-methyl, Ethanone, 1(2 furanyl), Acetic acid, Ethenyl pyrazine, 2-ethyl-5-methyl pyrazine, Pyrazine, 2,3-dimethyl; 2-Propanone, 1-hydroxy, Octanal, Acetoin, 2-pentyl-furan.

EXAMPLE 17: LEGHEMOGLOBIN CONTRIBUTION TO FLAVOR

Beef flavors and aromas can be created in non beef consumables by addition of heme proteins. Ground chicken (90% lean, 10% fat) was strained with cheesecloth and mixed with recombinant soy leghemoglobin or recombinant bovine myoglobin to a final

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concentration of 0.5-1.0% wt/wt. The recombinant heme proteins were expressed in *E. coli* and purified by nickel affinity purification as described in Example 1. Prior to being mixed with chicken, the heme proteins were reduced with 20 mM Na Dithionite. Na dithionite was removed from the sample with a Zeba desalting column (Thermo Scientific). Leghemoglobin was desalted into 20 mM potassium phosphate pH 7.4, 100 mM NaCl. Myoglobin was desalted into either 20 mM potassium phosphate pH 7.4, 100 mM NaCl or 20 mM Na citrate pH 6.0, 100 mM NaCl. The reduced heme protein samples were divided in two, and half the sample was bubbled with carbon monoxide (CO) for 2 minutes. After mixing the heme protein samples with ground chicken, the mixture was poured into nugget-shaped molds and incubated overnight at 4°C. The nuggets were oven baked or pan fried at 165°C until each nugget reached an internal temperature of 165°C. A panel of judges tasted nuggets containing chicken alone, chicken mixed with buffer, chicken mixed with either leghemoglobin or myoglobin +/- CO, or beef (90% lean, 10% fat). Judges filled out a survey to evaluate the aroma and flavor of each nugget. Judges rated the aroma and flavor of each nugget as follows: 1 = chicken, 2 = chicken + faint beef, 3 = 50/50 chicken + beef, 4 = beef + faint chicken, 5 = beef. Shown in Table 2 are the average scores received for each nugget. Percentages indicate the final concentration of heme protein wt/wt (abbreviations: KP = 20 mM potassium phosphate pH 7.4, 100 mM NaCl buffer. NC = 20 mM Na citrate pH 6.0, 100 mM NaCl buffer. n/d = not determined). Adding recombinant leghemoglobin or myoglobin to chicken resulted in an increased beef aroma and flavor. The perceived levels of beef flavor and aroma increased with the myoglobin and leghemoglobin content. Leghemoglobin and myoglobin provide the same benefit to the flavor and aroma.

TABLE 2

	Oven Baked		Pan Fried	
	Aroma	Flavor	Aroma	Flavor
Chicken	1	1	1	1
Chicken KP	1	1	2.5	1.2
Chicken NC	1.5	1.5	1.5	1
Chicken 0.5% legH KP	1.5	2.5	3.67	3.2
Chicken 0.5% legH+CO KP	2.5	2.5	2.67	2.2

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Chicken 0.5% Myo NC	2	2	1.5	2.4
Chicken 0.5% Myo+CO NC	2	2	2.5	3
Chicken 0.5% Myo+CO KP	2.5	2.5	2.33	2
Chicken 0.8% Myo+CO NC	2	3	4	2.6
Chicken 1% Myo NC	4.5	4	n/d	n/d
Chicken 1% legH NC	4	4	n/d	n/d
Beef	5	5	5	5

EXAMPLE 18: PREPARATION OF A NON-DAIRY CREAM LIQUOR

A cream liquor was made from a sunflower cream fraction, RuBsiCo and whiskey (Jameson). A sunflower cream fraction was made by blending sunflower seeds in 40 mM sodium phosphate pH 8.0, 400 mM sodium chloride buffer. Seed debris was pelleted by centrifugation at 5000g for 20 min, and the cream fraction collected. The cream fraction was resuspended in 10 mM potassium phosphate pH 7.4 buffer and collected by centrifugation at 5000g, 20 min. Rubsico was purified as described in Example 1 and used as 25mg/ml stock solution in 20mM K-phosphate pH 7.0, 150mM NaCl.

In one example, cream liquor was made as follows: 11.4% wt/v of sunflower cream fraction, 40% v/v Jameson whiskey, 0.4-1.6% wt/v Rubsico, 0.5% wt/v vanilla extract, 0.5% v/v espresso coffee, and 1.5% wt/v chocolate powder. The resulting mixture was homogenized at 5000 psi.

In another example, cream liquor was made from sunflower cream fraction and whiskey (Jameson) and sugar: 11.4% wt/v of sunflower cream fraction, 40% v/v Jameson whiskey, 0.5% v/v vanilla extract, 0.5% v/v espresso coffee, 1.5% wt/v chocolate powder and 8% wt/v sugar.

Beverages were served either at ambient room temperature or chilled. Resulting beverages were beige to light chocolate in color. The tasting results suggested that the beverage had a very creamy alcoholic flavor similar to dairy cream liquor. Chilled product was preferred. The emulsion was stable at room temperature for at least 1 week (maximum time tested).

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EXAMPLE 19 –CHOCOLATE SPREAD

A chocolate spread was made from 34% (wt/wt) cane sugar, 22 % (wt/wt) cocoa powder, 19 % (wt/v) pistachio cream fraction, and 12 % (v/v) almond milk. Cane sugar and cocoa powder (Ghirardelli) were commercially purchased. Almond skim milk was made in a following manner: Almonds were blanched by immersion into 100°C water for 30 sec. Blanched nuts were recovered and cooled by immersion into ice-cold water. Almonds were air dried. Almonds were then rehydrated by immersion in 2°C water for 16hr. Rehydrated almonds were drained, mixed with water at 1:2 wt/v ratio and blended in a Vitamix blender for 5 min. The blended slurry was collected in chilled container and stirred with a frozen cooling stick to cool. Once the slurry cooled to 10°C, the slurry was placed at 2°C for up to 12 hours. Almond skim and cream were separated by centrifugation at 7480g for 30min at 4°C. Almond milk separated into 3 layers, a dense pellet of insoluble solids, a clear to translucent aqueous layer (which is referred to as the "almond skim milk"), and a lighter, creamy, opaque layer (which is referred to as the "almond cream"). Almond milk was then was pasteurized at 75°C for 16 seconds, chilled and stored at 2°C.

A pistachio cream fraction was prepared by blending pistachios in 100 mM sodium carbonate pH 9.5 buffer with 400 mM sodium chloride and 1 mM EDTA, then centrifuging 5000xg for 20 min. The cream fraction was collected and washed into the same buffer once more. After centrifugation at 5000g for 20 min, the cream fraction was collected and washed into 20 mM sodium phosphate pH 7.4 buffer with 50 mM sodium chloride and 1 mM EDTA. After centrifugation at 5000g for 20 min, the cream fraction was collected an washed once more in the neutral (pH 7.4) buffer, centrifuged at 5000g for 20 min. Pistachio cream fraction was collected and stored at 4°C.

Chocolate spread was made in the following way. Cane sugar was melted in almond milk, cocoa powder was added to sugar-milk mixture with stirring and melted. Sugar, milk and cocoa were then added to the pistachio cream fraction and whisked together. Resulting mixture was then poured into molds and let sit for 24 hours at refrigeration and freezer temperatures.

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In another example, chocolate spread was made from 42% (wt/wt) cane sugar, 27 % (wt/wt) cocoa powder, 31 % (wt/v) sunflower cream fraction, and 23% (v/v) almond skim milk. All ingredients and procedures apart from sunflower cream fraction were as described above.

The sunflower cream fraction was created from blending sunflower seeds in 5 times the weight to volume of a solution of 40 mM potassium phosphate pH 8, with 400 mM NaCl, 1 mM EDTA, then cooled to 20°C, and the slurry was then centrifuged. The top cream layer was removed and is blended in the same buffer, followed by heating for 1 hour at 40°C. The slurry is cooled down to 20°C then centrifuged; the cream layer is removed and mixed with 5 times the weight to volume of 100 mM sodium carbonate pH 10 with 400 mM NaCl, then centrifuge. The top layer is then mixed with 5 times the weight to volume of water and centrifuged again, the resulting cream fraction is very creamy, white, and neutral tasting.

In another example, a chocolate spread was made from 37% (wt/wt) cane sugar, 23 % (wt/wt) cocoa powder, 13 % (wt/v) sunflower cream fraction, and 7 % (wt/wt) cocoa butter, and 20 % v/v almond skim milk.

In another example, a chocolate spread was made from 37% (wt/wt) cane sugar, 23 % (wt/wt) cocoa powder, 13 % (wt/v) sunflower cream fraction, and 7 % (wt/wt) coconut oil 20 % v/v almond skim milk.

In another example a chocolate spread was made from 37% (wt/wt) cane sugar, 23 % (wt/wt) cocoa powder, 13 % (wt/v) sunflower cream fraction, and 7 % (wt/wt) palm oil, and 20 % v/v almond skim milk.

In another example a chocolate spread was made from 1.8% (wt/wt) cane sugar, 1.13% (wt/wt) cocoa powder, 88 % (wt/v) pistachio cream fraction, and 9 % almond skim milk, by whisking equal amounts of spread described above and pistachio oil bodies.

In another example, a chocolate spread was made from 8.5 % (wt/wt) cane sugar, 5.4 % (wt/wt) cocoa powder, 81 % (wt/v) sunflower cream fraction, and 4.6 % (v/v) almond skim milk, by mixing chocolate spread described above with sunflower cream at ratio 2:1.

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Visual and textural inspection of all products suggested that they formed stable, solid, creamy spreads at room temperatures. All products were firm solids at refrigeration and freezer temperatures. Tasting results of all products suggested very pleasant, rich creamy texture with product melting in the mouth reviewed positively by tasters. Individual tasters preferences varied with respect to like or dislike of pistachio flavor, coconut flavor, preference for more or less sweet product and more or less cocoa flavor. One particular sample was described as being similar to a milk chocolate spread, as the sunflower cream fraction contributed a neutral flavor.

EXAMPLE 20 - Generation of Adipose Tissue Replica

Adipose tissue replicas were generated using the ingredients recited in Table 3.

TABLE 3

Adipose Tissue Replica	
Ingredient	**%**
Coconut oil	65
Pea vicillin protein in buffer	21.3
Cocoa butter	10
Buffer	2.7
Lecithin slurry, 50 mg/ml	1
Total	100

Lecithin (SOLEC™ F Deoiled Soy Lecithin, The Solae Company, St. Louis, MO) was prepared at a concentration of 50 mg/ml in 20 mM potassium phosphate, 100 mM NaCl, pH 8.0 buffer and sonicated (Sonifier Analog Cell Disruptor model 102C, BRANSON Ultrasonics Corporation, Danbury, Connecticut) for 30 seconds.

The pea vicilin protein was supplied as a liquid containing approximately 140 mg/g pea vicilin in 20 mM potassium phosphate, 100 mM NaCl, pH 8.0 buffer.

Coconut oil (Shay and Company, Milwaukie, OR) and cocoa butter (Cocoa Family, Duarte, CA) were melted by heating to 50 - 70°C and then combined and held warm until needed.

The buffered protein solution, additional buffer, and lecithin slurry were mixed in a 32 ounce sized metal beaker and equilibrated to room temperature. An emulsion was formed using a hand held homogenizer (OMNI model GLH fitted with G20-195ST

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20mm generator probe, OMNI International, Kennesaw, GA). The homogenizer probe was put into the protein lecithin mixture and turned on to speed 4. The warmed oil was then added slowly over the course of about 2 minutes while continuously moving the probe around in the mixture.

The emulsion was then heat set by placing the metal beaker into a 95°C waterbath. Using a clean spatula, the emulsion was stirred every 20 seconds for 3 minutes total. The beaker was then removed from the waterbath and stored at 4°C for several hours until thoroughly cooled.

EXAMPLE 21 - Generation of Raw Tissue Replica

A raw tissue replicawas generated using the ingredients recited in Table 4.

TABLE 4:

Raw Tissue Replica	
Ingredient	**%**
Buffer	41.6
Heme protein in buffer	26.7
Pea legumin in buffer, dried	12.1
Pea vicillin in buffer, dried	9.4
Flavor precursor mix, 17x	6.2
Transglutaminase preparation	4
Total	100

The buffer was 20 mM potassium phosphate, 100 mM NaCl, pH 7.4. The heme protein was prepared at a concentration of 55 mg/g in 20 mM potassium phosphate, 100 mM NaCl, pH 7.4 buffer. The 17x flavor precursor mix precursor is described in Example 27. The pea legumin was prepared in 20 mM potassium phosphate, 500 mM NaCl, pH 8 buffer and then freeze dried prior to use. The final protein concentration of the dried material was 746 mg/g. The pea vicilin was prepared in 20 mM potassium phosphate, 200mM NaCl, pH 8 buffer and then freeze dried prior to use. The final protein concentration of the dried material was 497 mg/g.

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The liquid ingredients (buffer, heme, and flavor precursor mix) were mixed in a plastic beaker. The dry pea legumin and pea vicilin were then added and allowed to fully rehydrate while gently stirring for 1 hour at room temperature. The dry transglutaminase preparation (ACTIVA® TI, Ajinomoto, Fort Lee, NJ) was then added and stirred for about 5 minutes until dissolved. The stirring was then turned off and the mixture was allowed to gel at room temperature until firm. After the gel had formed the raw tissue replicawas stored refrigerated until used.

EXAMPLE 22 - Hard Connective tissue replica

A hard connective tissue replica was made as following using soy protein isolate (Supro Ex38, Solae), wheat gluten (Cargill), and bamboo fiber (Alpha-Fiber B-200, The Ingredient House). The purified proteins were freeze-dried and milled using a standard coffee grinder. Commercially available powders of soy protein isolate and wheat gluten were used as received.

The connective tissue replica contained 49% soy protein isolate, 49% wheat gluten and 2% bamboo fiber. The ingredients were thoroughly mixed and loaded into the loading tube of the extruder's batch feeder. A twin-screw extruder (Nano 16, Leistritz Extrusion Corp.), with a high-pressure water injection pump (Eldex) and custom-made die nozzles (stainless steel tube, 3 mm ID, 15 cm length, pressure rating 3000+ PSI) attached using a Hy-Lok two-ferule tube fitting and a custom-made die with a threaded nozzle and 10 mm ID, 20 mm long flow channel was used.

The dry mixture was fed into the extruder at the rate 1 g/min. Water was fed by the pump into the second zone of the extruder's barrel. The rate of water feeding is adjusted to the rate of dry mixture feeding such as to provide 55% moisture level in the final extrudiate. A temperature gradient was maintained along the extruder barrel as follows: feed zone - 25°C, zone 1 – 30°C, zone 2 – 60°C, zone 3 – 130°C, zone 4 – 130°C. The die plate was neither actively heated, nor cooled. The die nozzle was actively cooled (by applying moist tissue replica) to maintain extrudate temperature below 100°C.

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Hard connective tissue replica obtained by this process was dark off-white ("cappuccino") colored material shaped into 3 mm thick filaments that had tensile strength similar to animal connective tissue (3 MPa).

Example 23. Soft Connective tissue replica

A soft connective tissue replica was made as in Example 22, except that the rate of water feeding was adjusted to the rate of dry mixture feeding to provide 60% moisture level in the final extrudate. A temperature gradient was maintained along the extruder barrel as follows: feed zone - 25°C, zone 1 – 30°C, zone 2 – 60°C, zone 3 – 115°C, zone 4 – 115°C. The die plate was neither actively heated, nor cooled. The die nozzle was actively cooled (by applying moist tissue) to maintain extrudate temperature below 100°C.

Soft connective tissue replica obtained by this process was light off-white colored material shaped into 3 mm thick filaments that had low tensile strength (< 0.1 MPa) and had a significant propensity to split longitudinally into bands and thin fibers

EXAMPLE 24. Thin connective tissue replica (zein fibers) process:

A thin connective tissue replica was prepared using zein protein powder, glycerol (FCC grade), polyethylene glycol (PEG 400 or PEG3350), ethanol, sodium hydroxide (FCC grade), and water. The zein powder and PEG3350 at a 35% w/w ratio to zein were dissolved in 85% aqueous ethanol to reach a final zein concentration of 57% w/w. The pH of the solution was adjusted to 7.0 with 1M solution of sodium hydroxide in ethanol. A syringe pump with A 1-12 ml syringe, spinning nozzle (hypodermic needle, 18-27 gauge, or a plastic nozzle, 18-24 gauge), heating silicon tape, and a heating fan was used, with a collector assembled using a computer-controlled motor rotating a Delrin rod, which serves as a collector.

This solution was loaded into the syringe, which was mounted onto the syringe pump, with an 18 gauge plastic tip attached. A silicon heating tape was looped around the tip to maintain it at elevated temperature. After solution was extruded out of the tip and forms a drop, it was picked up with a spatula and carefully transferred towards the

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collector rod to form a filament between the tip and the collector. The extrusion rate was optimized to produce even flow of material from the tip (5 ml/h for a 12 ml syringe and 18 gauge tip). The collector rotation speed was 3 RPM. The heating fan was positioned to blow hot air onto the spooling fiber. After spooling, the fibers were cured in a 120°C oven for 24 hours.

Thin connective tissue replica obtained by this process was semi-clear yellow colored material shaped into 300-micrometer thick fibers that were semi-flexible in air, and became very flexible and elastic in presence of water, maintaining high tensile strength similar to animal connective tissue (6 MPa)

EXAMPLE 25. Noodles

Noodles were prepared using purified pea vicilin (freeze-dried), and soy protein isolate (Supro EX38 by Solae, Solbar Q842 (CHS)) or soy protein concentrate (Hisolate, Harvest Innovations). To prepare the noodles, 67% soy protein concentrate or isolate, and 33% ground pea vicilin powder were thoroughly mixed and loaded into the loading tube of the extruder's batch feeder. The dry mixture was fed into the extruder at the rate in the range of 1-2 g/min. Water was fed by the pump into the second zone of the extruder's barrel at the rate 3.6-5.3 ml/min such as to maintain the final moisture content in the extrudate at 72.5%. A temperature gradient was maintained along the extruder barrel as follows: feed zone - 25 °C, zone 1 – 30 °C, zone 2 – 60 °C, zone 3 – 100 °C, zone 4 – 100 °C. Zone 1 temperature can be varied in the range of 25 – 45 °C. Zone 2 temperature can be varied in the range of 45 – 65°C. Zone 3 and 4 temperatures can be varied in the range of 95 – 100°C. The die plate was neither actively heated, nor cooled. The die nozzle was passively cooled by ambient air ensuring extrudate temperature below 100°C.

Noodles obtained by this process were light yellow colored material shaped into 1.5 mm thick filaments that had low tensile strength (< 0.1 MPa) and a moderately sticky texture.

Example 26. Sticky Tissue Replica Preparation

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Sticky tissue replica was prepared using purified pea vicilin (freeze-dried) and purified pea legumin (freeze-dried). To prepare the sticky tissue replica, 50% ground pea vicilin powder and 50% ground pea legumin powder were thoroughly mixed and loaded into the loading tube of the extruder's batch feeder. The dry mixture was fed into the extruder at the rate in the range 0.4 – 0.8 g/min. Water was fed by the pump into the second zone of the extruder's barrel at the rate in the range 1.6 – 3.2 ml/min, such that the final moisture content of the extrudate was maintained at 80%. As the total throughput increased from 2 g/min to 4 g/min, the screw speed was increased from 100 to 200 RPM. Larger die diameters (4 mm and above) are also helpful for preventing backflow at higher throughputs. A temperature gradient was maintained along the extruder barrel as follows: feed zone - 25 °C, zone 1 – 30 °C, zone 2 – 60 °C, zone 3 – 90 °C, zone 4 – 90 °C. The die plate was neither actively heated, nor cooled. The die nozzle was passively cooled by ambient air. The die nozzle is kept free of obstructions by solidifying gel material.

Sticky tissue replica obtained by this process was semi-transparent water-white colored material shaped into irregular 1-5 cm sized bulbs that had sticky paste-like texture.

Example 27. Preparation of the flavor precursor mix

A flavor precursor mix was prepared by mixing concentrated stock solutions of each additive to make a 17X solution. Table 5 contains the chemical composition of the mix and the mM concentration of each component in the final burger. The concentrated flavor precursor mix was sterile filtered and adjusted to pH 5.5 – 6.0 using NaOH, and used at a 1X concentration in burgers.

TABLE 5.

Chemical composition of the flavor precursor mix.

Chemical entity	mM
Alanine	5.6
Arginine	0.6
Asparagine	0.8

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Aspartate	0.8
Cysteine	0.8
Glutamic acid	3.4
Glutamine	0.7
Glycine	1.3
Histidine	0.6
Isoleucine	0.8
Leucine	0.8
Lysine	0.7
Methionine	0.7
Phenylalanine	0.6
Proline	0.9
Threonine	0.8
Tryptophan	0.5
Tyrosine	0.6
Valine	0.9
glucose	5.6
Ribose	6.7
Maltodextrin	5.0
Thiamine	0.5
GMP	0.2
IMP	0.6
Lactic acid	1.0
creatine	1.0
NaCl	10
KCl	10
Kphos pH 6.0	10

Example 28 - Freeze-alignment to produce texturized protein for use as muscle replicas

This example describes a non-extrusion based method to produce texturized protein materials that can be used in meat replicas.

A muscle tissue-replica was prepared by first preparing a gel of lentil proteins by mixing a 4.5% (w/v) solution of lentil proteins in 20mM potassium phosphate buffer pH 7.4 + 100mM sodium chloride with 20% (v/v) canola oil (from Jedwards International). The mixture was gelled by heating at 95°C for 15 minutes and slow cooled to room

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temperature (at the rate of 1°C/minute). The gel was then poured into a vessel and frozen at -40°C by placing above a liquid nitrogen bath until completely frozen. The frozen material was then dried in a freeze-dryer. When the material was completely dried, the material was stabilized by autoclaving (121°C, 15 minutes). The resulting material is a texturized muscle tissue replica formed with plant proteins.

The aligned muscle replica then was presoaked in water for 5 minutes, cut into pieces of length 3-4mm and then combined with 10g adipose-replica, 10g connective tissue replica and 5g cold-set gels to form 50g beef patty replicas. An in-house sensory panel attributed inclusion of freeze-aligned tissue to impart improved fibrous texture to the patty.

A muscle-tissue replica also was prepared by first forming freeze-aligned material as described above. After the tissue replica was steam cooked at 121°C for 10 minutes, the material was allowed to soak in a solution of heat-denatured pea vicilins (at 6% w/v in 20mM potassium phosphate buffer pH 7.4 + 100mM sodium chloride, heat denatured by heating at 95°C for 30 minutes), 1% equine myoglobin (w/v) (Sigma), and 40% (v/v) canola oil (from Jedwards International). Gelation of the medium was induced by addition of calcium chloride at 20 mM. The sample was allowed to sit for 5 minutes at room temperature to allow for gel formation. The resulting muscle-replica contained aligned material in a cold-set gel reminiscent of beef muscle in steak.

EXAMPLE 29 - Cold gelation of proteins for meat applications

In one example, a cold-set gel comprising myoglobin was prepared by first heat denaturing a 6% (w/v) solution of pea-vicilins in 20mM potassium phosphate buffer, pH 7.4 with 100mM sodium chloride at 100°C for 30 minutes. The solution was allowed to cool back to room temperature. Canola oil (from Jedwards International) and equine myoglobin (Sigma) were added to a final concentration of 20% (v/v) and 1% (w/v), respectively. Gel formation was induced by adding calcium chloride at 20mM. A 50g beef patty replica was formed by combining 5g of the cold gel with 10g of adipose tissue-replica, 10g of connective tissue-replica, and 25g of muscle tissue-replica. Five mls of a

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7% (w/w) solution of crude lentil protein was added to the mixture and patties were formed.

Example 30 - Binding materials in meat replicas

In one example, a beef replica was made by first preparing a coacervate from a 3% (w/v) solution of pea vicilins and legumins (vicilin:legumin ratio of 3:1) in 20 mM potassium phosphate pH 7.4 + 100 mM sodium chloride. Melted palm oil (from Jedwards International) was added to the solution to a final concentration of 5% and mixed by vortexing. The emulsion then was acidified by adding hydrochloric acid while stirring to a pH of 5. The slurry was then centrifuged at 5000 x g for 10 minutes and the liquid top layer was decanted from the coacervate.

A 50g beef patty replica was formed by combining the coacervate at 10% with adipose tissue-replica (20%), connective tissue-replica (20%) and muscle tissue-replica (50%). Five mls of a 7% solution of crude lentil protein was added to the mixture and patties were formed. Patties that included coacervate as binding material were observed to be more cohesive than patties that did not.

EXAMPLE 31 - Assembly of a sticky and noodle type ground tissue replica and burger replica

Ground tissue replica and burger replicas were prepared using the ingredients in Table 6. During all pre-processing steps, the temperature of the materials was maintained cold (4 – 15°C).

TABLE 6

Composition of sticky and noodle type formulation	
Ingredient	**%**
Adipose	26
Soft connective	20.6
Sticky	12
Raw	10
Noodles	10

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Hard connective	10
Flavor and heme solution	10
k-carrageenan	1.4
Total	100

Adipose tissue replica from Example 6 was chilled following the final heat-cool step into a solid block. Optionally 0.2% by weight of heme protein can be added as a 20 mg/ml liquid solution and manually worked into the adipose. The adipose was then crumbled manually into small bits 3 – 7 mm in diameter while cold.

Soft connective tissue replica from Example 23 was produced by an extrusion process as long string-like pieces. The soft connective was chopped with a mini chopper (Mini-Prep® Plus Processor model DLC-2L Cuisinart, Stamford, CT) in a single step process. Approximate 200g of soft connective was placed in the mini chopper and processed on the chop setting for 60 seconds to yield pieces of 1 – 3 mm in length with ragged edges.

Sticky tissue replica and noodles tissue replica (see Examples 25 and 26) were produced by an extrusion process as long noodle-like pieces or amorphous pieces, respectively. Raw tissue replica of Example 21 was provided from the enzymatic crosslinking process as a solid block. All three of these tissue replicas were manually broken down into pieces 1 - 3 cm in diameter.

Hard connective tissue replica from Example 22 was produced from an extrusion process as long string-like pieces. The hard connective tissue replica was chopped to three levels, named coarse, intermediate, and fine, in a mini chopper (Mini-Prep® Plus Processor model DLC-2L Cuisinart, Stamford, CT). 160 – 200 g of hard connective tissue replica was placed in the mini chopper and processed on the chop setting for 90 seconds. One third of the material was removed as the coarse chopped fraction. The material remaining in the chopper was then processed for an additional 60 seconds and one third of the original weight was removed as the intermediate chopped fraction. The material remaining in the chopper was then processed for an additional 30 seconds to produce the fine fraction.

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Leghemoglobin was freeze-dried and then reconstituted in a 17x flavor precursor mix (see Example 27) adjusted to pH 6.0 with 10 N NaOH to make the flavor and heme protein solution.

Following pre-processing described above, the soft connective, sticky, raw, noodles, hard connective, and 2/3 of the adipose were mixed by hand in a bowl. A typical batch size was 100 g to 2000 g. The flavor and heme solution was then dribbled onto the mixed tissue replicas and mixed gently by hand, then k-carrageenan powder was tsprinkled over the mixture and mixed in by hand. During the assembly, grinding, and forming all materials were kept cold (4 – 15°C). The mixture was ground using a stand mixer fitted with a food grinder attachment (KitchenAid® Professional 600 Series 6 Quart Bowl-Lift Stand Mixer model KP26M1XER and KitchenAid® Food Grinder model FGA, St. Joseph, MI) on speed setting 1. The food grinder material was fed by a screw conveyor past a rotating knife installed in front of a fixed hole plate.

The ground tissue replica mix was collected in a bowl and the remaining 1/3 of the crumbled adipose was then added to the ground tissue replica mix and mixed in by hand. Approximately 30 g or 90 g portions of ground tissue replica were then formed by hand into round patty shapes. Typical dimensions for 30 g patties were 50 mm x 12 mm. Typical dimensions for 90 g patties were 70 mm x 18 mm. Patties were refrigerated until cooked. Cooked patties had appearance, texture, and flavor similar to ground beef as judged by a trained sensory panel. In addition to cooking in patty format, the ground tissue replica can also be used in a variety of dishes such as taco filling, casseroles, sauces, toppings, soups, stews, or loaves.

EXAMPLE 32 - Assembly of a Wheat Gluten containing ground tissue replica and burger replicas

Ground tissue replica and burgers were prepared using the ingredients in Table 7.

TABLE 7:

Composition of Wheat Gluten Type formulation

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Ingredient	%
Adipose	25
Soft connective	30
Raw tissue replica with heme and flavor	35
Wheat gluten	5
Hard connective	5
Total	100

Adipose, soft connective, and hard connective were preprocessed as described in Example 31. During all pre-processing steps, the temperature of the materials was maintained cold (4 – 15°C).

Raw muscle with heme protein and flavor was then prepared as follows. Freeze-dried pea vicilin and pea legumin were dissolved in water and 16x flavor stock. The freeze-dried heme protein was dissolved in this mixture and the pH was adjusted with citric acid to 5.8. A dry transglutaminase preparation (ACTIVA® TI Ajinomoto Fort Lee, NJ) was then added and mixed for about 5 min to fully dissolve. The mixture was then allowed to stir for an additional 10 minutes until some increase in viscosity was observed. The soft connective and hard connective were then added and the mixture was allowed to sit for 1 hour at room temperature to cure and form a solid mass.

Wheat gluten powder (vital wheat gluten, Great Northern, item 131100, Giusto's Vita-Grain, South San Francisco, CA) then was added to the gelled raw muscle and mixed to distribute. This mixture was then immediately ground using a stand mixer fitted with a food grinder attachment as described in the previous example. Ground tissue replica was then chilled for 5 min at -20°C. Finally, the chopped adipose, pre-chilled to 4°C, was added to the chilled ground tissue replica.

Ground tissue replica mix with adipose tissue replica added was then formed by hand into two 90 g round patties. 90 g patties are typically 70 mm x 18 mm. A typical batch size was 180 - 200 g and produced two patties. The patties were then allowed to rest at room temperature for 30 minutes. After resting the patties can be cooked or refrigerated until ready to cook.

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EXAMPLE 33: Generation of beef flavor in replica burgers by the addition of Heme and flavor precursors.

Characteristic flavor and fragrance components in meat are mostly produced during the cooking process by chemical reactions molecules (precursors) including amino acids, fats and sugars that are found in plants as well as meat. Flavor precursors along with 1% Leghemoglobin were added to the muscle component of the burger replicas as indicated in Table 8. Three replicas, one with no precursors, and two different mixtures of precursors, along with 80:20 beef were cooked then served to a trained sensory panel to describe the flavor attributes shown in Table 9. The addition of precursors increased the beefy flavor, the bloody notes, overall flavor quanity, and decreased the off notes in the replica. The replicas and beef sample also were analyzed by GCMS by adding 3 grams of uncooked replica or beef into a GCMS vial. All samples were cooked at 150°C for 3 mins, cooled to 50°C to extract for 12 minutes using GCMS (SPME fiber sampling of headspace). A search algorithm analyzed the retention time and mass fingerprint information to assign chemical names to peaks. In the replica burger with 1% Leg hemeoglobin, and precursor mixture 2, 136 beef compounds were created. In Table 10, all the compounds created in the replica burger that were also identified by GCMS in the beef samples are indicated.

TABLE 8

Flavor precursors added to the beef replicas before cooking.

Samples	**767**	**804**	**929**
Additive (mm)	**Precursor Mix 1**	**No Precursors**	**Precursor Mix 2**
Alanine	5.61		5.61
Cysteine	0.83		0.83
Glutamic acid	3.40		3.40
Leucine	0.76		0.76
Lysine	0.68		0.68
Methionine	0.67		0.67
Tryptophan	0.49		0.49
Tyrosine	0.55		0.55
Valine	0.85		0.85

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Glucose	5.55		5.55
Ribose	6.66		6.66
Lactic acid	1.00		1.00
creatine	1.00		1.00
Thiamine	0.50		0.50
IMP + GMP	0.40		0.40
Sucrose			2.00
Fructose			2.00
Xylose			2.00
Maltodextrin	0.50%		0.50%

TABLE 9

The sensory score determined by the sensory panel for the replica burgers and 80:20 beef sample.

Sample #		**Beef**	**767**	**804**	**929**
Flavor Quality	mean	7.0	3.8	3.2	4.3
	STDEV	0.0	1.3	1.0	1.2
Flavor Intensity	mean	4.3	4.2	4.3	4.3
	STDEV	0.8	1.2	1.4	1.2
Flavor: Beefy	mean	5.8	3.3	2.3	4.2
	STDEV	1.0	1.2	0.8	1.2
Flavor: bloody/Metallic	mean	4.5	2.0	2.2	3.2
	STDEV	1.2	1.0	0.9	1.4
Flavor: Savory	mean	3.3	3.8	3.7	4.2
	STDEV	1.2	1.2	1.2	1.8
Off Flavors: chemical/oxidized/beany	mean	1.5	2.3	3.5	2.7
	STDEV	0.8	1.2	1.8	1.0

TABLE 10

Beef flavor compounds created in replica burger with 1% LegH and precursor mix 2 as detected by GCMS.

3-octen-2-one	octanoic acid	(Z)-2-decenal,
1-penten-3-ol	octane	carbon disulfide

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n-caproic acid vinyl ester	octanal	butyrolactone
2-acetylthiazole	nonanal	butanoic acid
thiophene	4,7-dimethyl-undecane,	3-methyl-butanal,
methyl-thiirane,	methyl ethanoate	2-methyl-butanal,
thiazole	methional	butanal
styrene	methacrolein	3,6,6-trimethyl-bicyclo[3.1.1]hept-2-ene,
	isovaleric acid	benzyl alcohol
pyrrole	isopropyl alcohol	1,3-dimethyl-benzene
pyridine	hexanoic acid	benzene
trimethyl-pyrazine	2,2,4,6,6-pentamethyl-heptane,	benzaldehyde
tetramethyl-pyrazine,	2-methyl-heptane,	acetophenone
methyl-pyrazine,	heptane	acetonitrile
ethyl-pyrazine	heptanal	acetone
3-ethyl-2,5-dimethyl-pyrazine	furfural	acetoin
2,5-dimethyl-pyrazine	furaneol	acetic acid ethenyl ester
2,3-dimethyl-pyrazine	3-methyl-furan	acetic acid
2-ethyl-5-methyl-pyrazine	2-propyl-furan	acetamide
2-ethenyl-6-methyl-pyrazine,	2-pentyl-furan	acetaldehyde
pyrazine	2-methyl-furan	4-methyl-5-thiazoleethanol
2-methyl-propanal,	2-ethyl-furan	6-methyl-5-hepten-2-one
propanal	furan	trans-2-(2-pentenyl)furan
phenylacetaldehyde	formamide	(E)-4-octene,
phenol	ethyl acetate	4-cyclopentene-1,3-dione
pentanoic acid	1-(2-furanyl)-ethanone	4-cyanocyclohexene
3-ethyl-2,2-dimethyl-pentane	1-(1H-pyrrol-2-yl)-ethanone	dihydro-2-methyl-3(2H)-furanone,
pentanal	dimethyl trisulfide	(E,E)-3,5-octadien-2-one
p-cresol	dimethyl sulfide	3,5-octadien-2-one
oxalic acid, butyl propyl ester	d-limonene	2,2-dimethyl-undecane
1-heptene	1-octen-3-ol	toluene
1-ethyl-5-methylcyclopentene	1-octanol	1-pentanol
1-butanol	1-hexanol	1-octen-3-one
1H-pyrrole-2-carboxaldehyde	2-methyl-1H-pyrrole	2-butanone
2-nonanone	3-methyl-2-butenal	2-thiophenecarboxaldehyde
2-n-butylacrolein	3-ethylcyclopentanone	2-pyrrolidinone
2-methyl-2-heptene	2(5H)-furanone	2-propenal
(E)-2-hexenal,	dihydro-5-pentyl-2(3H)-furanone	1-hydroxy-2-propanone

(E)-2-heptenal,	5-ethyldihydro-2(3H)-furanone	1-(acetyloxy)-2-propanone
6-methyl-2-heptanone	5-acetyldihydro-2(3H)-furanone	2-pentanone
2-heptanone	2,6-dimethylpyrazine	(E)-2-octenal
2-furanmethanol	(E,E)-2,4-nonadienal	2-octanone
3-ethyl-2-1,4-dioxin	(E,E)-2,4-heptadienal	(E)-2-nonenal
3-ethyl-2-methyl-1,3-hexadiene	(E,E)-2,4-decadienal	8-methyl-1-undecene
2-butenal	2,3-dimethyl-5-ethylpyrazine	1-propanol
1-penten-3-one		

EXAMPLE 34 - Removal of off-flavors from plant protein solutions

i) Synthesis of ligand modified LOX removal resin

A 1 mL settled volume of CM Sepharose resin (Sigma Aldrich Catalog # CCF100) was loaded into a BioRad minicolumn. 3 mL of 50 mM MES (2-morpholinoethanesulfonic acid) buffer preset to a pH range of 5.5 to 6 was allowed to pass through the resin bed. Separately, to 1 mL of the same buffer was added in succession, 0.044 mL of 4,7,10- trioxa-1,13-tridecanediamine, 0.030 mL of 12 N HCl, 23 mg of NHS (N-hydroxy-succinimide), and 38 mg of EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) with dissolution after each addition. The resulting solution was added to the top of the column bed and allowed to gravity flow through and the effluent was collected. The resulting effluent was returned to the top of the column bed. The cycle of addition of solution, elution and return was done four times. When the last elution was finished, 3 mL of 50 mM MES buffer preset to a pH of 5.5 to 6 was allowed to gravity flow through the column. Linoleic acid (0.03 mL) was dissolved in 0.5 mL DMF (N,N-dimethylformamide), followed in sequence by 12 mg NHS, 19 mg EDC, and 0.5 mL of 50 mM MES buffer preset to a pH of 5.5 to 6. The NHS/EDC mixture was shaken to mix, resulting in a two-phase liquid which was applied to the top of the column and eluted through the resin bed, allowed to gravity flow through the column and effluent collected. The effluent was returned to the top of the column bed. The cycle of addition of solution, elution and return was done four times. Once the last collection was finished a solution of 70% ethanol in water (5 mL) was added to the

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top of the column, followed by 3 mL of 0.1 M sodium hydroxide. This was followed by 0.1 M buffer of potassium phosphate preadjusted to pH 7 to 8.

ii) Removal of off-flavors from pea proteins using the ligand modified LOX removal resin.

A solution of pea proteins (30 mls at 20 mg/ml protein concentration in 20 mM potassium phosphate buffer pH 7.4, 100mM sodium chloride) was passed through a 100 ml resin bed described above. All unbound material was collected and the resin further washed with 200 mls of buffer. Both fractions were combined and resin was stripped of bound protein by washing with 2 bed volumes of 20 mM potassium phosphate, 1M sodium chloride. Resin bed was regenerated by washing with 2 bed volumes of 0.1M NaOH followed by 3 bed volumes of water and re-equilibration with buffer.

Depletion of LOX activity in the pooled unbound fraction was confirmed by assaying for enzyme activity. Sodium linoleate was used as substrate for LOX and formation of hydroperoxide intermediates was monitored by absorbance at 234nm. Assays were performed at pH9 in 50mM sodium borate buffer. Assays confirmed depletion of LOX activity in the pooled unbound fraction. LOX was removed from resin in washes with 0.5M and 1M sodium chloride.

Improvement in flavor in the LOX depleted protein solutions (both as-is and incubated with canola oil at 10%) was confirmed by a panel of 4 tasters. Samples of pea proteins at same concentration, but not depleted of LOX was used at control. Tasters described LOX -depleted samples as mild tasting and control samples as having beany, planty taste. In addition, GC-MS analysis of samples showed 5X reduction in overall volatiles in the LOX-depleted samples.

Reduction of off-flavors using dialysis and activated carbon

A solution of pea albumins (30 mls at 40 mg/ml in 20mM potassium phosphate buffer pH 7.4, 100 mM sodium chloride) was dialyzed against 100 volumes of buffer overnight at 4°C. The solution was then poured over a bed of activated carbon (100-mesh, Sigma-Aldrich) prewetted with buffer. The slurry was centrifuged at 5000 x g for

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10 minutes and the supernatant containing protein was decanted off. This solution was tested for improvement in flavor by both taste and GC-MS. Improved flavor (less bitter, less soapy flavor) was noted by tasters and GC-MS confirmed a >2X reduction in volatile compounds; in particular, samples treated with activated carbon showed a decrease in C6- and C7 compounds (e.g., 1-heptanal, 2-heptenal, or 2-heptanone) that are associated with green/grassy/planty flavors.

Reduction of off-flavors using antioxidants and/or LOX inhibitors

A 7% solution of pea-vicilins was heated with coconut oil (20%) to 95°C for 15 min in the presence of antioxidants or LOX inhibitors and compared for off-flavors sample against a control without any antioxidants or LOX inhibitors. In a similar experiment, soymilk was heated in the presence of antioxidants or LOX inhibitors and tasted in-house for off-flavors. Table 11 summarizes off-flavors noted by tasters. Both epigallocetechin gallate and propyl gallate were effective at minimizing off-flavors in pea samples. However, in case of soymilk, epigallocatechin gallate did not appear to reduce beaniness; propyl gallate and α-tocopherol were found to slightly improve flavor in soymilk.

Table 11

Compound	soymilk	Pea
α-tocopherol	Slightly improved flavor	Oxidized oil
caffeic acid (0.02%)	Beany	Oxidized oil
epigallocatechin gallate	Beany	Improved flavor
propyl gallate (0.02%)	Slightly improved flavor	Improved flavor
β-carotene	Beany	Oxidized oil

EXAMPLE 35 - ADIPOSE REPLICA WITH LECITHIN GRADIENT

Lecithin (SOLEC™ F Deoiled Soy Lecithin, The Solae Company, St. Louis, MO) was prepared at a concentration of 50 mg/mL in 20 mM potassium phosphate, 100 mM NaCl, pH 8.0 buffer and sonicated (Sonifier Analog Cell Disruptor model 102C,

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BRANSON Ultrasonics Corporation, Danbury, Connecticut) for 30 seconds. Moong protein was supplied as a liquid in 20 mM potassium phosphate, 100 mM NaCl, pH 8.0 buffer. Coconut oil was melted by heating to 50 - 70°C and held warm until needed. The coconut oil, buffered protein solution, additional buffer, and lecithin slurry were mixed at 70°C, and an emulsion was formed using a hand held homogenizer. The emulsion was then heat set by placing the tube into a 95°C waterbath for a total of 5 mins. The tube was then removed from the waterbath and stored at 4°C for twelve hours or longer prior to analysis.

To observe the effect of lecithin on the adipose tissue replica replica, a formulation was prepared that contained 1% w/v moong protein and 75% v/v coconut oil, with the lecithin amount increasing from 0%, 0.05%, 0.25%, 0.5%, and 1.0% w/v.

Properties of the adipose replica were measured by weighing small portions of the material and forming uniform round balls, which were then cooked on a non-stick pan with temperature slowly ramping up to 150°C. The temperature of the pan at which fat visibly released from the balls was measured as the fat release temperature. After cooking to completion, the point at which fat no longer released, the total fat released was measured.

An increase in the amount of lecithin in the adipose replica correlated with an increase in the percent fat released, and with a decrease in the fat release temperature (see FIGs. 2A and 2B). With 0% lecithin, there was an average of 40% fat released, and when lecithin was increased to 0.05%, there was an average of 82% fat released, which further increased to 88% with 0.25% lecithin, then leveled off to an average of 60% with further increases in lecithin. With 0% lecithin, a high temperature of 217°C was required to begin the fat release. The fat release temperature decreased to 122°C with 0.25% lecithin, then leveled off to an average of 62°C with further increases of lecithin.

Firmness of the adipose replica was measured by texture analyzer (TA XT plus). A probe penetrated a flat surface of the adipose replica, and the force at 2 mm penetration was recorded. A small amount of lecithin (0.05%) increased the firmness, and at 0.25% and above, the firmness of the adipose replica decreased. See FIG. 2C.

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EXAMPLE 36 - ADIPOSE REPLICA WITH VARYING VEGETABLE OIL TYPE

To observe the effect of vegetable oil type on the adipose replica, a formulation was prepared that contained a 1.5% w/v moong protein and 0.05% w/v lecithin, and 75% v/v of different vegetable oils (canola oil, cocoa butter, coconut oil, and olive oil) using the same methodology as Example 35. Properties of the adipose replica were measured by weighing small portions of the material and forming uniform round balls, which were then cooked on a non-stick pan with temperature slowly ramping up to 250°C. The temperature of the pan at which fat visibly released from the balls was measured as the fat release temperature. After cooking to completion, the point at which fat no longer released, the total fat released was measured.

Varying the vegetable oil type had a large effect on the adipose replica. Oils with a higher amount of unsaturated fats, including canola and rice bran oils, released very little fat (1 and 2% fat released, see FIG. 3), while oils with a higher amount of saturated fats, including cocoa butter and coconut oil released significantly more fat (30% and 50% fat released, see FIG. 4). The adipose replicas with canola and rice bran oils required a temperature greater than 250°C to melt (not measured), while the adipose replicas with cocoa butter and coconut oil released fat at a lower temperature (82°C and 137°C).

EXAMPLE 37 - ADIPOSE REPLICA MADE WITH COACERVATE

Lecithin (SOLECTM F Deoiled Soy Lecithin, The Solae Company, St. Louis, MO) was prepared at a concentration of 50 mg/ml in 20 mM potassium phosphate, 100 mM NaCl, pH 8.0 buffer and sonicated (Sonifier Analog Cell Disruptor model 102C, BRANSON Ultrasonics Corporation, Danbury, Connecticut) for 30 seconds. Pea legumin and pea vicilin proteins in 20 mM potassium phosphate, 100 mM NaCl, pH 8.0 buffer, were mixed to a 1:1 ratio. Cocoa butter was melted by heating to 70°C and held warm until needed. The cocoa butter was added to 2% and 10% w/v to the protein mixtures, and was added at 60°C to maintain cocoa butter in the liquid state. While the solutions were still warm, the mixtures were sonicated 1-3 minutes, until cocoa butter particles were visibly emulsified. The pH of the samples was adjusted to 5.5 with HCl,

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and the mixtures turned into a milky white color, then centrifuged at 5,000xg for ten minutes. After centrifugation, a pellet was collected, comprising a coacervate of protein and cocoa butter. The 2% fat coacervates were sticky and stretchy, while the 10% fat coacervates were fatty and pliable. The coacervates were sealed in plastic and subjected to high pressure processing (HPP). The sample was sealed in a heat-sealable food-saver plastic bag and then subject to high pressure processing (85k psi for 5 minutes in an Avure 2L Isostatic Food Press). After HPP, the 2% coacervate samples formed a semi-firm, cohesive material. The 10% coacervate samples were crumbly, soft, and oily.

Properties of the processed coacervate samples were measured by breaking off small portions of the material and cooking on a non-stick pan with temperature slowly ramping up to 250°C. The coacervate samples did not release any fat upon cooking to this temperature.

OTHER EMBODIMENTS

While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

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WHAT IS CLAIMED IS:

1. A consumable product comprising an isolated and purified plant protein, wherein the isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2°C and about 32°C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90°C and 110°C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM.

2. The consumable product of claim 1, wherein said product is a beverage, a protein supplement, a baked good, a condiment, a meat product, or a meat substitute product.

3. The consumable product of claim 2, wherein said beverage is an alcoholic beverage or a protein drink.

4. The consumable product of claim 3, wherein said alcoholic beverage is a cream liquor.

5. The consumable product of claim 4, said cream liquor further comprising a non-dairy lipid emulsion, wherein said cream liquor is free of animal products.

6. The consumable product of claim 3, wherein said protein drink is a meal replacement beverage, a beer supplemented with said protein, or distilled alcoholic beverage supplemented with said protein.

7. The consumable product of claim 2, wherein said condiment is a mayonnaise replica.

8. The consumable product of claim 2, wherein said meat product is a pate, a sausage replica, or a meat substitute.

9. The consumable product of any one of claims 1-8, wherein said isolated and purified plant protein is at least 10 kDa in size.

10. The consumable product of any one of claims 1-9, wherein said isolated and purified plant protein is not fully denatured.

11. The consumable product of any one of claims 1-10, wherein said isolated and purified plant protein is not derived from soy.

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12. The consumable product of any one of claims 1-11, wherein said isolated and purified plant protein comprises one or more of RuBisCo, Moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin.

13. The consumable product of any one of claims 1-12, wherein said isolated and purified plant protein comprises a dehydrin, a hydrophilin, an intrinsically disordered protein, or a protein identified based on its ability to stay soluble after boiling at a pH and a salt concentration comparable to a food.

14. The consumable product of any one of claims 1-13, said consumable product further comprising a plant derived lipid or a microbial-derived lipid.

15. The consumable product of any one of claims 1-14, further comprising a second isolated and purified protein.

16. The consumable product of any one of claims 1-15, further comprising a seasoning agent, a flavoring agent, an emulsifier, a gelling agent, a sugar, or a fiber.

17. A consumable product comprising a coacervate comprising one or more isolated and purified proteins.

18. The consumable product of claim 17, wherein said consumable product is a meat replica.

19. The consumable product of claim 17 or claim 18, said product further comprising a plant derived lipid or a microbial-derived lipid.

20. The consumable product of claim 19, wherein said plant derived lipid or microbial-derived lipid comprises lecithin and/or an oil.

21. The consumable product of claim 20, said product comprising up to about 1% lecithin by weight.

22. The consumable product of claims 20 or claim 21, wherein said product comprises lecithin and said oil.

23. The consumable product of any one of claims 20-22, wherein said oil is canola oil, palm oil, or cocoa butter.

24. The consumable product of any one of claims 20-23, said product comprising about 1% to about 9% of said oil.

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25. The consumable product of any one of claims 17-24, wherein said one or more isolated and purified proteins comprise plant proteins.

26. The consumable product of claim 25, wherein said one or more plant proteins comprise one or more pea proteins, chickpea proteins, lentil proteins, lupine proteins, other legume proteins, or mixtures thereof.

27. The consumable product of claim 26, wherein said one or more pea proteins are legumins, vicilin, a convicilin, or a mixture thereof.

28. A meat replica comprising a muscle replica, a connective tissue replica; an adipose tissue replica, and a coacervate comprising one or more isolated and purified proteins.

29. The meat replica of claim 28, wherein said coacervate further comprises a plant-derived lipid or microbial-derived lipid.

30. The meat replica of claim 29, wherein said plant derived lipid or microbial-derived lipid is lecithin and/or an oil.

31. The meat replica of any one of claims 28-30, wherein said meat replica is a ground beef replica.

32. A consumable product comprising a comprising a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt.

33. The consumable product of claim 32, wherein said isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin.

34. The consumable product claim 32, wherein said isolated and purified plant protein comprises a dehydrin, a hydrophilin, or an intrinsically disordered protein.

35. The consumable product of any one of claims 32-35, wherein said cold set gel further comprises a plant derived lipid or microbial derived lipid.

36. The consumable product of claim 35, wherein said plant derived lipid or microbial derived lipid is lecithin and/or oil.

37. An adipose tissue replica comprising one or more isolated plant proteins, one or more plant or algal derived oils, and optionally a phospholipid.

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38. The adipose tissue replica of claim 37, wherein said phospholipid is lecithin.

39. The adipose tissue replica of claim 37 or claim 38, wherein said plant based oils are selected from the group consisting of corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, and combinations thereof.

40. The adipose tissue replica of any one of claims 37-39, wherein the fat release temperature of said adipose tissue replica is between 23°C to 33°C, 34°C to 44°C, 45°C to 55°C, 56°C to 66°C, 67°C to 77°C, 78°C to 88°C, 89°C to 99°C, 100°C to 110°C, 111°C to 121°C, 122°C to 132°C, 133°C to 143°C, 144°C to 154°C, 155°C to 165°C, 166°C to 167°C, 168°C to 169°C, 170°C to 180°C, 181°C to 191°C, 192°C to 202°C, 203°C to 213°C, 214°C to 224°C, 225°C to 235°C, 236°C to 246°C, 247°C to 257°C, 258°C to 268°C, 269°C to 279°C, 280°C to 290°C, or 291°C to 301°C.

41. The adipose tissue replica of any one of claim 37-40, wherein the percent fat release of said adipose tissue replica is 0 to 10%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, 50% to 60%, 60% to 70%, 70% to 80%, 80% to 90%, or 90% to 100% upon cooking.

42. The adipose tissue replica of any one of claims 37-41, wherein said isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin.

43. The adipose tissue replica of any one of claims 37-42, wherein said adipose tissue replica comprises from about 40% to about 90% of said oil.

44. The adipose tissue replica of any one of claims 37-43, wherein said adipose tissue replica comprises from about 1% to about 6% of said isolated and purified plant protein.

45. The adipose tissue replica of any one of claims 37-44, wherein said adipose tissue replica comprises from about 0.05 to about 2% of said phospholipid.

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46. The adipose tissue replica of any one of claims 37-45, wherein the firmness of said adipose tissue replica is similar to that of beef adipose tissue.

47. A consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, wherein said consumable product does not comprise meat.

48. The consumable product of claim 47, wherein said heme-containing protein accounts for at least 0.01% of the composition.

49. The consumable product of claim 47 or claim 48, further comprising one or more ammonium, sodium, potassium, or calcium salts.

50. The consumable product or meat replica of any one of claims 17-49, wherein said isolated and purified proteins are crosslinked.

51. A consumable product comprising a gelled emulsion, said gelled emulsion comprising:

a) an isolated and purified protein;

b) a first lipid that when not in said consumable product is solid at a selected temperature range; and

c) a second lipid that when not in said consumable product is liquid at said selected temperature range; wherein the melting temperature of the mixture of said first and second lipids is similar to the melting temperature of lipids found in meat, and wherein said first and second lipids are plant derived lipids or microbial derived lipids.

52. A method for making a consumable product comprising:

a) preparing a solution comprising an isolated and purified plant protein, wherein said isolated and purified plant protein has (i) a solubility in said solution of at least 25 at a temperature between about 2°C and 32°C, wherein said solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in said solution of at least 1 mg/ml at a temperature of between 90°C and 110°C, wherein said solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM; and

b) adding said solution to a beverage.

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53. The method of claim 52 wherein said solution comprises two or more isolated and purified plant proteins.

54. The method of claim 52 or claim 53, wherein said beverage is clear.

55. The method of any one of claim 52-54, wherein said isolated and purified plant protein is at a concentration of at least 1% by weight in said solution.

56. The method of any one of claims 52-55, wherein said isolated and purified plant protein is selected from the group consisting of RuBisCo, a moong globulin, a soy globulin, a pea globulin, a pea albumin, a prolamin, a lentil protein, a dehydrin, a hydrophilin, and an intrinsically disordered protein.

57. The method of any one of claims 52-56, wherein said isolated and purified plant protein is lyophilized prior to making said solution.

58. The method of any one of claims 52-57, wherein said beverage has an improved mouthfeel compared to a corresponding beverage without said isolated and purified protein.

59. A method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions.

60. The method of claim 59, wherein said heme-containing protein comprises an amino acid sequence with at least 70% homology to an amino acid sequence set forth in any one of SEQ ID NOs: 1-27.

61. A method for making a meat replica comprising a cold set gel comprising:

a) denaturing a solution comprising at least one isolated and purified protein from a non-animal source under conditions wherein said isolated and purified protein does not precipitate out of said solution;

b) optionally adding any heat-labile components to said solution of denatured protein;

c) gelling said solution of denatured protein at about 4°C to about 25°C by increasing the ionic strength of said solution to form a cold set gel, and

d) incorporating said cold set gel into a meat replica.

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62. The method of claim 61, wherein gelling is induced using 5 to 100 mM sodium or calcium chloride.

63. The method of claim 61 or claim 62, wherein said heat-labile components are proteins or lipids, or mixtures thereof.

64. The method of claim 63, wherein said protein is a heme-containing protein.

65. The method of claim 63, wherein said cold set gel is formed in a matrix comprising a freeze-aligned plant protein.

66. The method of any one of claims 61-65, wherein said isolated and purified protein from a non-animal source is a plant protein.

67. The method of claim 66, wherein said plant protein is selected from the group consisting of RuBisCo, a moong globulin, a soy globulin, a pea globulin, a pea albumin, a prolamin, a lentil protein, a dehydrin, a hydrophilin, and an intrinsically disordered protein.

68. An adipose tissue replica, comprising

a) an isolated and purified non-animal protein;

b) a non-animal lipid; and

c. a three-dimensional matrix comprising fibers derived from non-animal sources, wherein said lipid and said protein are dispersed in said three-dimensional matrix, and wherein said three-dimensional matrix stabilizes the structure of the adipose tissue replica.

69. A connective tissue replica comprising one or more isolated and purified proteins assembled into fibrous structures by a solution spinning process.

70. The connective tissue replica of claim 69 wherein the fibrous structures are stabilized by a cross-linking agent.

71. A method for imparting a beef like flavor to a consumable product, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

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72. A method for making a poultry or a fish composition taste like beef, said method comprising adding a heme protein to said poultry or fish composition, respectively.

73. The method of claim 71 or claim 72, wherein said heme-containing protein has an amino acid sequence with at least 70% homology to any one of the amino acid sequences set forth in SEQ ID NOs: 1-27.

74. A method of making a coacervate, said method comprising

a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of sodium chloride; and

b) isolating said coacervate from said solution.

75. The method of claim 74, wherein said pH is between 4 and 5.

76. The method of claim 74 or claim 75, wherein said plant proteins comprise one or more pea proteins, chickpea proteins, lentil proteins, lupine proteins, other legume proteins, or mixtures thereof.

77. The method of claim 76, wherein said pea proteins comprise isolated and purified legumins, isolated and purified vicilins, isolated and purified convicilins, or combinations thereof.

78. The method of claim 77, wherein said isolated and purified pea proteins comprise isolated and purified vicilins and isolated and purified convicilins.

79. The method of any one of claims 74-78, wherein said acidifying step is done in the presence of a plant derived lipid or microbial derived lipid.

80. The method of claim 79, wherein said plant derived lipid or microbial derived lipid comprises oils and/or phospholipids.

81. A method of making an adipose tissue replica, said method comprising forming an emulsion comprising one or more isolated plant proteins, one or more plant or algal derived oils, and optionally a phospholipid.

82. The method of claim 81, wherein said phosoholipid is included, and is lecithin.

83. The method of claim 81 or claim 82, wherein said plant based oils are selected from the group consisting of corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil,

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sunflower oil, flax seed oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, and combinations thereof.

84. The method of any one of claims 81-83, wherein the fat release temperature of said adipose tissue replica is between 23°C to 33°C, 34°C to 44°C, 45°C to 55°C, 56°C to 66°C, 67°C to 77°C, 78°C to 88°C, 89°C to 99°C, 100°C to 110°C, 111°C to 121°C, 122°C to 132°C, 133°C to 143°C, 144°C to 154°C, 155°C to 165°C, 166°C to 167°C, 168°C to 169°C, 170°C to 180°C, 181°C to 191°C, 192°C to 202°C, 203°C to 213°C, 214°C to 224°C, 225°C to 235°C, 236°C to 246°C, 247°C to 257°C, 258°C to 268°C, 269°C to 279°C, 280°C to 290°C, or 291°C to 301°C.

85. The method of any one of claim 81-84, wherein the percent fat release of said adipose tissue replica is 0 to 10%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, 50% to 60%, 60% to 70%, 70% to 80%, 80% to 90%, or 90% to 100% upon cooking.

86. The method of any one of claims 81-85, wherein said isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin.

87. The method of any one of claims 81-86, wherein said emulsion comprises from about 40% to about 90% of said oil.

88. The method of any one of claims 81-87, wherein said emulsion comprises from about 1% to about 4% of said isolated and purified plant protein.

89. The method of any one of claims 81-88, wherein said adipose tissue replica comprises from about 0.05 to about 1% of said phospholipid.

90. The method of any one of claims 81-89, wherein the emulsion is formed by high-pressure homogenization, sonication, or hand homogenization.

91. A method of minimizing undesirable odors in a composition comprising plant proteins, said method comprising contacting said composition with a ligand having affinity for one or more lipoxygenases.

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92. A method of minimizing undesirable odors in a composition comprising plant proteins, said method comprising contacting said composition with activated carbon then removing the activated carbon from said composition.

93. A method of minimizing undesirable odors in a composition comprising plant proteins, said method comprising adding a lipoxygenase inhibitor and/or an antioxidant to said composition.

94. A chocolate flavored spread comprising:

a) sugar

b) a chocolate flavoring, and

c) a cream fraction from a plant based milk.

95. A method for altering the texture of a consumable during or after cooking comprising incorporating within the consumable one or more plant proteins with a low denaturation temperature.

96. The method of claim 95, wherein at least one of the one or more plant proteins is isolated and purified.

97. The method of claim 95, wherein the one or more plant proteins are selected from the group consisting of rubisco, pea proteins, lentil proteins, or other legume proteins.

98. The method of claim 97, wherein the pea proteins comprise pea albumin proteins.

99. The method of claim 95, wherein the consumable becomes firmer during or after cooking.

100. A tissue replica comprising a freeze-aligned non-animal protein.

101. The tissue replica of claim 100, wherein the non-animal protein is a plant protein.

102. The tissue replica of claim 101, wherein the non-animal protein is isolated and purified.

103. The tissue replica of claim 100, which is a muscle tissue replica.

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104. A meat replica comprising a tissue replica comprising a freeze-aligned non-animal protein.

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FIGURE 1

SEQ ID NO:1 *Vigna radiata*

MTTTLERGFTEEQEALVVKSWNVMKKNSGELGLKFFLKIFEIAPSAQKLFSFLRDSTVP
LEQNPKLKPHAVSVFVMTCDSAVQLRKAGKVTVRESNLKKLGATHFRTGVANEHFEVTK
FALLETIKEAVPEMWSPAMKNAWGEAYDQLVDAIKYEMKPPSS

SEQ ID NO:2 *Methylacidiphilum infernorum*

MIDQKEKELIKESWKRIEPNKNEIGLLFYANLFKEEPTVSVLFQNPISSQSRKLMQVLG
ILVQGIDNLEGLIPTLQDLGRRHKQYGVVDSHYPLVGDCLLKSIQEYLGQGFTEEAKAA
WTKVYGIAAQVMTAE

SEQ ID NO:3 *Aquifex aeolicus*

MLSEETIRVIKSTVPLLKEHGTEITARMYELLFSKYPKTKELFAGASEEQPKKLANAII
AYATYIDRLEELDNAISTIARSHVRRNVKPEHYPLVKECLLQAIEEVLNPGEEVLKAWE
EAYDFLAKTLITLEKKLYSQP

SEQ ID NO:4 *Glycine max*

MGAFTEKQEALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLSNGVDPSNPKL
TGHAEKLFGLVRDSAGQLKANGTVVADAALGSIHAQKAITDPQFVVVKEALLKTIKEAV
GDKWSDELSSAWEVAYDELAAAIKKAF

SEQ ID NO:5 *Hordeum vulgare*

MSAAEGAVVFSEEKEALVLKSWAIMKKDSANLGLRFFLKIFEIAPSARQMFPFLRDSDV
PLETNPKLKTHAVSVFVMTCEAAAQLRKAGKITVRETTLKRLGGTHLKYGVADGHFEVT
RFALLETIKEALPADMWGPEMRNAWGEAYDQLVAAIKQEMKPAE

Magnaporthe oryzae, (SEQ ID NO:6)

```
  1 mdgavrldwt gldltgheih dgvpiasrvq vmvsfplfkd qhiimsskes psrksstigq
 61 strngscqad tqkgqlppvg ekpkpvkenp mkklkemsqr plptqhgdgt yptekkltgi
121 gedlkhirgy dvktllamvk sklkgeklkd dktmlmervm qlvarlptes kkraeltdsl
181 inelwesldh pplnylgpeh syrtpdgsyn hpfnpqlgaa gsryarsvip tvtppgalpd
241 pglifdsimg rtpnsyrkhp nnvssilwyw atiiihdifw tdprdintnk sssyldlapl
301 ygnsqemqds irtfkdgrmk pdcyadkrla gmppgvsvll imfnrfhnhv aenlalineg
361 grfnkpsdll egeareaawk kydndlfqva rlvtsglyin itlvdyvrni vnlnrvdttw
421 tldprqdaga hvgtadgaer gtgnavsaef nlcyrwhsci sekdskfvea qfqnifgkpa
481 sevrpdemwk gfakmeqntp adpgqrtfgg fkrgpdgkfd dddlvrcise avedvagafg
541 arnvpqamkv vetmgiiqgr kwnvaglnef rkhfhlkpys tfedinsdpg vaealrrlyd
601 hpdnvelypg lvaeedkqpm vpgvgiapty tisrvvlsda vclvrgdrfy ttdftprnlt
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 661 nwgykevdyd lsvnhgcvfy klfirafpnh fkqnsvyahy pmvvpsenkr ilealgradl
 721 fdfeapkyip prvnitsygg aeyiletqek ykvtwheglg flmgegglkf mlsgddplha
 781 qqrkcmaaql ykdgwteavk afyagmmeel lvsksyflgn nkhrhvdiir dvgnmvhvhf
 841 asqvfglplk taknptgvft eqemygilaa ifttiffdld psksfplrtk trevcqklak
 901 lveanvklin kipwsrgmfv gkpakdepls iygktmikgl kahglsdydi awshvvptsg
 961 amvpnqaqvf aqavdyylsp agmhyipeih mvalqpstpe tdalllgyam egirlagtfg
1021 syreaavddv vkedngrqvp vkagdrvfvs fvdaardpkh fpdpevvnpr rpakkyihyg
1081 vgphaclgrd asqiaitemf rclfrrrnvr rvpgpqqgelk kvprpggfyv ymredwgglf
1141 pfpvtmrvmw dde
```

Fusarium oxysporum (SEQ ID NO:7)

```
   1 mkgsatlafa lvqfsaasql vwpskwdeve dllymqggfn krgfadalrt cefgsnvpgt
  61 qntaewlrta fhdaithdak agtggldasi ywessrpenp gkafnntfgf fsgfhnprat
 121 asdltalgtv lavgacngpr ipfragrida ykagpagvpe pstnlkdtfa aftkagftke
 181 emtamvacgh aiggvhsvdf peivgikadp nndtnvpfqk dvssfhngiv teylagtskn
 241 plvasknatf hsdkrifdnd katmkklstk agfnsmcadi ltrmidtvpk svqltpvlea
 301 ydvrpyitel slnnknkihf tgsvrvritn nirdnndlai nliyvgrdgk kvtvptqqvt
 361 fqggtsfgag evfanfefdt tmdakngitk ffiqevkpst katvthdnqk tggykvddtv
 421 lyqlqqscav leklpnaplv vtamvrdara kdaltlrvah kkpvkgsivp rfqtaitnfk
 481 atgkkssgyt gfqaktmfee qstyfdivlg gspasgvqfl tsqampsqcs
```

Fusarium graminearum (SEQ ID NO:8)

```
   1 masatrqfar aatratrngf aiaprqvirq qgrryyssep aqksssawiw ltgaavagga
  61 gyyfygnsas satakvfnps kedyqkvyne iaarleekdd yddgsygpvl vrlawhasgt
 121 ydketgtggs ngatmrfape sdhganagla aardflqpvk ekfpwitysd lwilagvcai
 181 qemlgpaipy rpgrsdrdvs gctpdgrlpd askrqdhlrg ifgrmgfndq eivalsgaha
 241 lgrchtdrsg ysgpwtfspt vltndyfrll veekwqwkkw ngpaqyedks tkslmmlpsd
 301 ialiedkkfk pwvekyakdn daffkdfsnv vlrlfelgvp faqgtenqrw tfkpthqe
```

SEQ ID NO: 9 *Chlamydomonas eugametos*

MSLFAKLGGREAVEAAVDKFYNKIVADPTVSTYFSNTDMKVQRSKQFAFLAYALGGASE
WKGKDMRTAHKDLVPHLSDVHFQAVARHLSDTLTELGVPPEDITDAMAVVASTRTEVLN
MPQQ

SEQ ID NO:10 *Tetrahymena pyriformis*

MNKPQTIYEKLGGENAMKAAVPLFYKKVLADERVKHFFKNTDMDHQTKQQTDFLTMLLG
GPNHYKGKNMTEAHKGMNLQNLHFDAIIENLAATLKELGVTDAVINEAAKVIEHTRKDM
LGK

SEQ ID NO:11 *Paramecium caudatum*

MSLFEQLGGQAAVQAVTAQFYANIQADATVATFFNGIDMPNQTNKTAAFLCAALGGPNA
WTGRNLKEVHANMGVSNAQFTTVIGHLRSALTGAGVAAALVEQTVAVAETVRGDVVTV

SEQ ID NO:12 *Aspergillus niger*

MPLTPEQIKIIKATVPVLQEYGTKITTAFYMNMSTVHPELNAVFNTANQVKGHQARALA
GALFAYASHIDDLGALGPAVELICNKHASLYIQADEYKIVGKYLLEAMKEVLGDACTDD
ILDAWGAAYWALADIMINREAALYKQSQG

SEQ ID NO:13 *Zea mays*

MALAEADDGAVVFGEEQEALVLKSWAVMKKDAANLGLRFFLKVFEIAPSAEQMFSFLRD
SDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRETTLKRLGATHLRYGVADGHF
EVTGFALLETIKEALPADMWSLEMKKAWAEAYSQLVAAIKREMKPDA

SEQ ID NO:14 *Oryza sativa subsp. japonica*

MALVEGNNGVSGGAVSFSEEQEALVLKSWAIMKKDSANIGLRFFLKIFEVAPSASQMFS
FLRNSDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRDTTLKRLGATHFKYGVG
DAHFEVTRFALLETIKEAVPVDMWSPAMKSAWSEAYNQLVAAIKQEMKPAE

SEQ ID NO:15 *Arabidopsis thaliana*

MESEGKIVFTEEQEALVVKSWSVMKKNSAELGLKLFIKIFEIAPTTKKMFSFLRDSPIP
AEQNPKLKPHAMSVFVMCCESAVQLRKTGKVTVRETTLKRLGASHSKYGVVDEHFEVAK
YALLETIKEAVPEMWSPEMKVAWGQAYDHLVAAIKAEMNLSN

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SEQ ID NO:16 *Pisum sativum*

MGFTDKQEALVNSSWESFKQNLSGNSILFYTIILEKAPAAKGLFSFLKDTAGVEDSPKL
QAHAEQVFGLVRDSAAQLRTKGEVVLGNATLGAIHVQRGVTDPHFVVVKEALLQTIKKA
SGNNWSEELNTAWEVAYDGLATAIKKAMT

SEQ ID NO:17 *Vigna unguiculata*

MVAFSDKQEALVNGAYEAFKANIPKYSVVFYTTILEKAPAAKNLFSFLANGVDATNPKL
TGHAEKLFGLVRDSAAQLRASGGVVADAALGAVHSQKAVNDAQFVVVKEALVKTLKEAV
GDKWSDELGTAVELAYDELAAAAIKKAY

SEQ ID NO:18 Bos taurus

MGLSDGEWQLVLNAWGKVEADVAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGNTVLTALGGILKKKGHHEAEVKHLAESHANKHKIPVKYLEFISDAIIHVLHA
KHPSDFGADAQAAMSKALELFRNDMAAQYKVLGFHG

SEQ ID NO:19 *Sus scrofa*
MGLSDGEWQLVLNVWGKVEADVAGHGQEVLIRLFKGHPETLEKFDKFKHLKSEDEMKAS
EDLKKHGNTVLTALGGILKKKGHHEAELTPLAQSHATKHKIPVKYLEFISEAIIQVLQS
KH PGDFGADAQGAMSKALELFRNDMAAKYKELGFQG

SEQ ID NO: 20 *Equus caballus*

MGLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHVLHS
KH PGDFGADAQGAMTKALELFRNDIAAKYKELGFQG

SEQ ID NO: 21 Nicotiana benthamiana
MSSFTEEQEALVVKSWDSMKKNAGEWGLKLFLKIFEIAPSAKKLFSFLKDSNVPL
EQNAKLKPHSKSVFVMTCEAAVQLRKAGKVVVRDSTLKKLGATHFKYGVADE
HFEVTKFALLETIKEAVPEMWSVDMKNAWGEAFDQLVNAIKTEMK

SEQ ID NO: 22 Bacillus subtilis
MGQSFNAPYEAIGEELLSQLVDTFYERVASHPLLKPIFPSDLTETARKQKQFLTQY
LGGPPLYTEEHGHPMLRARHLPFPITNERADAWLSCMKDAMDHVGLEGEIREFL
FGRLELTARHMVNQTEAEDRSS

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SEQ ID NO: 23 Corynebacterium glutamicum

MTTSENFYDSVGGEETFSLIVHRFYEQVPNDDILGPMYPPDDFEGAEQRLKMFLS
QYWGGPKDYQEQRGHPRLRMRHVNYPIGVTAAERWLQLMSNALDGVDLTAEQ
REAIWEHMVRAADMLINSNPDPHA

SEQ ID NO: 24 Synechocystis PCC6803
MSTLYEKLGGTTAVDLAVDKFYERVLQDDRIKHFFADVDMAKQRAHQKAFLTY
AFGGTDKYDGRYMREAHKELVENHGLNGEHFDAVAEDLLATLKEMGVPEDLIA
EVAAVAGAPAHKRDVLNQ

SEQ ID NO: 25 Synechococcus sp. PCC 7335
MDVALLEKSFEQISPRAIEFSASFYQNLFHHHPELKPLFAETSQTIQEKKLIFSLAAI
IENLRNPDILQPALKSLGARHAEVGTIKSHYPLVGQALIETFAEYLAADWTEQLA
TAWVEAYDVIASTMIEGADNPAAYLEPELTFYEWLDLYGEESPKVRNAIATLTH
FHYGEDPQDVQRDSRG

SEQ ID NO: 26 Nostoc commune
MSTLYDNIGGQPAIEQVVDELHKRIATDSLLAPVFAGTDMVKQRNHLVAFLAQIF
EGPKQYGGRPMDKTHAGLNLQQPHFDAIAKHLGERMAVRGVSAENTKAALDR
VTNMKGAILNK

SEQ ID NO: 27 Bacillus megaterium
MREKIHSPYELLGGEHTISKLVDAFYTRVGQHPELAPIFPDNLTETARKQKQFLT
QYLGGPSLYTEEHGHPMLRARHLPFEITPSRAKAWLTCMHEAMDEINLEGPER
DELYHRLILTAQHMINSPEQTDEKGFSH

FIGURE 2A

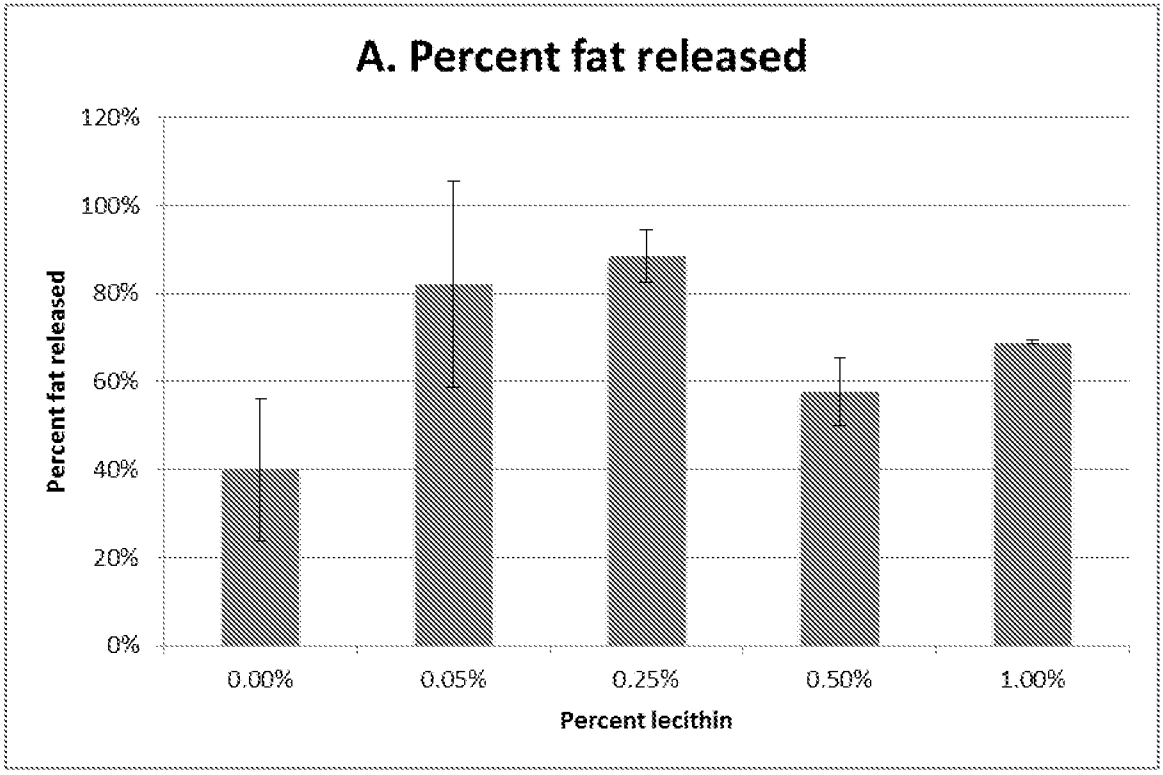


FIGURE 2B

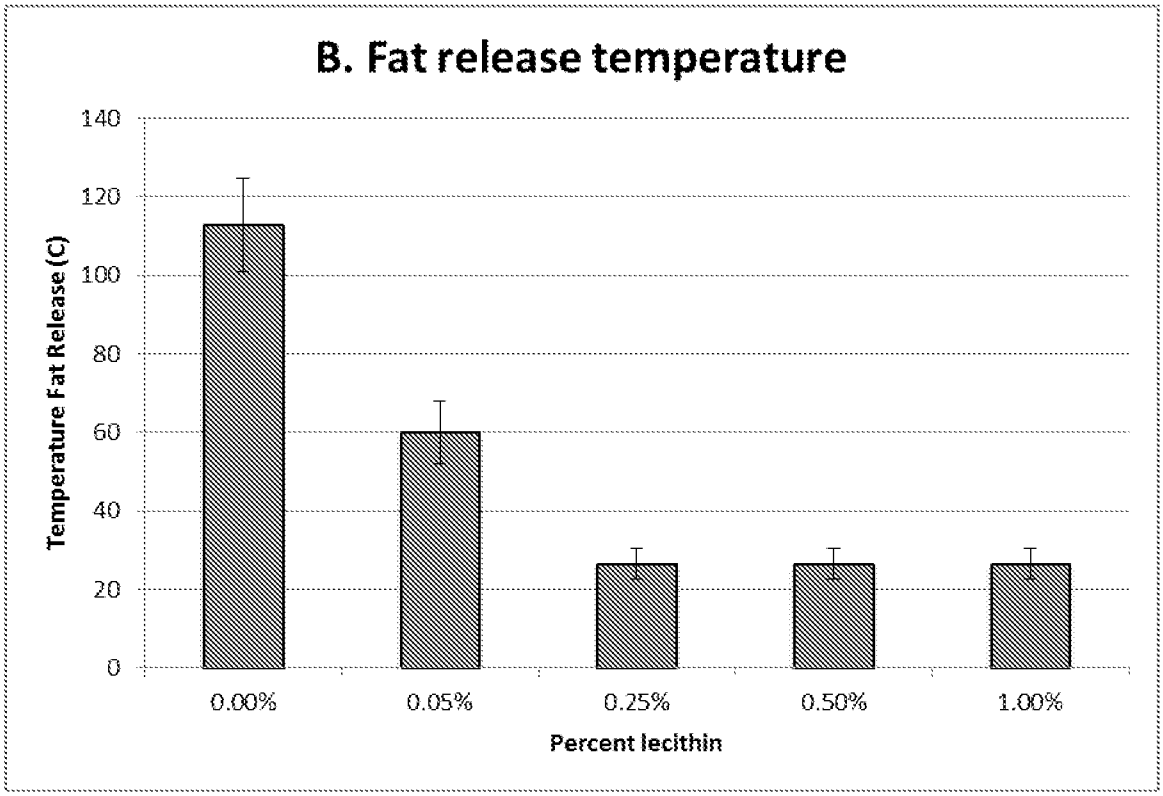


FIGURE 2C

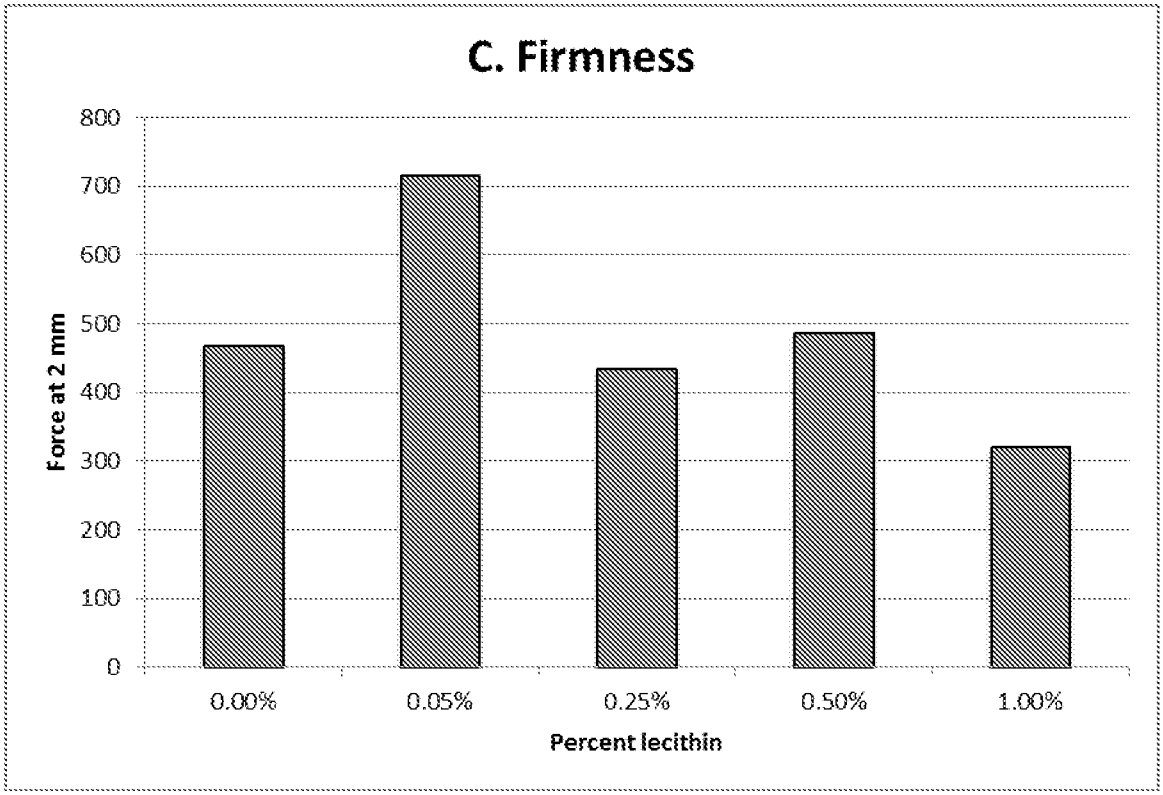

C. Firmness
Force at 2 mm
800
700
600
500
400
300
200
100
0
0.00%
0.05%
0.25%
0.50%
1.00%
Percent lecithin

FIGURE 3

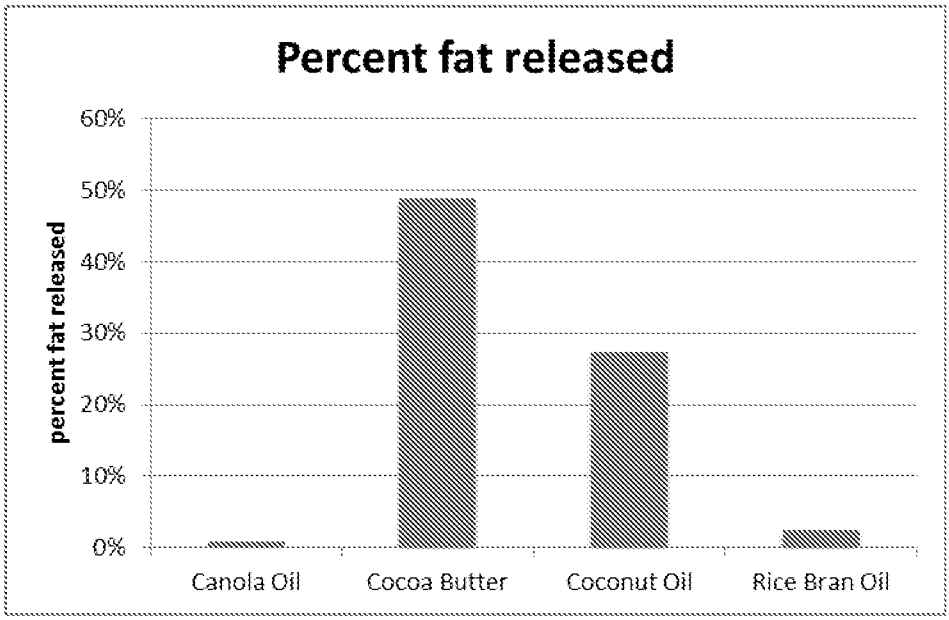
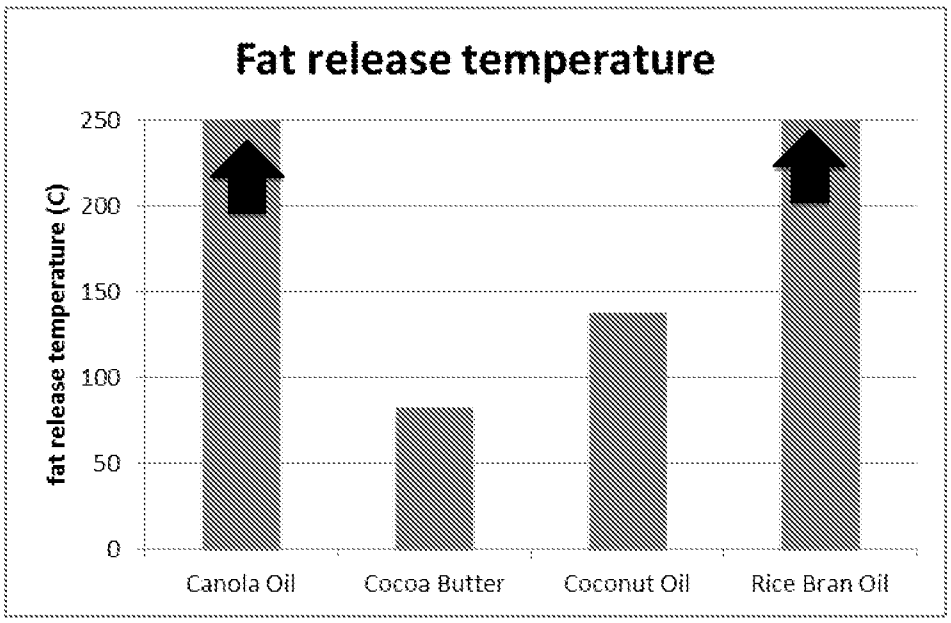

Percent fat released
percent fat released
60%
50%
40%
30%
20%
10%
0%
Canola Oil
Cocoa Butter
Coconut Oil
Rice Bran Oil

FIGURE 4



60902413.doc

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EXHIBIT 1E

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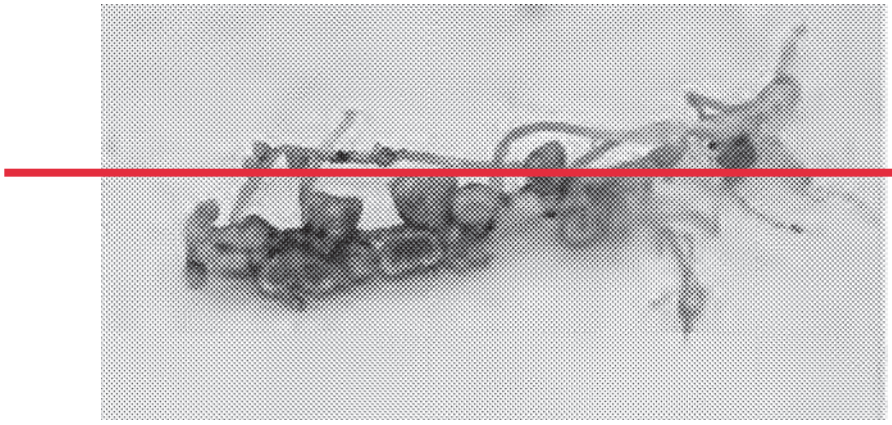


Figure 1

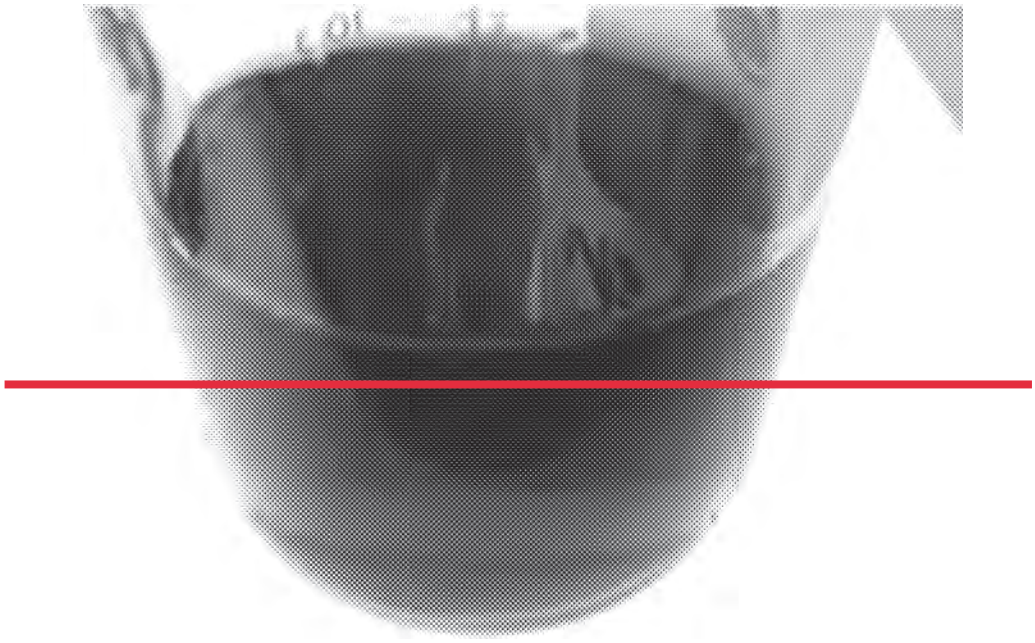


Figure 2

EXHIBIT 1E

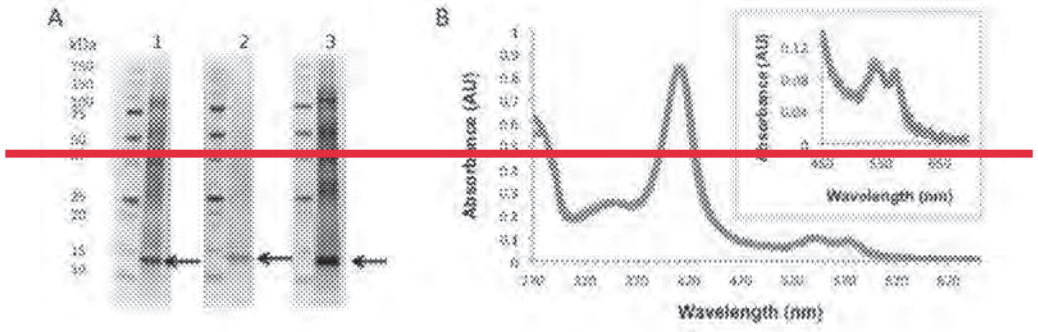


Figure 3

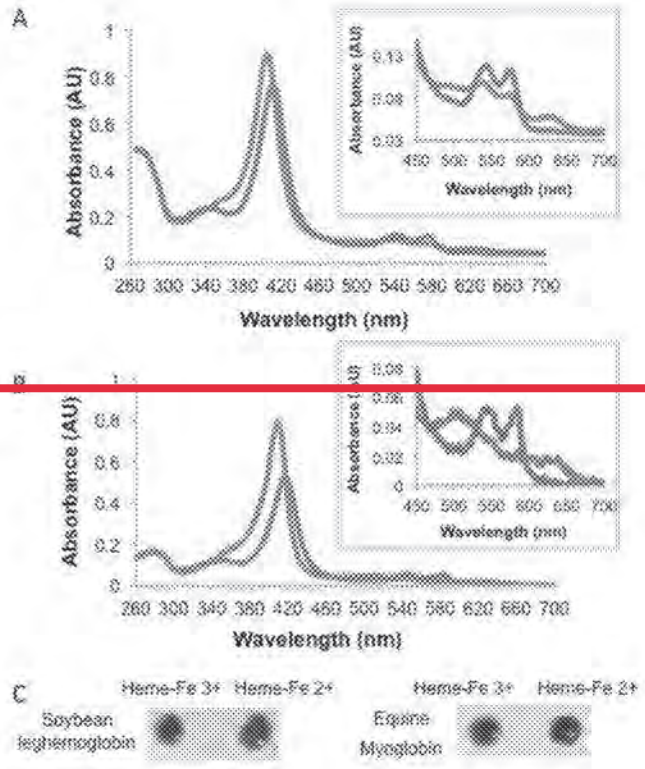


Figure 4

EXHIBIT 1E

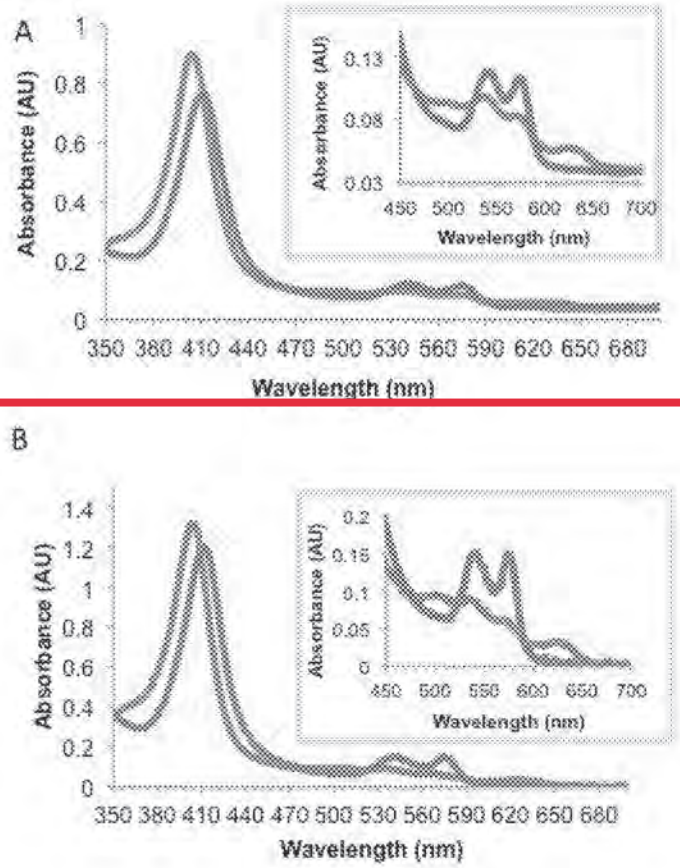


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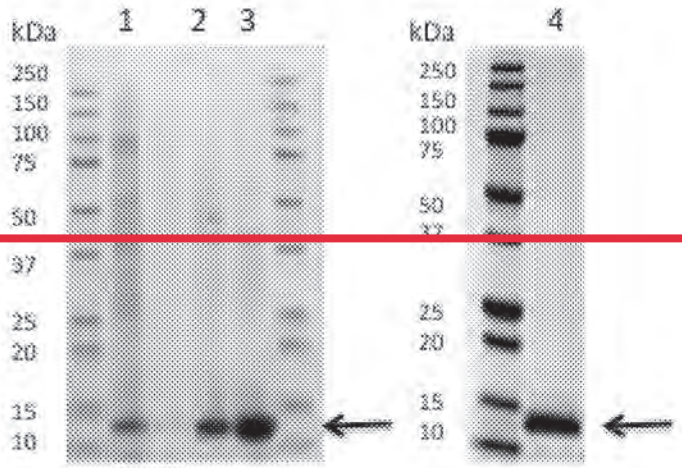


Figure 6

EXHIBIT 1E

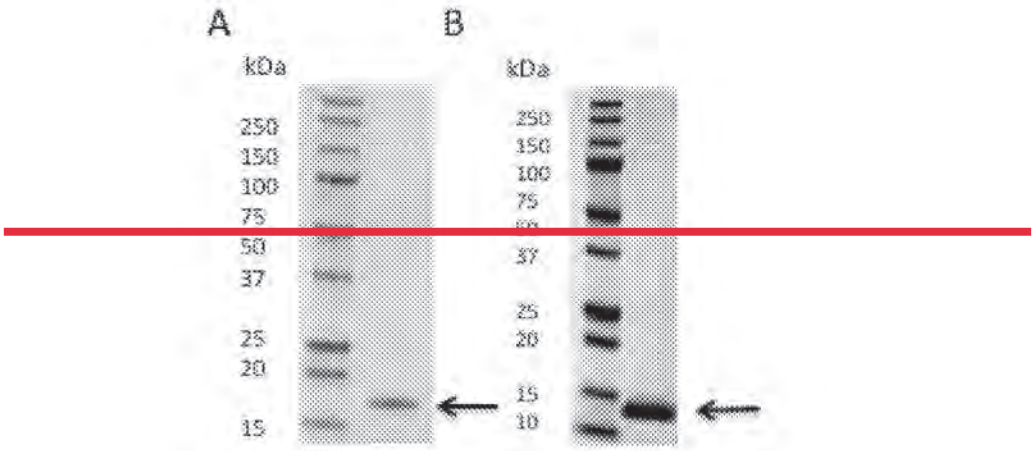


Figure 7

EXHIBIT 1E



Figure 8

EXHIBIT 1E

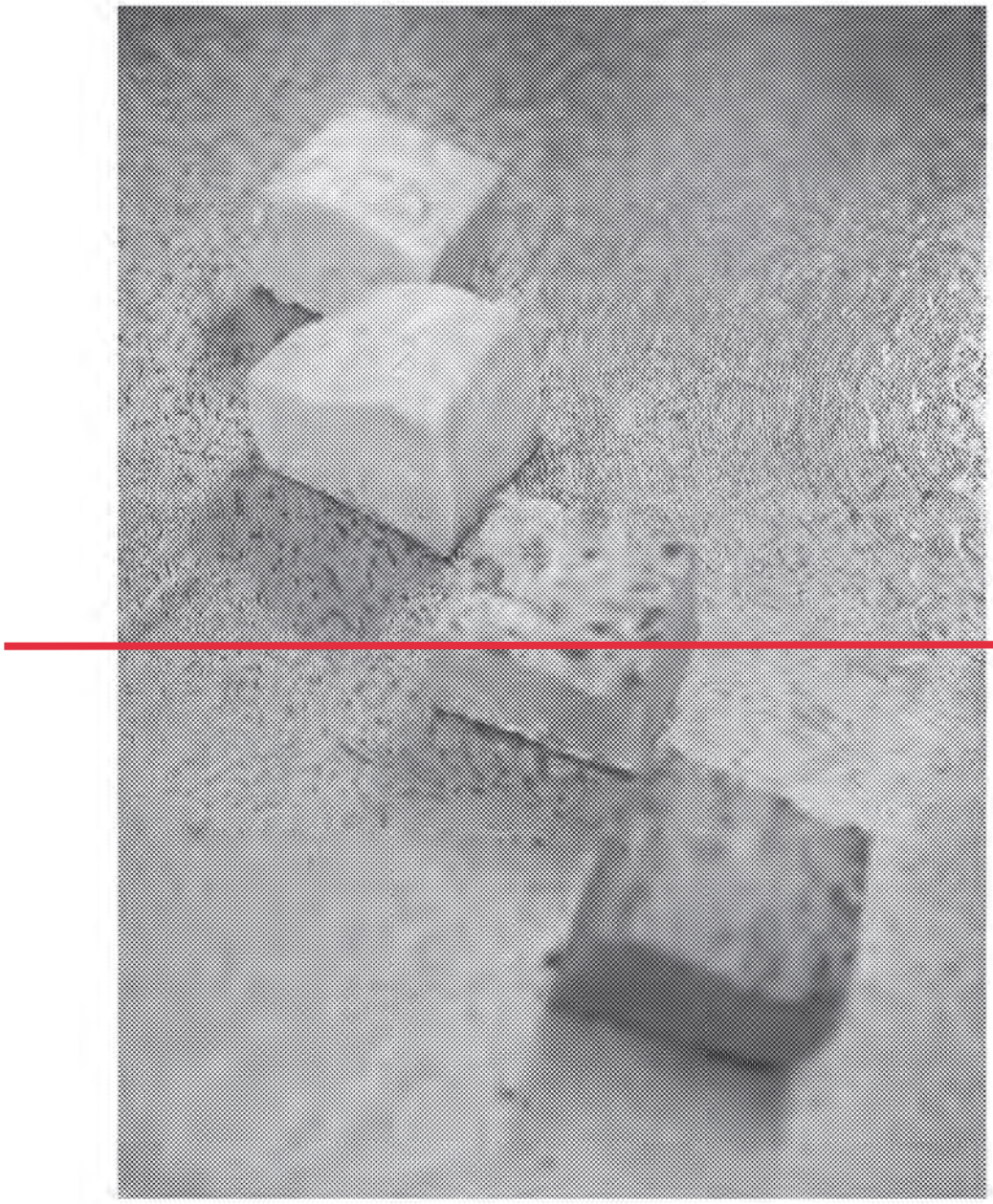


Figure 9

EXHIBIT 1E

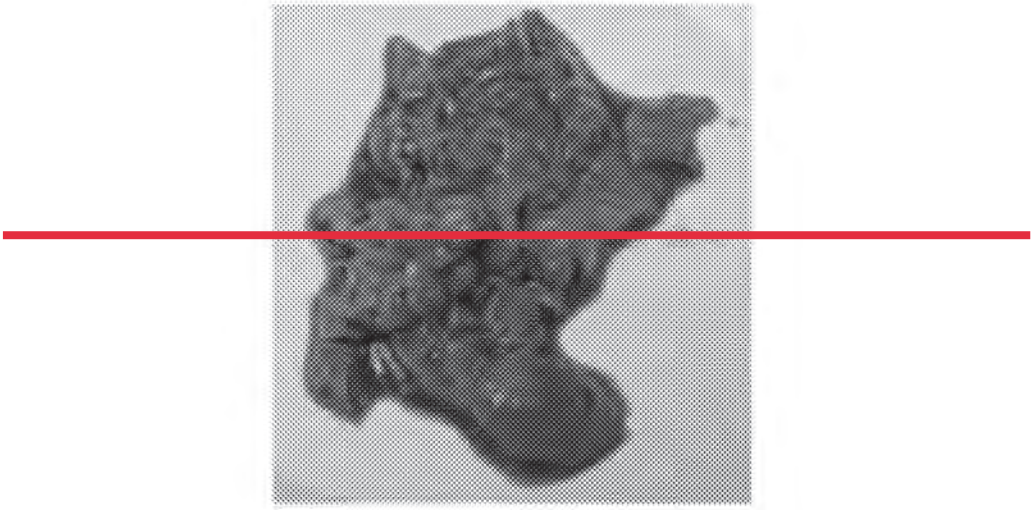


Figure 10

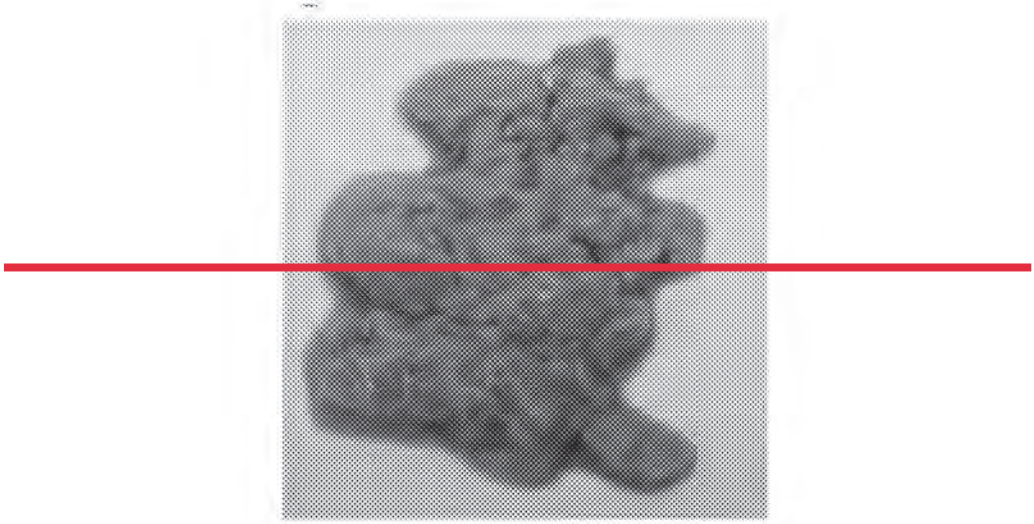


Figure 11

EXHIBIT 1E

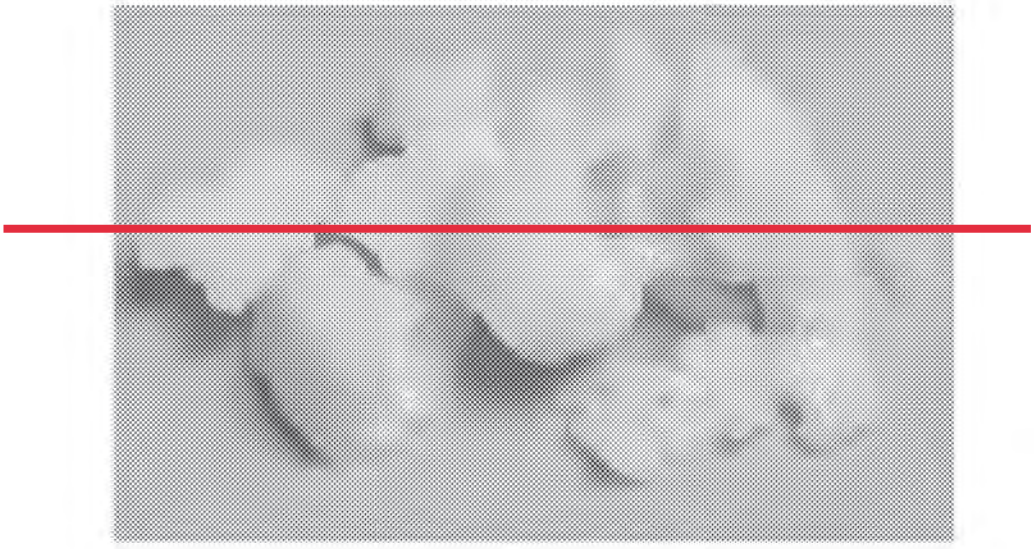


Figure 12

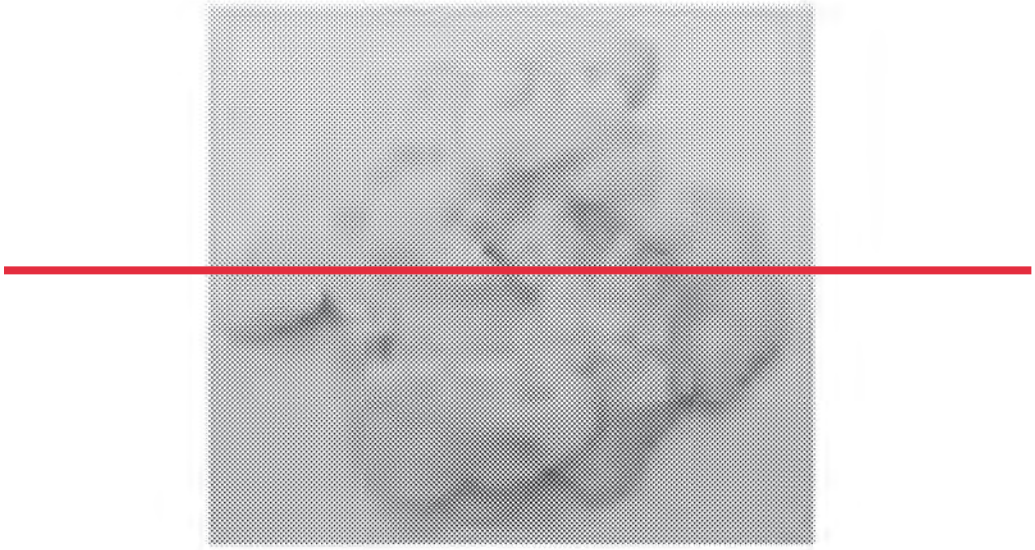


Figure 13

EXHIBIT 1E

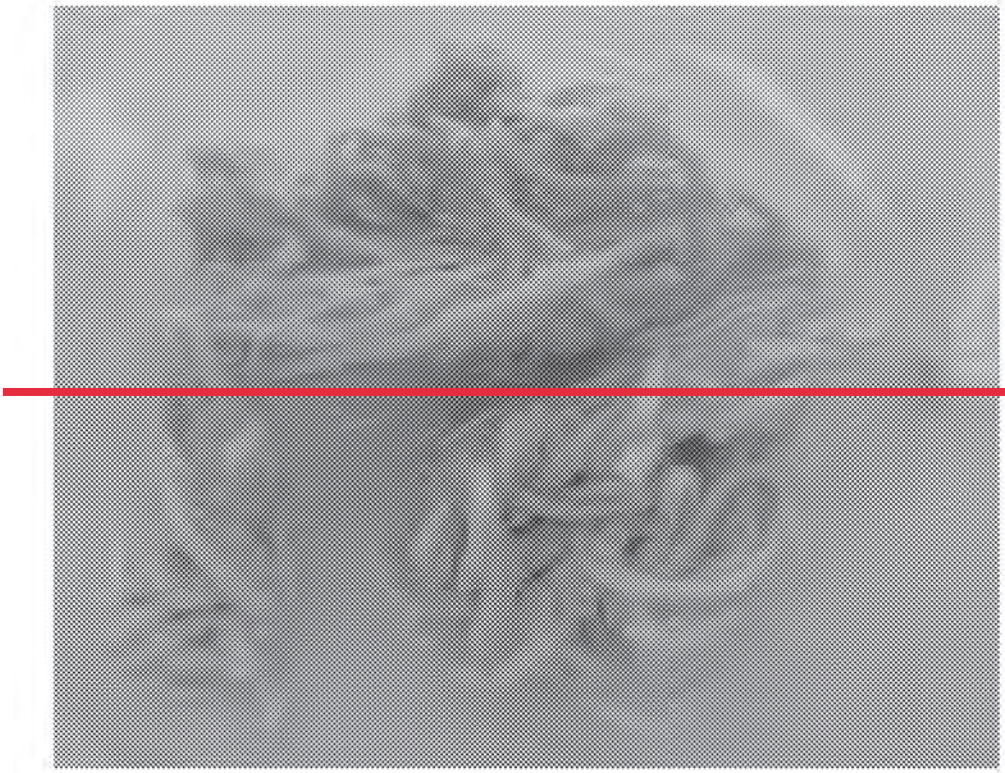


Figure 14

EXHIBIT 1E

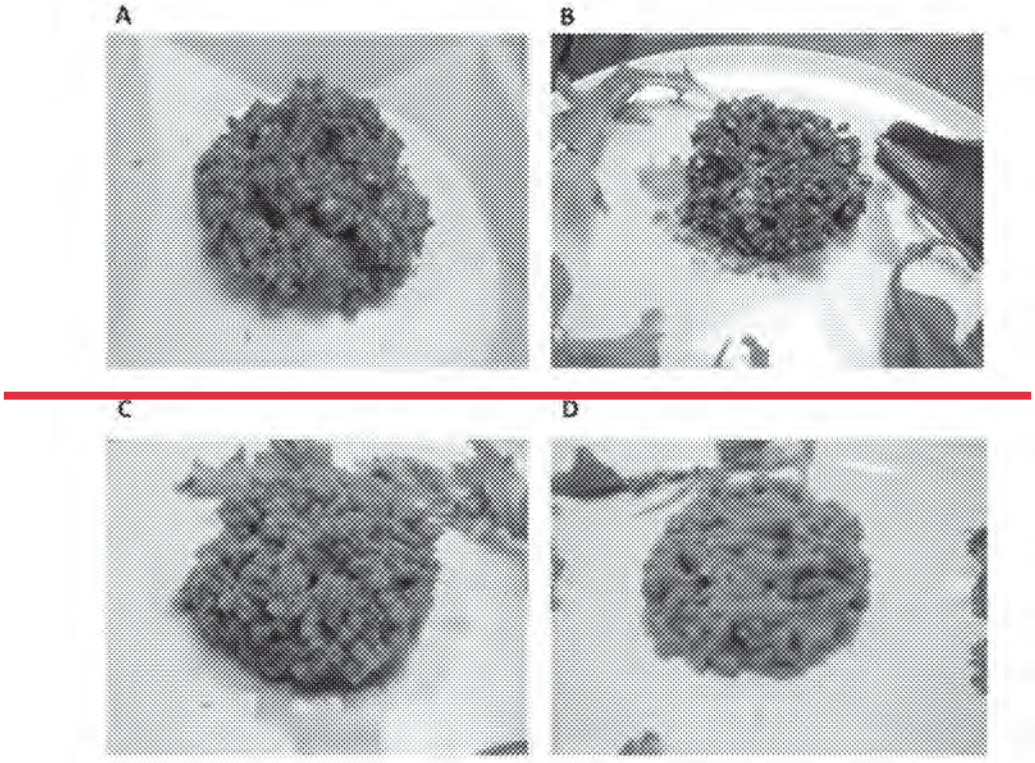


Figure 15

EXHIBIT 1E

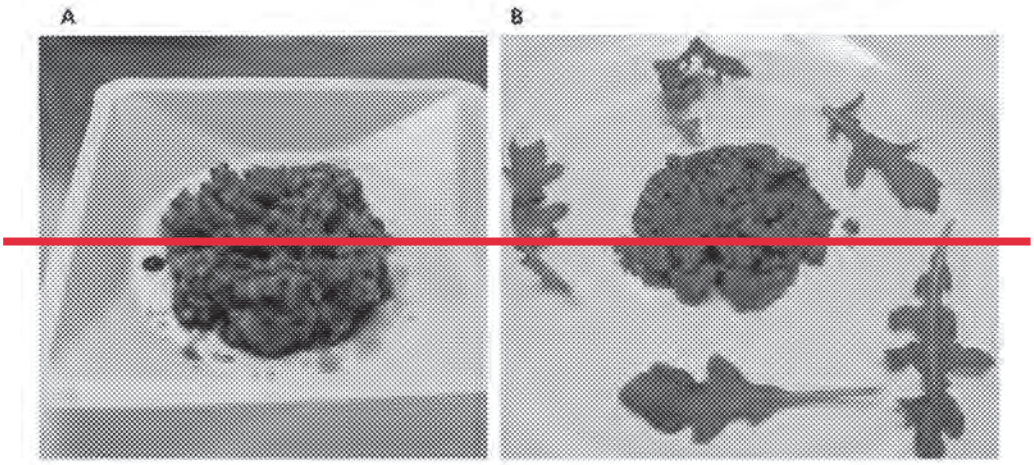


Figure 16

FIGURE 1

SEQ ID NO:1 *Vigna radiata*

MTTTLERGFTEEQEALVVKSWNVMKKNSGELGLKFFLKIFEIAPSAQKLFSFLRDSTVP
LEQNPKLKPHAVSVFVMTCDSAVQLRKAGKVTVRESNLKKLGATHFRTGVANEHFEVTK
FALLETIKEAVPEMWSPAMKNAWGEAYDQLVDAIKYEMKPPSS

SEQ ID NO:2 *Methylacidiphilum infernorum*

MIDQKEKELIKESWKRIEPNKNEIGLLFYANLFKEEPTVSVLFQNPISSQSRKLMQVLG
ILVQGIDNLEGLIPTLQDLGRRHKQYGVVDSHYPLVGDCLLKSIQEYLGQGFTEEAKAA
WTKVYGIAAQVMTAE

SEQ ID NO:3 *Aquifex aeolicus*

MLSEETIRVIKSTVPLLKEHGTEITARMYELLFSKYPKTKELFAGASEEQPKKLANAII
AYATYIDRLEELDNAISTIARSHVRRNVKPEHYPLVKECLLQAIEEVLNPGEEVLKAWE
EAYDFLAKTLITLEKKLYSQP

SEQ ID NO:4 *Glycine max*

MGAFTEKQEALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLSNGVDPSNPKL
TGHAEKLFGLVRDSAGQLKANGTVVADAALGSIHAQKAITDPQFVVVKEALLKTIKEAV
GDKWSDELSSAWEVAYDELAAAIKKAF

SEQ ID NO:5 *Hordeum vulgare*

MSAAEGAVVFSEEKEALVLKSWAIMKKDSANLGLRFFLKIFEIAPSARQMFPFLRDSDV
PLETNPKLKTHAVSVFVMTCEAAAQLRKAGKITVRETTLKRLGGTHLKYGVADGHFEVT
RFALLETIKEALPADMWGPEMRNAWGEAYDQLVAAIKQEMKPAE

Magnaporthe oryzae, (SEQ ID NO:6)

```
   1 mdgavrldwt gldltgheih dgvpiasrvq vmvsfplfkd qhiimsskes psrksstigq
  61 strngscqad tqkgqlppvg ekpkpvkenp mkklkemsqr plptqhgdgt yptekkltgi
 121 gedlkhirgy dvktllamvk sklkgeklkd dktmlmervm qlvarlptes kkraeltdsl
 181 inelwesldh pplnylgpeh syrtpdgsyn hpfnpqlgaa gsryarsvip tvtppgalpd
 241 pglifdsimg rtpnsyrkhp nnvssilwyw atiiihdifw tdprdintnk sssyldlapl
 301 ygnsqemqds irtfkdgrmk pdcyadkrla gmppgvsvll imfnrfhnhv aenlalineg
 361 grfnkpsdll egeareaawk kydndlfqva rlvtsglyin itlvdyvrni vnlnrvdttw
 421 tldprqdaga hvgtadgaer gtgnavsaef nlcyrwhsci sekdskfvea qfqnifgkpa
 481 sevrpdemwk gfakmeqntp adpggrtfgg fkrgpdgkfd dddlvrcise avedvagafg
 541 arnvpqamkv vetmgiiqgr kwnvaglnef rkhfhlkpys tfedinsdpg vaealrrlyd
 601 hpdnvelypg lvaeedkqpm vpgvgiapty tisrvvlsda vclvrgdrfy ttdftprnlt
```

```
661 nwgykevdyd lsvnhgcvfy klfirafpnh fkqnsvyahy pmvvpsenkr ilealgradl
721 fdfeapkyip prvnitsygg aeyiletqek ykvtwheglg flmgegglkf mlsgddplha
781 qqrkcmaaql ykdgwteavk afyagmmeel lvsksyflgn nkhrhvdiir dvgnmvhvhf
841 asqvfglplk taknptgvft eqemygilaa ifttiffdld psksfplrtk trevcqklak
901 lveanvklin kipwsrgmfv gkpakdepls iygktmikgl kahglsdydi awshvvptsg
961 amvpnqaqvf aqavdyylsp agmhyipeih mvalqpstpe tdalllgyam egirlagtfg
1021 syreaavddv vkedngrqvp vkagdrvfvs fvdaardpkh fpdpevvnpr rpakkyihyg
1081 vgphaclgrd asqiaitemf rclfrrrnvr rvpgpqqelk kvprpggfyv ymredwgglf
1141 pfpvtmrvmw dde
```

Fusarium oxysporum (SEQ ID NO:7)

```
1 mkgsatlafa lvqfsaasql vwpskwdeve dllymqggfn krgfadalrt cefgsnvpgt
61 qntaewlrta fhdaithdak agtggldasi ywessrpenp gkafnntfgf fsgfhnprat
121 asdltalgtv lavgacngpr ipfragrida ykagpagvpe pstnlkdtfa aftkagftke
181 emtamvacgh aiggvhsvdf peivgikadp nndtnvpfqk dvssfhngiv teylagtskn
241 plvasknatf hsdkrifdnd katmkklstk agfnsmcadi ltrmidtvpk svqltpvlea
301 ydvrpyitel slnnknkihf tgsvrvritn nirdnndlai nliyvgrdgk kvtvptqqvt
361 fqggtsfgag evfanfefdt tmdaknqitk ffiqevkpst katvthdnqk tggykvddtv
421 lyqlqqscav leklpnaplv vtamvrdara kdaltlrvah kkpvkgsivp rfqtaitnfk
481 atgkkssgyt gfqaktmfee qstyfdivlg gspasgvqfl tsqampsqcs
```

Fusarium graminearum (SEQ ID NO:8)

```
1 masatrqfar aatratrngf aiaprqvirq qgrryyssep aqksssawiw ltgaavagga
61 gyyfygnsas satakvfnps kedyqkvyne iaarleekdd yddgsygpvl vrlawhasgt
121 ydketgtggs ngatmrfape sdhganagla aardflqpvk ekfpwitysd lwilagvcai
181 qemlgpaipy rpgrsdrdvs gctpdgrlpd askrqdhlrg ifgrmgfndq eivalsgaha
241 lgrchtdrsg ysgpwtfspt vltndyfrll veekwqwkkw ngpaqyedks tkslmmlpsd
301 ialiedkkfk pwvekyakdn daffkdfsnv vlrlfelgvp faqgtenqrw tfkpthqe
```

SEQ ID NO: 9 *Chlamydomonas eugametos*

MSLFAKLGGREAVEAAVDKFYNKIVADPTVSTYFSNTDMKVQRSKQFAFLAYALGGASE
WKGKDMRTAHKDLVPHLSDVHFQAVARHLSDTLTELGVPPEDITDAMAVVASTRTEVLN
MPQQ

SEQ ID NO:10 *Tetrahymena pyriformis*

MNKPQTIYEKLGGENAMKAAVPLFYKKVLADERVKHFFKNTDMDHQTKQQTDFLTMLLG
GPNHYKGKNMTEAHKGMNLQNLHFDAIIENLAATLKELGVTDAVINEAAKVIEHTRKDM
LGK

SEQ ID NO:11 *Paramecium caudatum*

MSLFEQLGGQAAVQAVTAQFYANIQADATVATFFNGIDMPNQTNKTAAFLCAALGGPNA
WTGRNLKEVHANMGVSNAQFTTVIGHLRSALTGAGVAAALVEQTVAVAETVRGDVVTV

SEQ ID NO:12 *Aspergillus niger*

MPLTPEQIKIIKATVPVLQEYGTKITTAFYMNMSTVHPELNAVFNTANQVKGHQARALA
GALFAYASHIDDLGALGPAVELICNKHASLYIQADEYKIVGKYLLEAMKEVLGDACTDD
ILDAWGAAYWALADIMINREAALYKQSQG

SEQ ID NO:13 *Zea mays*

MALAEADDGAVVFGEEQEALVLKSWAVMKKDAANLGLRFFLKVFEIAPSAEQMFSFLRD
SDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRETTLKRLGATHLRYGVADGHF
EVTGFALLETIKEALPADMWSLEMKKAWAEAYSQLVAAIKREMKPDA

SEQ ID NO:14 *Oryza sativa subsp. japonica*

MALVEGNNGVSGGAVSFSEEQEALVLKSWAIMKKDSANIGLRFFLKIFEVAPSASQMFS
FLRNSDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRDTTLKRLGATHFKYGVG
DAHFEVTRFALLETIKEAVPVDMWSPAMKSAWSEAYNQLVAAIKQEMKPAE

SEQ ID NO:15 *Arabidopsis thaliana*

MESEGKIVFTEEQEALVVKSWSVMKKNSAELGLKLFIKIFEIAPTTKKMFSFLRDSPIP
AEQNPKLKPHAMSVFVMCCESAVQLRKTGKVTVRETTLKRLGASHSKYGVVDEHFEVAK
YALLETIKEAVPEMWSPEMKVAWGQAYDHLVAAIKAEMNLSN

SEQ ID NO:16 *Pisum sativum*

MGFTDKQEALVNSSWESFKQNLSGNSILFYTIILEKAPAAKGLFSFLKDTAGVEDSPKL
QAHAEQVFGLVRDSAAQLRTKGEVVLGNATLGAIHVQRGVTDPHFVVVKEALLQTIKKA
SGNNWSEELNTAWEVAYDGLATAIKKAMT

SEQ ID NO:17 *Vigna unguiculata*

MVAFSDKQEALVNGAYEAFKANIPKYSVVFYTTILEKAPAAKNLFSFLANGVDATNPKL
TGHAEKLFGLVRDSAAQLRASGGVVADAALGAVHSQKAVNDAQFVVVKEALVKTLKEAV
GDKWSDELGTAVELAYDELAAAIKKAY

SEQ ID NO:18 Bos taurus

MGLSDGEWQLVLNAWGKVEADVAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGNTVLTALGGILKKKGHHEAEVKHLAESHANKHKIPVKYLEFISDAIIHVLHA
KHPSDFGADAQAAMSKALELFRNDMAAQYKVLGFHG

SEQ ID NO:19 *Sus scrofa*
MGLSDGEWQLVLNVWGKVEADVAGHGQEVLIRLFKGHPETLEKFDKFKHLKSEDEMKAS
EDLKKHGNTVLTALGGILKKKGHHEAELTPLAQSHATKHKIPVKYLEFISEAIIQVLQS
KH PGDFGADAQGAMSKALELFRNDMAAKYKELGFQG

SEQ ID NO: 20 *Equus caballus*

MGLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHVLHS
KH PGDFGADAQGAMTKALELFRNDIAAKYKELGFQG

SEQ ID NO: 21 Nicotiana benthamiana
MSSFTEEQEALVVKSWDSMKKNAGEWGLKLFLKIFEIAPSAKKLFSFLKDSNVPL
EQNAKLKPHSKSVFVMTCEAAVQLRKAGKVVVRDSTLKKLGATHFKYGVADE
HFEVTKFALLETIKEAVPEMWSVDMKNAWGEAFDQLVNAIKTEMK

SEQ ID NO: 22 Bacillus subtilis
MGQSFNAPYEAIGEELLSQLVDTFYERVASHPLLKPIFPSDLTETARKQKQFLTQY
LGGPPLYTEEHGHPMLRARHLPFPITNERADAWLSCMKDAMDHVGLEGEIREFL
FGRLELTARHMVNQTEAEDRSS

SEQ ID NO: 23 Corynebacterium glutamicum

MTTSENFYDSVGGEETFSLIVHRFYEQVPNDDILGPMYPPDDFEGAEQRLKMFLS
QYWGGPKDYQEQRGHPRLRMRHVNYPIGVTAAERWLQLMSNALDGVDLTAEQ
REAIWEHMVRAADMLINSNPDPHA

SEQ ID NO: 24 Synechocystis PCC6803
MSTLYEKLGGTTAVDLAVDKFYERVLQDDRIKHFFADVDMAKQRAHQKAFLTY
AFGGTDKYDGRYMREAHKELVENHGLNGEHFDAVAEDLLATLKEMGVPEDLIA
EVAAVAGAPAHKRDVLNQ

SEQ ID NO: 25 Synechococcus sp. PCC 7335
MDVALLEKSFEQISPRAIEFSASFYQNLFHHHPELKPLFAETSQTIQEKKLIFSLAAI
IENLRNPDILQPALKSLGARHAEVGTIKSHYPLVGQALIETFAEYLAADWTEQLA
TAWVEAYDVIASTMIEGADNPAAYLEPELTFYEWLDLYGEESPKVRNAIATLTH
FHYGEDPQDVQRDSRG

SEQ ID NO: 26 Nostoc commune
MSTLYDNIGGQPAIEQVVDELHKRIATDSLLAPVFAGTDMVKQRNHLVAFLAQIF
EGPKQYGGRPMDKTHAGLNLQQPHFDAIAKHLGERMAVRGVSAENTKAALDR
VTNMKGAILNK

SEQ ID NO: 27 Bacillus megaterium
MREKIHSPYELLGGEHTISKLVDAFYTRVGQHPELAPIFPDNLTETARKQKQFLT
QYLGGPSLYTEEHGHPMLRARHLPFEITPSRAKAWLTCMHEAMDEINLEGPER
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FIGURE 2A

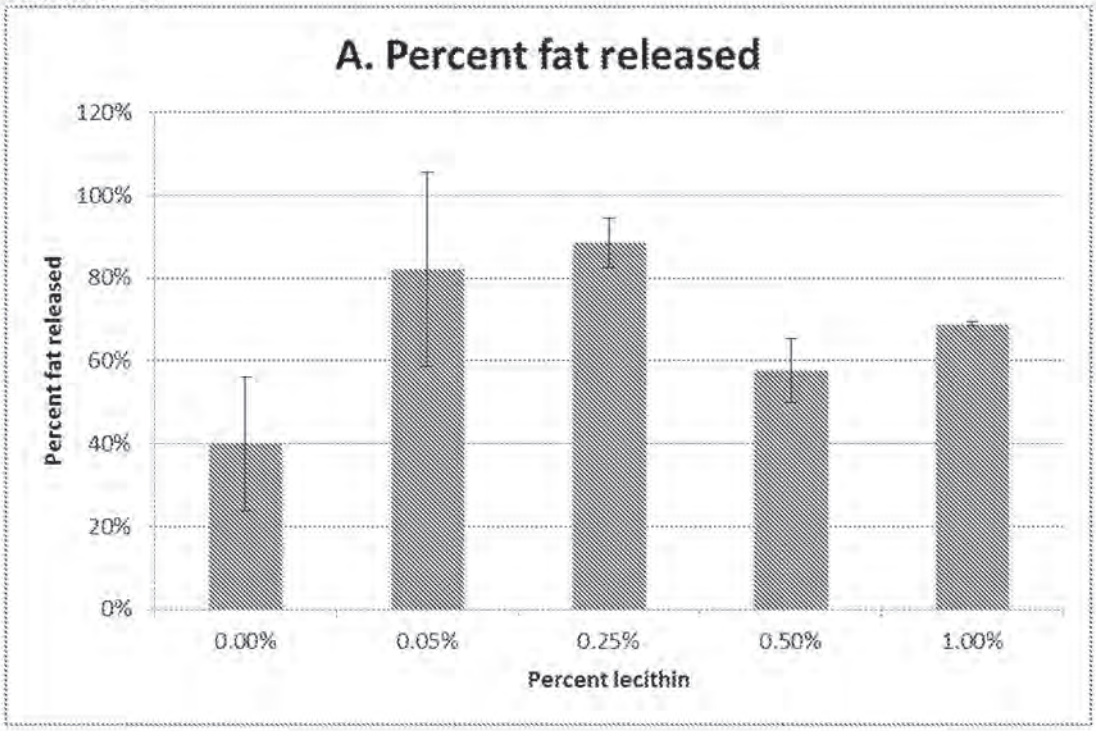


FIGURE 2B

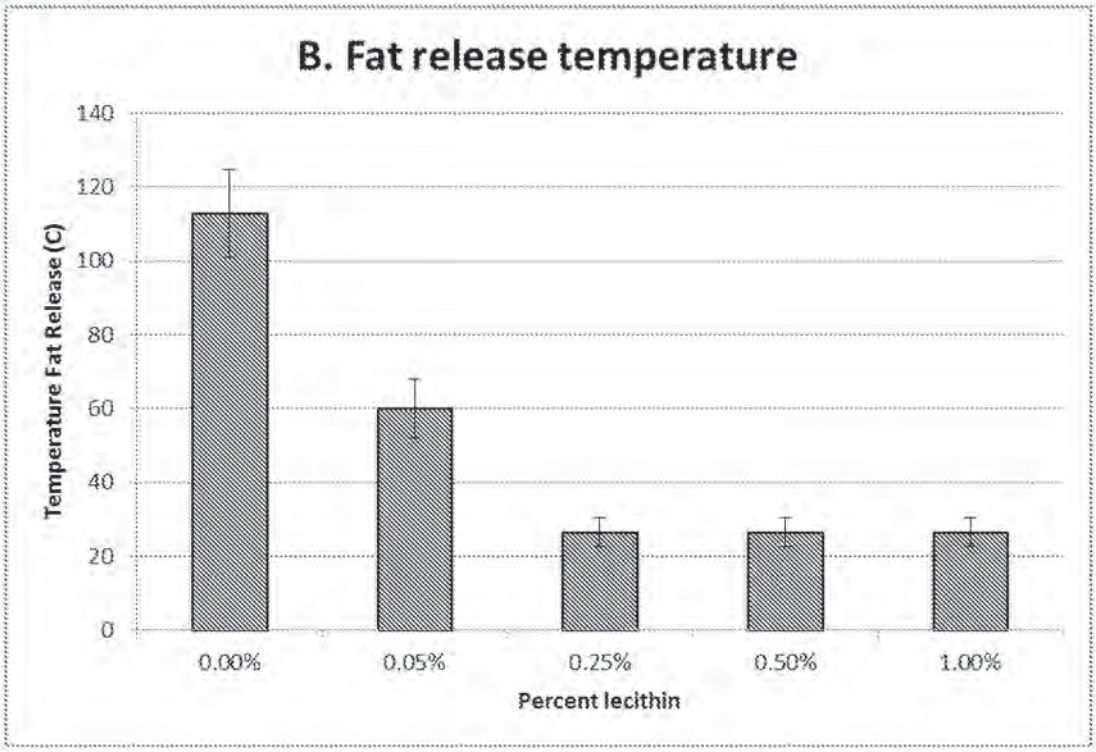


FIGURE 2C

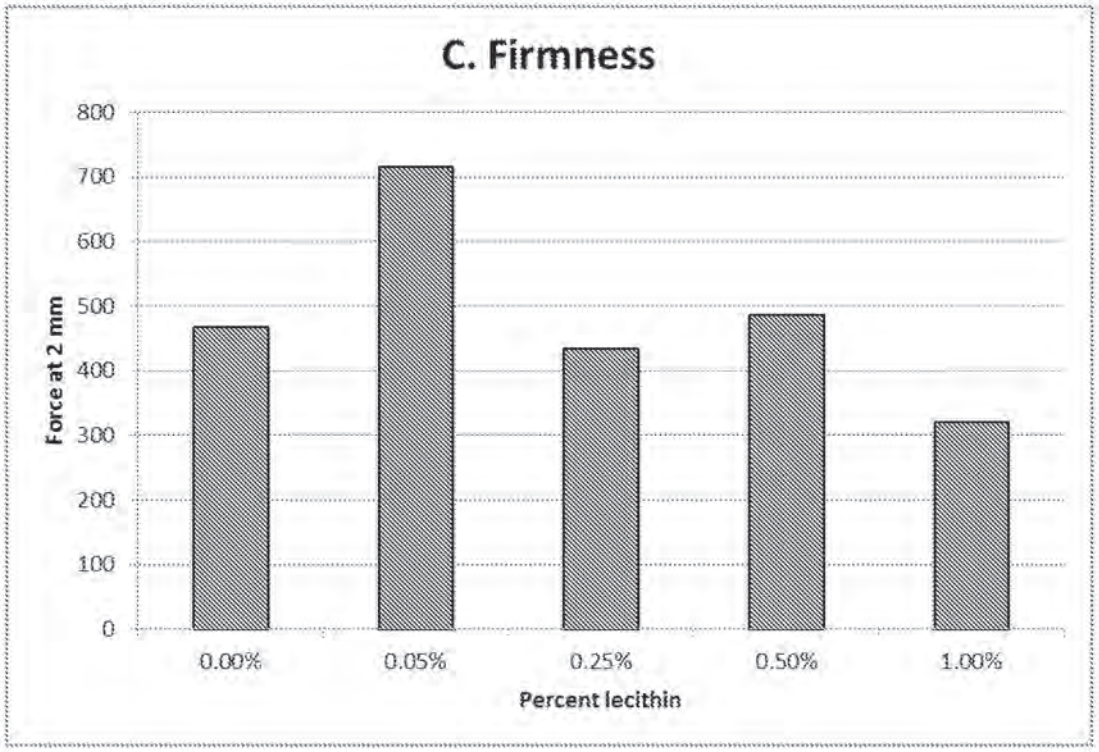

C. Firmness
800
700
600
500
400
300
200
100
0
Force at 2 mm
0.00%
0.05%
0.25%
0.50%
1.00%
Percent lecithin

FIGURE 3

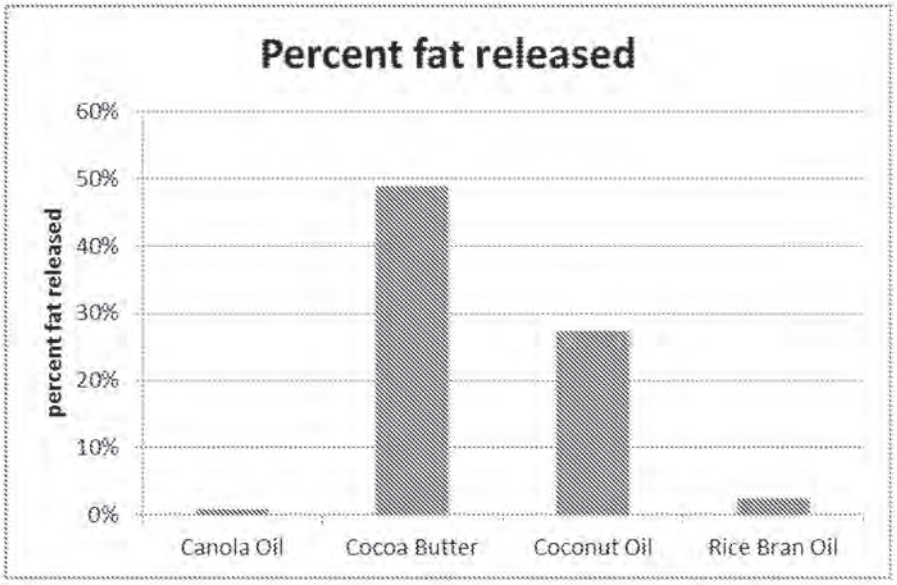
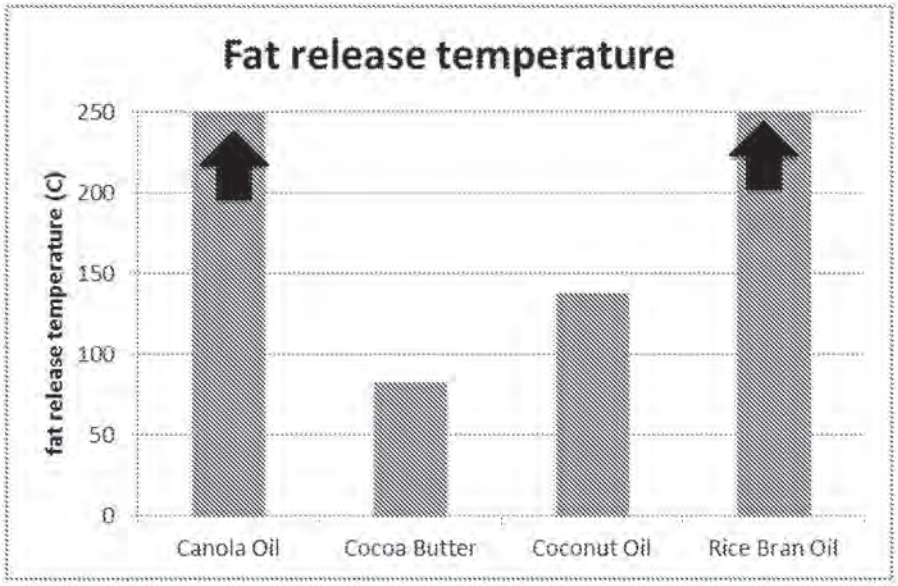

Percent fat released
percent fat released
60%
50%
40%
30%
20%
10%
0%
Canola Oil
Cocoa Butter
Coconut Oil
Rice Bran Oil

FIGURE 4


Fat release temperature
fat release temperature (C)
250
200
150
100
50
0
Canola Oil
Cocoa Butter
Coconut Oil
Rice Bran Oil

Disclosed herein are improved methods and compositions which more accurately replicate the characteristics that consumers value in the preparation and consumption of meat and which overcome the shortcomings and drawbacks of current meat substitutes.

METHOD AND COMPOSITIONS FOR CONSUMABLES

CROSS REFERENCE

[0001] This application is a continuation in part of International Application No. PCT/US2012/046560, filed Jul. 12, 2012, which claims the benefit of priority of U.S. Provisional Application 61/572,205, filed Jul. 12, 2011 and is a non provisional of and claims the benefit of U.S. Provisional Application 61/671,069, filed Jul. 12, 2012, all of which are incorporated herein by reference.

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(63) Continuation-in-part of application No. PCT/US2012/046560, filed on Jul. 12, 2012.

(60) Provisional application No. 61/671,069, filed on Jul. 12, 2012, provisional application No. 61/572,205, filed on Jul. 12, 2011.

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(57) ABSTRACT

METHODS AND COMPOSITIONS FOR CONSUMABLES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 13/941,211, filed July 12, 2013, U.S. Application Serial No. 61/908,634, filed November 25, 2013, and to U.S. Application Serial No. 61/751,816, filed January 11, 2013; and is related to the following co-pending patent applications: Application Serial No. PCT/US12/46560; Application Serial No PCT/US12/46552; Application Serial No. 61,876,676, filed September 11, 2013; Application Serial No. 61/751,818, filed January 11, 2013, and Application Serial No. 61/611,999, filed March 16, 2012, all of which are incorporated herein by reference.

TECHNICAL FIELD

This invention relates to consumable products and more particularly, to non-animal based replicas of animal-based food products, that can be produced, in some embodiments, by breaking down non-animal materials into their constituent parts and reassembling those parts into the consumables.

BACKGROUND OF THE INVENTION

[0002] Animal farming has a profound negative environmental impact. Currently it is estimated that 30% of Earth's land surface is dedicated to animal farming and that livestock account for 20% of total terrestrial animal biomass. Due to this massive scale, animal farming accounts for more than 18% of net greenhouse gas emissions. Animal farming may be the largest human source of water pollution, and animal farming is by far

the world's largest threat to biodiversity. It has been estimated that if the ~~worlds~~ world's human population could shift from a meat containing diet to a diet free of animal products, 26% of Earth's land surface would be freed for other uses. Furthermore the shift to a vegetarian diet would massively reduce water and energy consumption.

~~[0003]~~ The consumption of meat has a profound negative impact on human health. The ~~heath~~ health benefits of a vegetarian diet are well established. If the human population would shift to a more vegetarian diet ~~the cost savings~~, there would be a decrease in health care ~~would be significant~~costs.

[0004] Hunger is a worldwide problem, yet the world's 4 major commodity crops (soybeans, maize, wheat, and rice) already supply more than 100% of the human population's requirements for calories and protein, including every essential amino acid.

[0005] Plant based meat substitutes have largely failed to cause a shift to a vegetarian diet. The current state of the art for meat substitute compositions involves the extrusion of soy/ soy/grain mixture, resulting in products which largely fail to replicate the experience of cooking and eating meat. Common limitations of these products are a texture and mouthfeel that are more homogenous than that of equivalent meat products.

Furthermore, as the products must largely be sold precookedpre-cooked, with artificial flavors and aromas built in, they fail to replicate aromas, flavors, and other key features associated with cooking meat. As a result, these products appeal mainly to a limited consumer base that is already committed to veg etarianism/veganismvegetarianism/veganism, but have failed to appeal to the larger consumer segment accustomed to eating meat.

[0006] Disclosed herein are improved methods and compositions which more accurately replicate the characteristics that consumers value in the preparation and consumption of meat and which overcome the shortcomings and drawbacks of current meat substitutes.

SUMMARY OF THE INVENTION

[0007] In some aspects, the invention provides a meat substitute composition comprising a protein content, wherein one or more isolated and purified proteins accounts for 10% or more of said protein content by weight, wherein said meat substitute composition accurately mimics the taste, texture, or color of a meat product derived from animal sources.

~~[0008] In one embodiment, the meat substitute composition accurately mimics the color of said meat product in its raw state and in a cooked state after cooking.~~

~~[0009] In another embodiment, the one or more isolated and purified proteins accounts for 25% or more of said protein content by weight.~~

~~[0010] In another embodiment, the one or more isolated and purified proteins accounts for 50% or more of said protein content by weight.~~

~~[0011] In another embodiment, the one or more isolated and purified proteins accounts for 75% or more of said protein content by weight.~~

~~[0012] In another embodiment, the one or more isolated and purified proteins accounts for 90% or more of said protein content by weight.~~

~~[0013] In another embodiment, gluten does not account for 10% or more of said protein content by weight.~~

~~[0014] In another embodiment, each of said isolated, purified proteins is separately isolated and purified.~~

~~[0015] In another embodiment, the meat substitute composition comprises 1-7~~ Food is any substance that is either eaten or drunk by any animal, including humans, for nutrition or pleasure. It is usually of plant or animal origin, and contains essential nutrients, such as carbohydrates, fats, proteins, vitamins, or minerals. The substance is ingested by an organism and assimilated by the organism's cells in an effort to produce energy, maintain life, or stimulate growth.

Food typically has its origin in a photosynthetic organism, typically from plants. Some food is obtained directly from plants; but even animals that are used as food sources are raised by feeding them food derived from plants. Edible fungi and bacteria are used to transform materials from plants or animals into other food products, mushrooms, bread, yogurt and the like.

In most cases, the plant or animal is fractionated into a variety of different portions, depending upon the purpose of the food. Often, certain portions of the plant, such as the seeds or fruits, are more highly prized by humans than others and these are selected for human consumption whilst other less desirable portions, such as the stalks of grasses, are typically used for feeding animals.

Animals are typically butchered into smaller cuts of meat with specific flavor and handling properties before consumption.

While many foods can be eaten raw, many also undergo some form of preparation for reasons of safety, palatability, texture, or flavor. At the simplest level, this may involve washing, cutting, trimming, or adding other foods or ingredients. It may also involve mixing, heating or cooling or fermentation and individual foods may be combined with other food products to achieve the desired mix of properties.

In recent years, attempts have been made to bring scientific rigor to the process of food preparation, under the fields of food science and molecular gastronomy. Food science broadly studies the safety, microbiology, preservation, chemistry, engineering and physics of food preparation, whereas molecular gastronomy focuses on the use of scientific tools such as liquid nitrogen, emulsifying agents such as soy lecithin and gelling agents such as calcium alginates to transform food products into unexpected forms.

However, the raw material is typically an entire organism (plant or animal) or an isolated tissue such as a steak, the fruiting body of a fungus, or the seed of a plant. In some cases, the isolated tissue is modified before food preparation, such as making flour or isolating oils and bulk proteins from seeds.

isolated and purified proteins.**[0016]** In another embodiment, said 1-7 isolated and purified proteins are each isolated from different plant species. **[0017]** In some embodiments the meat substitute comprises less than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 individual proteins isolated from one or more plant species.

[0018] In another embodiment, said protein content comprises no more than trace amounts of any other proteins derived from the one or more plant species.

[0019] In another embodiment, said one or more isolated and purified proteins are selected from the group consisting of leghemoglobin, non symbiotic hemoglobin, hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyano globin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglo bins, ribosomal proteins, actin, hexokinase, lactate dehydro genase, fructose bisphosphate aldolase, phosphofructoki nases, trio se phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde 3 phosphate dehydrogenases, pyru vate decarboxylases, actin, translation elongation factors, ribulose 1,5 bisphosphate carboxylase oxygenase (rubisco), ribulose 1,5 bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

[0020] In another embodiment, said one or more isolated and purified proteins are not isolated from an animal.

[0021] In another embodiment, said one or more isolated and purified proteins are isolated from a single plant source. **[0022]** In another embodiment, said one or more isolated and purified proteins are isolated from multiple plant sources. **[0023]** In another embodiment, wherein said one or more isolated, purified proteins are isolated from a genetically modified organism.

[0024] In some embodiments, said genetically modified organism is a genetically modified bacteria or yeast organism.

[0025] In some embodiments, said isolated, purified protein has been formed into fibers.

[0026] In particular embodiments, said fibers resemble skeletal muscle fibers.

[0027] In yet more particular embodiments, said fibers are asymmetric fibers.

[0028] In some embodiments, the meat substitute composition further comprises one or more isolated and purified iron-containing proteins.

[0029] In some embodiments, said one or more isolated and purified iron containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuro-globin,

cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

[0030] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSV-VFYTSILEKAPAAKDLF SFLANGVDPTNPKLTGHAE KLFALVRDSAGQLKASGTVVADAALGS-VHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDE LSRAWEVAYDELAAAIKKA.

[0031] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 2. SEQ ID NO 2: MIDQKEKELI KESWKRIEPN KNEIGLLFYA NLFKEEPTVS VLFQN PISSQ SRKLMQVLGI LVQGIDNLEG LIPTLQDLGR RHKQYGVVDS HYPLVGDCLL KSIQEYLGQG FTEEA KAAWT KVYGIAAQVM TAE. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 80% homology to SEQ ID NO 2. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 90% homology to SEQ ID

- In some embodiments said iron-containing protein comprises an amino acid sequence with at least 98% homology to SEQ ID NO 2.

[0032] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 3. SEQ ID NO 3: MRKQPTVFEK LGGQAAMHAA VPLFYKKVLA DDRVKHYFKN TNMEHQAKQQ EDFLTMLLGG PNHYKGKNMA EAH KGMNLQN SHFDAIIENL AATLKELGVS DQI IGEAAKV IEHTRKDCLG K. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 80% homology to SEQ ID NO 3. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 90% homology to SEQ ID

- In some embodiments said iron-containing protein comprises an amino acid sequence with at least 98% homology to SEQ ID NO 3.

[0033] In some embodiments, the isolated and purified proteins are assembled into one or more gels.

[0034] In some embodiments, the meat substitute composition further comprises one or more fats.

[0035] In particular embodiments, said one or more fats are derived from a plant source.

[0036] In another aspect, the invention provides a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources.

[0037] In some embodiments, said indicator is a visual indicator that accurately mimics the color transition of a meat product during said cooking progression.

[0038] In one embodiment, said color transition is from red to brown.

[0039] In one embodiment, said color transition is from pink to white or tan.

[0040] In one embodiment, said visual indicator transitions from a translucent to opaque color during said cooking progression.

[0041] In some embodiments, the indicator is an olfactory indicator that indicates cooking progression.

[0042] In one embodiment, said olfactory indicator is one or more volatile odorants released during cooking. **[0043]** In some embodiments, said indicator comprises one or more isolated, purified iron-containing proteins. **[0044]** In particular embodiments, said one or more isolated, purified iron-containing proteins is in a reduced state before cooking.

[0045] In one embodiment, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuro-globin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohe-moglobins.

[0046] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSV-VFYTSILEKAPAAKDLF SFLANGVDPTNPKLTGHAE KLFALVRDSAGQLKASGTVVADAALGS-VHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDE LSRAWEVAYDELAAAIKKA.

[0047] In some embodiments, said one or more isolated and purified iron-containing proteins are not isolated from an animal. In some embodiments compositions of the invention do not contain any proteins from an animal.

[0048] In particular embodiments, said one or more isolated and purified iron-containing proteins are isolated from one or more plant sources.

[0049] In yet more particular embodiments, said one or more isolated, purified proteins are isolated from the root nodules, roots, seeds, leaves, or stems of said one or more plant sources.

[0050] In other particular embodiments, said one or more plant sources are soy or pea plants.

[0051] In one embodiment, said one or more plant sources comprises one or more plants of the legume family. **[0052]** In some embodiments, said one or more isolated and purified iron carrying proteins in a reduced or oxidized state has a similar UV-VIS profile to a myoglobin protein derived from an animal source when in an equivalent reduced or oxidized state.

[0053] In a particular embodiment, the difference between the peak absorbance wavelength of said one or more isolated and purified iron-containing proteins and the peak absorbance wavelength of myoglobin derived from an animal source is less than 5%.

[0054] In some embodiments, said one or more isolated, purified proteins are isolated from a genetically modified organism.

[0055] In one embodiment, said genetically modified organism is a genetically modified bacteria or yeast organism.

[0056] In some embodiments, the meat substitute product contains no methylcellulose, no carrageenan, no caramel color,

~~no konjac flour, no gum arabic, and no acacia gum. **[0057]** In particular embodiments, the meat substitute product additionally contains less than 1% wheat gluten. **[0058]** In a more particular embodiment, said meat substitute product contains no wheat gluten.~~
~~**[0059]** In other particular embodiments, said meat substitute product contains no soy protein isolate.~~
~~**[0060]** In other particular embodiments, said meat substitute product contains no soy protein concentrate.~~
~~**[0061]** In other particular embodiments, said meat substitute product contains no soy protein.~~
~~**[0062]** In a more particular embodiment, said meat substitute product contains less than 5% carbohydrates.~~
~~**[0063]** In other particular embodiments, said meat substitute product contains no tofu.~~
~~**[0064]** In some embodiments, said meat substitute product contains no tofu, and no wheat gluten.~~
~~**[0065]** In some embodiments, said meat substitute product contains no soy protein, and no wheat gluten.~~
~~**[0066]** In some embodiments, said meat substitute product contains no animal products and less than 5% carbohydrates. **[0067]** In some embodiments, said meat substitute product contains less than 1% cellulose.~~
~~**[0068]** In some embodiments, said meat substitute product contains less than 5% insoluble carbohydrates.~~
~~**[0069]** In some embodiments, said meat substitute product contains no soy protein, and less than 1% cellulose.~~
~~**[0070]** In some embodiments, said meat substitute product contains no soy protein, and less than 5% insoluble carbohydrates.~~
~~**[0071]** In some embodiments, said meat substitute product contains no wheat gluten, and less than 1% cellulose.~~
~~**[0072]** In some embodiments, said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.~~
~~**[0073]** In another aspect, the invention provides a muscle tissue replica comprising a protein content, wherein said protein content comprises one or more isolated and purified proteins, wherein said muscle tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source.~~
~~**[0074]** In some embodiments, said one or more isolated and purified proteins accounts for at least 50% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 40% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 30% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 20% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 10% of said protein content by weight.~~
~~**[0075]** In some embodiments, said one or more isolated and purified proteins accounts for at least 50% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 40% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 30% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 20% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 10% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 5% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 1% of said composition content by weight.~~
~~**[0076]** In some embodiments, said protein content is derived from one or more non-animal sources.~~
~~**[0077]** In particular embodiments, said one or more non-animal sources is a plant source.~~
~~**[0078]** In other particular embodiments, said one or more non-animal sources is a genetically modified yeast or bacteria.~~
~~**[0079]** In some embodiments, each of said one or more isolated proteins is isolated and purified separately.~~

Despite that fact that all of these items comprise a mixture of proteins, carbohydrates, fats, vitamins and minerals, the physical arrangement of these materials in the original plant or animal determines the use to which the plant or animal tissue will be put. Disclosed herein are improved methods and composition for the production of consumables.

SUMMARY

Provided herein are consumable products and methods of making the same. The consumables can be non-animal based consumable goods, e.g., containing mainly plant or entirely-plant based proteins and/or fats, and can be in the form of a beverage (e.g., an alcoholic beverage such as cream liquor, or a protein drink), a protein supplement, a

baked good (e.g., a bread or a cookie), a condiment (e.g., a mayonnaise, a mustard), a meat product, or a meat substitute product (e.g., a ground beef product). For example, the protein drink can be a meal replacement beverage, a beer supplemented with the

protein, or a distilled alcoholic beverage (e.g., vodka or rum) supplemented with the protein. The condiment can be mayonnaise. The meat product can be a pate, a sausage, or a meat substitute that can include a muscle replica, plant-based adipose and/or connective tissue. Coacervates that include one or more proteins can be used to help bind the ingredients to each other in the consumable products (e.g., a ground beef product).

Accordingly, provided herein is a consumable product comprising an isolated and purified plant protein, wherein the isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2°C and about 32°C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90°C and 110°C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM. In some embodiments, the consumable product is a beverage, a protein supplement, a baked good, a condiment, a meat product, or a meat substitute product. In some embodiments, the beverage is an alcoholic beverage or a protein drink. In some embodiments, the alcoholic beverage is a cream liquor. The cream liquor can further include a non-dairy lipid emulsion, where the cream liquor is free of animal products. In some embodiments, the protein drink is a meal replacement beverage, a beer supplemented with said protein, or a distilled alcoholic beverage supplemented with the protein. A condiment can be a mayonnaise replica. In some embodiments, the meat product can be a pate, a sausage replica, or a meat substitute. In some embodiments, the isolated and purified plant protein is at least 10 kDa in size. In some embodiments, the isolated and purified plant protein is not fully denatured. In some cases, the isolated and purified plant protein is not derived from soy. In some embodiments, the isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin.

In some embodiments, the isolated and purified plant protein comprises a dehydrin, a hydrophilin, an intrinsically disordered protein, or a protein identified based on its ability to stay soluble after boiling at a pH and a salt concentration comparable to a food. In some embodiments, the consumable product further comprises a plant derived

lipid or a microbial-derived lipid. In some embodiments, the consumable product further includes a second isolated and purified protein, and/or a seasoning agent, a flavoring agent, an emulsifier, a gelling agent, a sugar, or a fiber.

The disclosure also provides a consumable product comprising a coacervate comprising one or more isolated and purified proteins. In some embodiments, the consumable product is a meat replica. In some embodiments, the consumable product further includes a plant derived lipid or a microbial-derived lipid. The plant derived lipid or microbial-derived lipid can comprise lecithin and/or an oil. The product can include up to about 1% lecithin by weight. The product can include lecithin and the oil. In some embodiments, the oil is canola oil, palm oil, or cocoa butter. The product can include about 1% to about 9% of the oil. The one or more isolated and purified proteins can comprise plant proteins. The one or more plant proteins can comprise one or more pea proteins, chickpea proteins, lentil proteins, lupine proteins, other legume proteins, or mixtures thereof In some embodiments, the one or more pea proteins are legumins, vicilin, a convicilin, or a mixture thereof

The disclosure also provides a meat replica comprising a muscle replica, a connective tissue replica, an adipose tissue replica, and a coacervate comprising one or more isolated and purified proteins. The coacervate further can comprise a plant-derived lipid or microbial-derived lipid. The plant derived lipid or microbial-derived lipid can be lecithin and/or an oil. The meat replica can be a ground beef replica.

Also provided is a consumable product comprising a comprising a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt. In some embodiments, the isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin. In some embodiments, the isolated and purified plant protein comprises a dehydrin, a hydrophilin, or an intrinsically disordered protein. In some embodiments, the cold set gel further comprises a plant derived lipid or microbial derived lipid. In some embodiments, the plant derived lipid or microbial derived lipid is lecithin and/or oil.

The disclosure further provides an adipose tissue replica comprising one or more isolated plant proteins, one or more plant or algal derived oils, and optionally a

phospholipid. In some embodiments, the phospholipid is lecithin. In some embodiments, the plant based oils are selected from the group consisting of corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, palm oil, palm kernel oil, coconut oil, babassu oil, Shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, and combinations thereof In some embodiments, the fat release temperature of the adipose tissue replica is between 23°C to 33°C, 34°C to 44°C, 45°C to 55°C, 56°C to 66°C, 67°C to 77°C, 78°C to 88°C, 89°C to 99°C, 100°C to 110°C, 111°C to 121°C, 122°C to 132°C, 133°C to 143°C, 144°C to 154°C, 155°C to 165°C, 166°C to 167°C, 168°C to 169°C, 170°C to 180°C, 181°C to 191°C, 192°C to 202°C, 203°C to 213°C, 214°C to 224°C, 225°C to 235°C, 236°C to 246°C, 247°C to 257°C, 258°C to 268°C, 269°C to 279°C, 280°C to 290°C, or 291°C to 301°C. In some embodiments, the percent fat release of the adipose tissue replica is 0 to 10%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, 50% to 60%, 60% to 70%, 70% to 80%, 80% to 90%, or 90% to 100% upon cooking. In some embodiments, the isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin.

In some embodiments, the adipose tissue replica comprises from about 40% to about 90% of the oil. In some embodiments, the adipose tissue replica comprises from about 1% to about 6% of the isolated and purified plant protein. In some embodiments, the adipose tissue replica comprises from about 0.05 to about 2% of the phospholipid. In some embodiments, the firmness of the adipose tissue replica is similar to that of beef adipose tissue.

Also provided is a consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, wherein the consumable product does not comprise meat. In some embodiments, the heme-containing protein accounts for at least 0.01% of the composition. In some embodiments, the consumable product further comprises one or more ammonium, sodium, potassium, or calcium salts. In some embodiments, the isolated and purified proteins are crosslinked.

Further provided is a consumable product comprising a gelled emulsion, wherein the gelled emulsion comprises:

a) an isolated and purified protein;

b) a first lipid that when not in the consumable product is solid at a selected temperature range; and

c) a second lipid that when not in the consumable product is liquid at the selected temperature range; wherein the melting temperature of the mixture of the first and second lipids is similar to the melting temperature of lipids found in meat, and wherein the first and second lipids are plant derived lipids or microbial derived lipids.

The disclosure also provides a method for making a consumable product comprising:

a) preparing a solution comprising an isolated and purified plant protein, wherein the isolated and purified plant protein has (i) a solubility in the solution of at least 25 at a temperature between about 2°C and 32°C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in the solution of at least 1 mg/ml at a temperature of between 90°C and 110°C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM; and

b) adding the solution to a beverage.

In some embodiments, the solution comprises two or more isolated and purified plant proteins. In some embodiments, the beverage is clear. In some embodiments, the isolated and purified plant protein is at a concentration of at least 1% by weight in the solution. In some embodiments, the isolated and purified plant protein is selected from the group consisting of RuBisCo, a moong globulin, a soy globulin, a pea globulin, a pea albumin, a prolamin, a lentil protein, a dehydrin, a hydrophilin, and an intrinsically disordered protein. In some embodiments, the isolated and purified plant protein is lyophilized prior to making said solution. In some embodiments, the beverage has an improved mouthfeel compared to a corresponding beverage without the isolated and purified protein.

Also provided is a method for extending the shelf-life of a meat-free consumable product, the method comprising adding a heme-containing protein to the consumable product, wherein the heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions. In some embodiments, the heme-containing protein comprises an amino acid sequence with at least 70% homology to an amino acid sequence set forth in any one of SEQ ID NOs: 1-27.

Further provided is a method for making a meat replica comprising a cold set gel, wherein the method includes:

a) denaturing a solution comprising at least one isolated and purified protein from a non-animal source under conditions wherein the isolated and purified protein does not precipitate out of the solution;

b) optionally adding any heat-labile components to the solution of denatured protein;

c) gelling the solution of denatured protein at about 4°C to about 25°C by increasing the ionic strength of the solution to form a cold set gel; and

d) incorporating the cold set gel into a meat replica.

In some embodiments, the gelling is induced using 5 to 100 mM sodium or calcium chloride. In some embodiments, the heat-labile components are proteins or lipids, or mixtures thereof. In some embodiments, the protein is a heme-containing protein. In some embodiments, the cold set gel is formed in a matrix comprising a freeze-aligned plant protein.

In some embodiments, the isolated and purified protein from a non-animal source is a plant protein. In some embodiments, the plant protein is selected from the group consisting of RuBisCo, a moong globulin, a soy globulin, a pea globulin, a pea albumin, a prolamin, a lentil protein, a dehydrin, a hydrophilin, and an intrinsically disordered protein.

Further provided is an adipose tissue replica, comprising

a) an isolated and purified non-animal protein;

b) a non-animal lipid; and

c. a three-dimensional matrix comprising fibers derived from non-animal sources, wherein the lipid and the protein are dispersed in the three-dimensional matrix, and wherein the three-dimensional matrix stabilizes the structure of the adipose tissue replica.

Also provided is a connective tissue replica comprising one or more isolated and purified proteins assembled into fibrous structures by a solution spinning process. In some embodiments, the fibrous structures are stabilized by a cross-linking agent.

Provided herein is a method for imparting a beef like flavor to a consumable product, comprising adding to the consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to the consumable composition.

Also provided is a method for making a poultry or a fish composition taste like beef, the method comprising adding a heme protein to the poultry or fish composition, respectively.

In some embodiments, the heme-containing protein has an amino acid sequence with at least 70% homology to any one of the amino acid sequences set forth in SEQ ID NOs: 1-27.

Further provided is a method of making a coacervate, the method comprising

a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, wherein the solution comprises 100 mM or less of sodium chloride; and

b) isolating the coacervate from the solution. In some embodiments, the pH is between 4 and 5. In some embodiments, the plant proteins comprise one or more pea proteins, chickpea proteins, lentil proteins, lupine proteins, other legume proteins, or mixtures thereof. In some embodiments, the pea proteins comprise isolated and purified legumins, isolated and purified vicilins, isolated and purified convicilins, or combinations thereof. In some embodiments, the isolated and purified pea proteins comprise isolated and purified vicilins and isolated and purified convicilins. In some embodiments, the acidifying step is done in the presence of a plant derived lipid or microbial derived lipid. In some embodiments, the plant derived lipid or microbial derived lipid comprises oils and/or phospholipids.

Provided herein is a method of making an adipose tissue replica, the method comprising forming an emulsion comprising one or more isolated plant proteins, one or more plant or algal derived oils, and optionally a phospholipid. In some embodiments, when the phosoholipid is included, it is lecithin. In some embodiments, the plant based oils are selected from the group consisting of corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, palm oil, palm kernel oil, coconut oil, babassu oil, Shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, and combinations thereof In some embodiments, the fat release temperature of the adipose tissue replica is between 23°C to 33°C, 34°C to 44°C, 45°C to 55°C, 56°C to 66°C, 67°C to 77°C, 78°C to 88°C, 89°C to 99°C, 100°C to 110°C, 111°C to 121°C, 122°C to 132°C, 133°C to 143°C, 144°C to 154°C, 155°C to 165°C, 166°C to 167°C, 168°C to 169°C, 170°C to 180°C, 181°C to 191°C, 192°C to 202°C, 203°C to 213°C, 214°C to 224°C, 225°C to 235°C, 236°C to 246°C, 247°C to 257°C, 258°C to 268°C, 269°C to 279°C, 280°C to 290°C, or 291°C to 301°C. In some embodiments, the percent fat release of the adipose tissue replica is 0 to 10%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, 50% to 60%, 60% to 70%, 70% to 80%, 80% to 90%, or 90% to 100% upon cooking. In some embodiments, the isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin. In some embodiments, the emulsion comprises from about 40% to about 90% of the oil. In some embodiments, the emulsion comprises from about 1% to about 4% of the isolated and purified plant protein. In some embodiments, the adipose tissue replica comprises from about 0.05 to about 1% of the phospholipid. In some embodiments, the emulsion is formed by high-pressure homogenization, sonication, or hand homogenization.

Further provided is a method of minimizing undesirable odors or flavors in a composition comprising plant proteins, the method comprising contacting the composition with a ligand having affinity for one or more lipoxygenases.

Also provided is a method of minimizing undesirable odors or flavors in a composition comprising plant proteins, the method comprising contacting the

composition with activated carbon then removing the activated carbon from the composition.

Also provided is a method of minimizing undesirable odors or flavors in a composition comprising plant proteins, the method comprising adding a lipoxygenase inhibitor and/or an antioxidant to the composition.

The disclosure further provides a chocolate flavored spread comprising:

a) sugar

b) a chocolate flavoring, and

c) a cream fraction from a plant based milk.

Provided herein is a method for altering the texture of a consumable during or after cooking comprising incorporating within the consumable one or more plant proteins with a low denaturation temperature. In some embodiments, at least one of the one or more plant proteins is isolated and purified. In some embodiments, the one or more plant proteins are selected from the group consisting of rubisco, pea proteins, lentil proteins, or other legume proteins. In some embodiments, the pea proteins comprise pea albumin proteins. In some embodiments, the consumable becomes firmer during or after cooking.

Also provided is a tissue replica, which comprises a freeze-aligned non-animal protein. In some embodiments, the non-animal protein is a plant protein. In some embodiments the non-animal protein is isolated and purified. In some embodiments the tissue replica is amuscle tissue replica.

The disclosure also provides a meat replica which includes a tissue replica comprising a freeze-aligned non-animal protein.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims. The word "comprising" in the claims may be replaced by "consisting essentially of or with "consisting of," according to standard practice in patent law.

DESCRIPTION OF THE DRAWING

FIG. 1 contains amino acid sequences of exemplary heme-containing proteins.

FIG. 2A is a bar graph depicting the percent fat release based on the amount of lecithin.

FIG. 2B is a bar graph depicting the temperature of the fat release based on the amount of lecithin.

FIG. 2C is a bar graph depicting the firmness of adipose replicas based on the amount of lecithin.

FIG. 3 is a bar graph depicting the percent fat release of adipose replicas containing different oils (canola oil, cocoa butter, coconut oil, or rice bran oil).

FIG. 4 is a bar graph depicting the fat release temperature of adipose replicas containing different oils (canola oil, cocoa butter, coconut oil, or rice bran oil).

DETAILED DESCRIPTION

I. Consumables

Methods and compositions for producing consumables are described herein. In some cases, the consumables are non-animal based replicas of animal-based food products that can be produced by breaking down non-animal materials into their constituent parts and reassembling those parts into the consumables. In certain instances, the consumables are not intended to replicate an animal-based food and instead have their own unique characteristics desirable as a food. Additionally, the consumables may, in some instances, act as nutraceuticals or carriers for pharmaceutical compositions rather than serving a primary function as food.

The advantages of the consumables described herein can include, for example, the using less energy or water in the production of the consumable compared to similar food products, using no animals in the production of the consumable, making a healthier product, using raw materials that would otherwise be discarded, or allowing for the elimination (or lack of incorporation) of certain components (e.g., allergens) from the consumables. The consumables also may have a higher degree of production consistency, allowing for improved quality control of the products. Another advantage is that the consumables can be designed intentionally to have desirable characteristics for food preparation that are superior to traditional food products.

[0080] In some embodiments, said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyano-globin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglo-bins, ribosomal proteins, actin, hexokinase, lactate dehydro-genase, fructose bisphosphate aldolase, phosphofructoki-nases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde 3-phosphate dehydrogenases, pyru-vate decarboxylases, actin, translation elongation factors, ribulose 1,5 bisphosphate carboxylase oxygenase (rubisco), ribulose 1,5 bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

[0081] In one embodiment, said seed storage protein is moong bean 8S protein.

[0082] In some embodiments, said protein content is suspended in a gel.

[0083] In some embodiments, said protein content is in the form of a gel.

[0084] In one embodiment, said gel comprises an isolated, purified cross-linking enzyme.

[0085] In some embodiments, said isolated, purified cross-linking enzyme is selected from the group consisting of trans-glutaminase, lysyl oxidases, and amine oxidases.

[0086] In a particular embodiment, said isolated, purified cross-linking enzyme is transglutaminase.

[0087] In some embodiments, said protein content has been assembled into fibers.

[0088] In particular embodiments, said fibers are arranged isotropically.

[0089] In one embodiment, said fibers are asymmetric fibers.

[0090] In some embodiments, the muscle tissue replica further comprises one or more isolated and purified iron-containing proteins.

[0091] In some embodiments, said one or more isolated and purified iron containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuro-globin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohe-moglobins.

[0092] In a particular embodiment, said one or more isolated and purified iron containing proteins comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKA-NIPQYSVVFYTSILEKAPAAKDLF S-FLANGVDPTNPKLTGHAE KLFALVRDSAGQLKAS GTVVADAALGSVHAQKAVTDPQFVVVKEALLKTIKA AVGDKWSDE LSRAWEVAYDELAAAIKKA. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 80% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron containing proteins comprises an amino acid sequence with at least 90% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron containing proteins comprises an amino acid sequence with at least 95% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron containing proteins comprises an amino acid sequence with at least 98% homology to SEQ ID NO 1.

[0093] In particular embodiments, the muscle tissue replica comprises a protein content, wherein (i) one isolated and purified protein that is not an iron containing protein accounts for 40-95% of said protein content, (ii) one or more isolated and purified iron containing proteins accounts for 1-20% of said protein content, and (iii) one or more cross linking reagents accounts for 0.1-35% of said protein content. **[0094]** In one embodiment, said protein content accounts for 5-50% of said replica by weight or by weight/volume. **[0095]** In one embodiment, said one isolated and purified protein is moong bean 8S protein.

[0096] In one embodiment, said one or more isolated and purified iron-containing proteins is leghemoglobin.

[0097] In one embodiment, said one or more cross-linking reagents is transglutaminase.

[0098] In some embodiments, the muscle tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum. **[0099]** In particular embodiments, the muscle tissue replica additionally contains less than 1% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 5% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 10% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 0.1% wheat gluten. **[0100]** In a more particular embodiment, said muscle tissue replica contains no wheat gluten.

[0101] In other particular embodiments, said muscle tissue replica contains no soy protein isolate.

[0102] In other particular embodiments, said muscle tissue replica contains no soy protein concentrate.

[0103] In other particular embodiments, said muscle tissue replica contains no soy protein.

[0104] In a more particular embodiment, said muscle tissue replica contains less than 5% carbohydrates.

[0105] In other particular embodiments, said muscle tissue replica contains no tofu.

[0106] In some embodiments, said muscle tissue replica contains no tofu, and no wheat gluten.

[0107] In some embodiments, said muscle tissue replica contains no soy protein, and no wheat gluten.

[0108] In some embodiments, said muscle tissue replica contains no animal products and less than 5% carbohydrates. **[0109]** In some embodiments, said muscle tissue replica contains less than 1% cellulose.

[0110] In some embodiments, said muscle tissue replica contains less than 5% insoluble carbohydrates.

[0111] In some embodiments, said muscle tissue replica contains no soy protein, and less than 1% cellulose.

[0112] In some embodiments, said muscle tissue replica contains no soy protein, and less than 5% insoluble carbohydrates.

[0113] In some embodiments, said muscle tissue replica contains no wheat gluten, and less than 1% cellulose. **[0114]** In some embodiments, said muscle tissue replica contains no wheat gluten, and less than 5% insoluble carbohydrates.

[0115] In some embodiments, the muscle tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum. **[0116]** In particular embodiments, the meat replica contains less than 1% wheat gluten. In particular embodiments, the meat replica contains less than 5% wheat gluten. In particular embodiments, the meat replica contains less than 10% wheat gluten. In particular embodiments, the meat replica contains less than 0.1% wheat gluten.

[0117] In a more particular embodiment, the meat replica contains no wheat gluten.

[0118] In other particular embodiments, the meat replica contains no soy protein isolate.

[0119] In other particular embodiments, the meat replica contains no soy protein concentrate.

[0120] In other particular embodiments, the meat replica contains no soy protein.

[0121] In a more particular embodiment, the meat replica contains less than 5% carbohydrates.

[0122] In other particular embodiments, the meat replica contains no tofu.

[0123] In some embodiments, the meat replica contains no tofu, and no wheat gluten.

[0124] In some embodiments, the meat replica contains no soy protein, and no wheat gluten.

[0125] In some embodiments, the meat replica contains no animal products and less than 5% carbohydrates. **[0126]** In some embodiments, the meat replica contains less than 1% cellulose. In some embodiments, the meat replica contains less than 0.1% cellulose. In some embodiments, the meat replica contains less than 10% cellulose. In some embodiments, the meat replica contains less than 5% cellulose.

[0127] In some embodiments, the meat replica contains less than 5% insoluble carbohydrates.

[0128] In some embodiments, the meat replica contains no soy protein, and less than 1% cellulose.

[0129] In some embodiments, the meat replica contains no soy protein, and less than 5% insoluble carbohydrates.

[0130] In some embodiments, the meat replica contains no wheat gluten, and less than 1% cellulose.

[0131] In some embodiments, the meat replica contains no wheat gluten, and less than 5% insoluble carbohydrates.

[0132] In another aspect, the invention provides a fat tissue replica comprising a gelled emulsion, said gelled emulsion comprising a protein solution with fat droplets suspended therein.

[0133] In some embodiments, said fat droplets are derived from a non-animal source.

[0134] In some embodiments, said fat droplets are comprised of one or more plant oils.

[0135] In some embodiments, said one or more plant oils is selected from the group consisting of corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, Shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, -triglycerides, monoglycerides, diglycer-ides, sphingosides, glycolipids, lecithin, lysolecithin, phos-phatidic acids, lysophosphatidic acids, oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachi-donic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lau-ric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachi-donic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyc-eride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosa-pentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid.

[0136] In one embodiment, said one or more plant oils is rice bran oil or canola oil.

[0137] In some embodiments, said protein solution comprises one or more isolated, purified proteins.

[0138] In some embodiments, said one or more isolated, purified proteins accounts for 75% or more of the protein in said protein solution.

[0139] In some embodiments, said one or more isolated, purified proteins are derived from a non-animal source.

[0140] In some embodiments, said non-animal source is a plant source.

[0141] In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

[0142] In some embodiments, each of said one or more isolated proteins is isolated and purified separately.

~~**[0143]** In some embodiments, said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyano globin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglo bins, ribosomal proteins, actin, hexokinase, lactate dehydro genase, fructose bisphosphate aldolase, phosphofructoki~

nases, trio se phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde 3 phosphate dehydrogenases, pyru vate decarboxylases, actin, translation elongation factors, ribulose 1,5 bisphosphate carboxylase oxygenase (rubisco), ribulose 1,5 bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

[0144] In some embodiments, said one or more isolated, purified proteins is an albumin protein, a seed storage protein, or pea globulin protein.

[0145] In particular embodiments, said albumin protein is isolated pea albumin protein.

[0146] In some embodiments, said seed storage protein is moong bean 8S protein.

[0147] In some embodiments, said gelled emulsion comprises a protein solution comprising 1-3 isolated and purified proteins, wherein said solution accounts for 30-70% of the volume of said emulsion; a plant oil, wherein said plant oil accounts for 30-70% of the volume of said emulsion; and an isolated, purified cross-linking enzyme, wherein said cross-linking enzyme accounts for 0.5-5% of said emulsion by wt/volume; wherein said plant oil is emulsified in said protein solution, wherein said emulsion is formed into a gel by said cross-linking enzyme.

[0148] In other embodiments said gelled emulsion comprises a protein solution comprising 1-3 isolated and purified proteins, wherein said solution accounts for 1-30% of the volume of said emulsion; a plant oil, wherein said plant oil accounts for 70-99% of the volume of said emulsion; and an isolated, purified cross-linking enzyme, wherein said cross-linking enzyme accounts for 0.5-5% of said emulsion by wt/volume; wherein said plant oil is emulsified in said protein solution, wherein said emulsion is formed into a gel by said cross-linking enzyme.

[0149] In some embodiments, the fat replica further comprises a cross-linking enzyme.

[0150] In some embodiments, said cross-linking enzyme is transglutaminase.

[0151] In some embodiments, one of said 1-3 isolated and purified proteins is moong bean 8S protein, pea albumin protein, or pea globulin protein.

[0152] In particular embodiments, said plant oil is rice bran oil or canola oil.

[0153] In some embodiments, the fat tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[0154] In particular embodiments, the fat tissue replica additionally contains less than 1% wheat gluten.

[0155] In a more particular embodiment, said fat tissue replica contains no wheat gluten.

[0156] In other particular embodiments, said fat tissue replica contains no soy protein isolate.

[0157] In other particular embodiments, said fat tissue replica contains no soy protein concentrate.

[0158] In other particular embodiments, said fat tissue replica contains no soy protein.

[0159] In a more particular embodiment, said fat tissue replica contains less than 5% carbohydrates.

[0160] In other particular embodiments, said fat tissue replica contains no tofu.

[0161] In some embodiments, said fat tissue replica contains no tofu, and no wheat gluten.

[0162] In some embodiments, said fat tissue replica contains no soy protein, and no wheat gluten.

[0163] In some embodiments, said fat tissue replica contains no animal products and less than 5% carbohydrates.

[0164] In some embodiments, said fat tissue replica contains less than 1% cellulose.

[0165] In some embodiments, said fat tissue replica contains less than 5% insoluble carbohydrates.

[0166] In some embodiments, said fat tissue replica contains no soy protein, and less than 1% cellulose.

[0167] In some embodiments, said fat tissue replica contains no soy protein, and less than 5% insoluble carbohydrates.

[0168] In some embodiments, said fat tissue replica contains no wheat gluten, and less than 1% cellulose.

[0169] In some embodiments, said fat tissue replica contains no wheat gluten, and less than 5% insoluble carbohydrates.

[0170] In another aspect, the invention provides a connective tissue replica, comprising a protein content comprising one or more isolated, purified proteins, wherein said protein content has been assembled into structures approximating the texture and visual appearance of connective tissue or skin. **[0171]** In some embodiments, said protein content is derived from non-animal source.

[0172] In some embodiments, said non-animal source is a plant source.

[0173] In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

[0174] In some embodiments, said one or more isolated proteins account for 50% or more of said protein content by weight.

[0175] In some embodiments, said one isolated and purified protein accounts for 90% or more of said protein content by weight.

[0176] In some embodiments, each of said one or more isolated proteins is isolated and purified separately.

[0177] In some embodiments, said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyano-globin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglo-bins, ribosomal proteins, actin, hexokinase, lactate dehydro-genase, fructose bisphosphate aldolase, phosphofructoki-nases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyru-vate decarboxylases, actin, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

[0178] In some embodiments, said one or more isolated and purified proteins is a prolamin family protein.
[0179] In some embodiments, said one or more isolated and purified proteins is zein.
[0180] In some embodiments, said protein content is suspended in a gel.
[0181] In some embodiments, said gel comprises an isolated, purified cross-linking enzyme.
[0182] In some embodiments, said isolated, purified cross-linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.
[0183] In some embodiments, said isolated, purified cross-linking enzyme is transglutaminase.
[0184] In some embodiments, said protein content is formed into a fiber.
[0185] In some embodiments, said fiber is produced by an extrusion process.
[0186] In some embodiments, said fiber is stabilized by protein cro-s-slinks-.
[0187] In some embodiments, fiber contains an isolated, purified cross-linking enzyme.
[0188] In some embodiments, said isolated, purified cross-linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.
[0189] In some embodiments, said isolated, purified cross-linking enzyme is transglutaminase.
[0190] In another aspect, the invention provides a meat substitute product, comprising a muscle replica; a fat tissue replica; and a connective tissue replica; wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat.
[0191] In some embodiments, the meat substitute product comprises two or more of said muscle replica, fat tissue replica, and connective tissue replica.
[0192] In some embodiments of the meat substitute product, said muscle replica accounts for 40-90% of said product by weight, said fat tissue replica accounts for 1-60% of said product by weight, and said connective tissue replica accounts for 1-30% of said product by weight.
[0193] In some embodiments, the meat substitute product comprises 60-90% water; 5-30% protein content; and 1-20% of a fat or fat replica; wherein said protein content comprises one or more isolated, purified plant proteins.
[0194] In some embodiments, said protein content is derived from non-animal source.
[0195] In some embodiments, non-animal source is a plant source.
[0196] In some embodiments, said non-animal source is a genetically modified yeast or bacteria.
[0197] In some embodiments, 50% or more of said protein content by weight are one or more isolated purified proteins. **[0198]** In some embodiments, each of said one or more isolated proteins is isolated and purified separately from different plant species.
[0199] In some embodiments, one or more of said isolated proteins is selected from the group consisting of: hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyano-globin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglo-bins, ribosomal proteins, actin, hexokinase, lactate dehydro-genase, fructose bisphosphate aldolase, phosphofructoki-nases, trio se phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyru-
vate decarboxylases, actin, translation elongation factors, ribulose-1,5 bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5 bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.
[0200] In some embodiments, the meat substitute product further comprises one or more isolated and purified iron-containing proteins.
[0201] In some embodiments, said one or more isolated and purified iiron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuro-globin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins. In some embodiments, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO: 1. SEQ ID NO: 1: MVAFTEKQDALVSSSFEAFKA-NIPQYSVVFYTSILEKAPAAKDLF S-
FLANGVDPTNPKLTGHAE KLFALVRDSAGQLKAS GTVVADAALGSVHAQKAVTDPQFVVVKEALLKTIKA AVGDKWSDE LSRAWEVAYDELAAAIKKA
[0202] In some embodiments, the meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum. **[0203]** In particular embodiments, the meat substitute product additionally contains less than 1% wheat gluten. **[0204]** In a more particular embodiment, said meat substitute product contains no wheat gluten.
[0205] In other particular embodiments, said meat substitute product contains no soy protein isolate.
[0206] In other particular embodiments, said meat substitute product contains no soy protein concentrate.
[0207] In other particular embodiments, said meat substitute product contains no soy protein.
[0208] In a more particular embodiment, said meat substitute product contains less than 5% carbohydrates.
[0209] In other particular embodiments, said meat substitute product contains no tofu.
[0210] In some embodiments, said meat substitute product contains no tofu, and no wheat gluten.
[0211] In some embodiments, said meat substitute product contains no soy protein, and no wheat gluten.
[0212] In some embodiments, said meat substitute product contains no animal products and less than 5% carbohydrates. **[0213]** In some embodiments, said meat substitute product contains less than 1% cellulose.

~~**[0214]** In some embodiments, said meat substitute product contains less than 5% insoluble carbohydrates.~~
~~**[0215]** In some embodiments, said meat substitute product contains no soy protein, and less than 1% cellulose.~~
~~**[0216]** In some embodiments, said meat substitute product contains no soy protein, and less than 5% insoluble carbohydrates.~~
~~**[0217]** In some embodiments, said meat substitute product contains no wheat gluten, and less than 1% cellulose.~~
~~**[0218]** In some embodiments, said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.~~

[0219] In another aspect, the invention provides a food product comprising one or more isolated, purified iron containing proteins, wherein said food product is configured for consumption by an animal.

[0220] In some embodiments, said one or more isolated, purified iron containing proteins is derived from a non animal source.

[0221] In some embodiments, said non animal source is a plant source.

[0222] In some embodiments, said plant source comprises one or more plants of the legume family.

[0223] In some embodiments, said one or more plants of the legume family is a soy or pea plant.

[0224] In some embodiments, said non animal source is a genetically modified yeast or bacteria.

[0225] In some embodiments, said iron containing protein is selected from the group consisting of hemoglobin, myo globin, leghemoglobin, non symbiotic hemoglobin, chloro eruorin, erythroeruorin, neuroglobin, cytoglobin, protoglo bin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

[0226] In one embodiment, said iron containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSV-VFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAE KLFALVRDSAGQLKASGTVVADAALGS-VHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDE LSRAWEVAYDELAAAIKKA

[0227] In some embodiments, the food product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[0228] In particular embodiments, the food product additionally contains less than 1% wheat gluten.

[0229] In a more particular embodiment, said food product contains no wheat gluten.

[0230] In other particular embodiments, said food product contains no soy protein isolate.

[0231] In other particular embodiments, said food product contains no soy protein concentrate.

[0232] In other particular embodiments, said food product contains no soy protein.

[0233] In a more particular embodiment, said food product contains less than 5% carbohydrates.

[0234] In other particular embodiments, said food product contains no tofu.

[0235] In some embodiments, said food product contains no tofu, and no wheat gluten.

[0236] In some embodiments, said food product contains no soy protein, and no wheat gluten.

[0237] In some embodiments, said food product contains no animal products and less than 5% carbohydrates. **[0238]** In some embodiments, said food product contains less than 1% cellulose.

[0239] In some embodiments, said food product contains less than 5% insoluble carbohydrates.

[0240] In some embodiments, said food product contains no soy protein, and less than 1% cellulose.

[0241] In some embodiments, said food product contains no soy protein, and less than 5% insoluble carbohydrates. **[0242]** In some embodiments, said food product contains no wheat gluten, and less than 1% cellulose.

[0243] In some embodiments, said food product contains no wheat gluten, and less than 5% insoluble carbohydrates. **[0244]** In another aspect, the invention provides a method of making a meat substitute composition, comprising isolating and purifying one or more proteins; and assembling said one or more proteins into a physical organization that approximates the physical organization of meat.

[0245] In another aspect, the invention provides a method of making a muscle tissue replica, comprising isolating and purifying one or more proteins; and assembling said one or more proteins into a physical organization that approximates the physical organization of skeletal muscle.

[0246] In another aspect, the invention provides a method of making a fat tissue replica, comprising isolating and purifying one or more proteins; preparing a solution comprising one or more proteins; emulsifying one or more fats in said solution; and stabilizing said solution into a gelled emulsification with one or more cross linking reagents.

[0247] In another aspect, the invention provides a method of making a connective tissue replica, comprising isolating and purifying one or more proteins; and precipitating said one or more proteins, wherein said precipitating results in said one or more proteins forming physical structures approximating the physical organization of connective tissue.

[0248] In some embodiments, said precipitating comprises solubilizing said one or more proteins in a first solution; and extruding said first solution into a second solution, wherein said one or more proteins is insoluble in said second solution, wherein said extruding induces precipitation of said one or more proteins.

[0249] In another aspect, the invention provides a food product comprising one or more isolated, purified iron containing proteins, wherein said food product is configured for consumption by an animal.

[0250] In another aspect, the invention provides a food product comprising one or more isolated, purified iron containing proteins, wherein said food product is configured for consumption by humans.

[0251] In another aspect, the invention provides a food product comprising one or more isolated, purified iron containing proteins, wherein said food product is configured for consumption by an animal. In another aspect, the invention provides a food product comprising one or more isolated, purified iron containing proteins, wherein said food product is configured for consumption by humans.

[0252] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0253] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0254] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0255] FIG. **1** depicts a portion of the root of a pea plant *(Pisum sativum)* with the root nodules sliced open to demonstrate the red color conferred by leghemoglobin contained therein. The sliced open rood nodule appears red.

[0256] FIG. **2** depicts leghemoglobin isolated from 1 oz of pea roots. The red color commonly attributed to meat is evident in the color photo.

[0257] FIG. **3** shows that leghemoglobins from different species are homologs and have similar color properties. In FIG. **3,** panel A shows an SDS_PAGE gels of lysed root nodules of three legume plant species (1) Fava bean (2) English Pea (3) Soybean. Arrows mark respective leghemo-globins. Panel B shows the similarity of UV-VIS spectral profile of leghemoglobins from two different plant species (Favabean and Soybean).

[0258] FIG. **4** shows a comparison of reduced (heme iron 2+) and oxidized (heme iron 3+) soybean leghemoglobin (FIG. **4** panel A) and equine heart muscle myoglobin (FIG. **4** panel B) showing similarity of UV-VIS absorption profiles of two proteins. We purified soybean leghemoglobin from soybean root nodules using here described protocol. Purified equine myoglobin was purchased from SigmaAldrich. Soybean leghemoglobin (FIG. **4** panel A) and equine myoglobin (FIG. **4** panel B) were reduced with 1 mm sodium hydro sulfite. Shown are UV-VIS absorption spectra of heme Fe3+

(blue linethe higher peak in FIGS. **4** and **5)** and heme Fe2+ (red line) of soybean leghemoglobin (FIG. **4** panel A) and equine myoglobin (FIG. **4** panel B). Insets show a zoom in of UV-VIS spectra in 450 nm to 700 nm region. (FIG. **4** panel C) Images of 10 ul liquid droplet of a 40 mg/ml solution of soybean leghemoglobin in the heme Fe3+ state (left droplet) showing characteristic rusty red color and a 40 mg/ml solution of soybean leghemoglobin solution in the heme Fe2+ state (right droplet) showing characteristic red color of and (right image) corresponding samples of equine myoglobin. **[0259]** FIG. **5** depicts examples of successful reduction of leghemoglobin heme iron with sodium hydrosulfite and titanium citrate. In FIG. **5** the UV-VIS spectrogram of purified soybean leghemoglobin in which the heme iron is in the oxidized (+3) state is represented by the blue curves in each panel (the blue curves have the higher peaks on the main graphs). The red curves in each panel represent the UV-VIS spectra of the same leghemoglobin species after reduction to the (+2) state (red lines) by addition of (Panel A) 1 mM sodium hydrosulfite or (Panel B) 0.24% (wt/v) titanium citrate in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride buffer. The Insets show a zoom in of UV-VIS spectra in 450-700 nm region. For this example, leghemoglobin was purified from soybean root nodules using 60/90% ammonium sulfate fractionation and exchanged into 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride buffer. Sodium hydrosulfite stock solution was prepared by dissolving 100 mM sodium hydro sulfite in 1 mM sodium hydroxide in water. Titanium citrate stock solution was prepared from 20% (wt/v) Ti-chloride in hydrochloric acid by mixing it with 0.2M sodium citrate (1:10 v/v). pH was adjusted using sodium carbonate to pH 7.0.

[0260] FIG. **6** depicts an example of the leghemoglobin purification flow from soybean root nodules. The figure shows SDS-PAGE fractionation of different soybean leghe-moglobin purification steps (Lane 1) Soybean root nodule lysate; (Lane 2) Soybean root nodule lysate purified by 60/90% (wt/v) ammonium sulfate fractionation. Shown is the protein content of 90% ammonium sulfate fractionated pro-

tein pellet resuspended in 20 mm potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mM EDTA buffer; Proteins from 90% ammonium sulfate pellet were further purified by anion exchange chromatography (FFQ GE Healthcare) in 20 mM potassium phosphate ph 7.4, 100 mM sodium chloride. Leghemoglobin collected in the flowthrough is shown in Lane 3. Anion-exchange flowthrough was fractionated using size exclusion chromatography (Sephacryl S-100 GE Healthcare) and resulting leghemoglobin fraction is shown in Lane 4. Leghemoglobin content at different purification steps was determined by determining the fraction of leghemoglo-bin band on SDS-PAGE gel in a respective sample using ImageDoc analysis software (BioRad). Purity (partial abundance) of leghemoglobin at respective steps in the purification steps was: lysate: 32.7% (lane 1), 60/90% (wt/v) ammonium sulfate fractionation 78% (lane 2), anion exchange chromatography ~ 83% (lane 3), and size-exclusion chromatography to ~ 95% (lane 4).

[0261] FIG. 7 shows stained SDS-PAGE gel analysis of (A) soybean leghemoglobin expressed and purified using recom-binant protein technology and (B) soybean leghemoglobin purified from soybean root nodules. (A) Recombinant Soybean leghemoglobin A carrying His-tag and TEV protease His-tag removal site was expressed in *E. coli* BL21 strain and purified using His-tag affinity chromatography (Talon resin, CloneTech). The left lane contains molecular weight standards, the right lane contains purified recombinant soybean leghemoglobin A (arrow). Expected molecular weight of the recombinant soybean leghemoglobin A is 17.1 kDa. (B) SDS-PAGE gel of purified Soybean leghemoglobin from root nodules. The left lane contains molecular weight standards, the right lane contains purified soybean leghemoglobin A (arrow). Mass spectrometry analysis of purified material determined that all four soybean leghemoglobin isoforms are present, and are full length (data not shown). Expected molecular weights (MW) of soybean leghemoglobin iso-forms range from MW15.4 to 15.8 kDa.

[0262] FIG. 8 shows an example of 6 cubes of a commercial meat analog (Quorn chicken analog), about 1 cm on a side, 4 of which (Left and lower right) have been soaked in a solution of about 10 mg/ml soybean leghemoglobin in 20 mM Potassium phosphate pH 7.4 and 100 mM NaCl; the remaining two (Upper right) were soaked in the same buffer without leghe-moglobin.

~~A deep pink color of the leghemoglobin-infused cubes is apparent in color photos contrasting the pale tan color of the un-infused cubes.~~

~~[0263] FIG. 9 shows the 4 leghemoglobin-infused cubes of Quorn chicken analog in the process of cooking in a pan at 350° C. The two lower cubes have been turned over to expose the grilled surface, which has turned brown. In the upper two cubes that the heated portion has turned grey-brown, while the cooler top surface retains its pink color. In some embodiments the consumable is injected with a heme-containing solution, for instance a leghemoglobin solution, until the consumable is the color of uncooked meat.~~

~~[0264] FIG. 10 depicts 43 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 37 ml of leghemoglobin solution (46.5 mg/ml leghemoglobin and 20 mg/ml of other soybean root nodule protein) in 20 mM potassium phosphate, 100 mM NaC1, pH 7.3). 20 ml of trans-glutaminase solution (20% w/w) were added, solutions thoroughly mixed, divided into two 50 ml Falcon tubes and incubated overnight at room temperature. Final protein con~~

centrations were 65 mg/ml for moong bean protein, 18 mg/ml of leghemoglobin, 91 mg/ml total protein.

[0265] FIG. 11 depicts "White" muscle analog prepared by mixing 43 ml moong bean protein solution (150 mg/ml) with 45 ml of 11.7 mg/ml solution of leghemoglobin and 0.8% (wt/v) of transglutaminase solution. Final protein concentrations were 63 mg/ml for moong bean protein, 5.2 mg/ml of leghemoglobin, 68 mg/ml total protein.

[0266] FIG. 12 depicts a fat tissue analog based on moong beans and prepared in eppendorf tubes formed an opaque gel of off-white color, smooth uniform texture, with no visible discernible liquid that was not incorporated into the gel. The gel was freely standing, elastic and springy. The gel has a slight, pleasant aroma and a mild and pleasant flavor. The taste is mildly salty.

[0267] FIG. 13 depicts at tissue analog based on pea globulin and prepared in eppendorf tubes very similar to moong bean-based fat analog, except that it gave up a little of oil upon compression.

[0268] FIG. 14 shows connective tissue analog strands that were created using a 1:3 ratio in 70% ethanol, loaded into a syringe with a 23 gauge needle (ID 0.337 mm). The solution was slowly extruded from the bottom of a 5 inch high vessel into an excess of 5 M NaC1 solution. The ethanol zein solution being less dense than the NaC1 solution, floated upward, drawing out a fibrous stand of solidifying zein. The NaC1 was constantly stirred as the strands began to develop to assist in the strand lengthening. The strands bunch together and become a hard, dense mass.

[0269] FIG. 15 depicts a ground beef prototype patty was made by combining 62% (wt/wt) muscle analog (62% (wt/ wt) "dark muscle analog" and 38% (wt/wt) "white muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (FIG. 15 panel A). A ground beef prototype patty was made by combining 62% muscle analog (62% "dark muscle analog" and 38% "white muscle analog), 29% fat tissue analog (from moong bean seed 8S protein and rice bran oil), 5% connective tissue analog (FIG. 15 panel B). A ground beef prototype patty was made by combining 71% (wt/wt) muscle tissue analog (composed of 60% "white" muscle analog, 40% "dark" muscle analog), 23% fat tissue (from pea seed globulin proteins and canola oil) (FIG. 15 panel C). A ground beef prototype patty was made by combining 67% "White" muscle analog, with 28% fat tissue analog (from pea globulins and rice bran oil), (FIG. 15, panel D)

[0270] FIG. 16 depicts a ground beef patty analog was made by combining 62% (wt/wt) muscle tissue analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog. The panel on the left shows the patty before cooking and the panel on the right shows the same patty after cooking for about 2 minutes. Observers described the aroma of the cooking ground beef replica as distinctly "beefy".

DETAILED DESCRIPTION OF THE INVENTION

[0271] Methods and compositions for the production of consumables are described herein. The consumables can be for animal consumption. For example the consumable can be food fit for human consumption. The consumable can be approved by suitable regulatory authorities. The consumables can be sold in grocery stores or prepared in restaurants, schools, hospitals, military facilities, prisons, shelters, long-term care facilities, similar to already existing human foods. The consumables could also The consumables can be for animal consumption, including human consumption. The consumables can be food for domestic animals. For instance (e.g., dog food could be produced according to the present inventions. The consumables may also be food for) or wild animals. For instance, the consumables could be provided to (e.g., food for non-domesticated predatory animals.).

The consumables can be sold in grocery stores, convenience stores, mass merchandisers, and club stores or prepared in restaurants, including fast food restaurants, schools, event locations, hospitals, military facilities, prisons, shelters, or long-term care facilities, similar to already existing human foods.

The consumable can be approved by suitable regulatory authorities. For example, the consumable could be prepared to be suitable for the U.S. Food and Drug Administration. Methods of the invention can include steps necessary to satisfy regulatory agencies.

The consumables of the present invention can replicate, compete with,

supplement or replace conventional food products (herein referred to as "food products"). Food products can be any foods which presently exist. The consumables of the invention can be made to replicate the food products, e.g., an equivalent meat product. The equivalent meat product can be a white meat or a dark meat. The equivalent meat product can be derived from any animal. Non-limiting examples of animals used to derive the equivalent meat product include farmed animals such as, e.g., cattle, sheep, pig, chicken, turkey, goose, duck, horse, dog or game animals (whether wild or farmed) such as, e.g., rabbit, deer, bison, buffalo, boar, snake, pheasant, quail, bear, elk, antelope,

pigeon, dove, grouse, fox, wild pig, goat, kangaroo, emu, alligator, crocodile, turtle, groundhog, marmot, possum, partridge, squirrel, raccoon, whale, seal, ostrich, capybara, nutria, guinea pig, rat, mice, vole, any variety of insect or other arthropod, or seafood such as, e.g., fish, crab, lobster, oyster, muscle, scallop, abalone, squid, octopus, sea urchin, tunicate and others.

Many meat products are typically derived from skeletal muscle of an animal but it is understood that meat can also come from other muscles or organs of the animal. In some embodiments, the equivalent meat product is a cut of meat derived from skeletal muscle. In other embodiments, the equivalent meat product is an organ such as, e.g., kidney, heart, liver, gallbladder, intestine, stomach, bone marrow, brain, thymus, lung, or tongue. Accordingly, in some embodiments, the compositions of the present invention are consumables similar to skeletal muscle or organs.

A consumable (e.g., a meat substitute) can comprise one or more of a first composition comprising a muscle tissue replica, a second composition comprising an adipose tissue replica, and/or a third composition comprising a connective tissue replica, wherein the one or more compositions are combined in a manner that recapitulates the physical organization of meat. The present invention also provides distinct compositions for a muscle tissue replica (herein referred to as "muscle replica"), an adipose tissue replica (herein referred to as an "adipose replica" or "fat replica"), and a connective tissue replica (herein referred to as "connective tissue replica"). In some embodiments, these compositions are principally or entirely composed of ingredients derived from non-animal sources (e.g., 10% or less of the ingredients are from animal sources). In alternative embodiments, the muscle, fat, and/or connective tissue replica, or the meat substitute products comprising one or more of the replicas, are partially derived from animal sources but supplemented with ingredients derived from non-animal sources. In some embodiments, as much as 90% of the food product is derived from animal sources. In some embodiments about 75% of the food product is derived from animal sources. In some embodiments, about 50% of the food product is derived from animal sources. In some embodiments about 10% of the food product is derived from animal sources. In yet other alternative embodiments, the invention provides meat products substantially

derived from animal sources (e.g., a beef, chicken, turkey, or a pork product) that are supplemented with one or more of a muscle tissue replica, a fat replica, and/or a connective tissue replica, wherein the replicas are derived substantially or entirely from non-animal sources. A non-limiting example of such a meat product is an ultra-lean ground beef product supplemented with a non-animal derived fat replica which improves texture and mouthfeel while preserving the health benefits of a consumable low in animal fat. Such alternative embodiments can result in products with properties that more closely recapitulate key features associated with preparing and consuming meat but which are less costly and associated with a lesser environmental impact, less animal welfare impact, or improved health benefits for the consumer.

Examples of other food products which the consumable can replicate or replace include: beverages (e.g., cream liquor or milk), protein drinks (e.g., RuBisCo can be used as a protein supplement in beer, distilled alcohol beverages such as vodka, fruit juices, meal replacement beverages, or water), pastes (e.g. Nutella™, cream, nacho cheese or mayonnaise replicas), pate, blood sausage, meat extenders, eggs, fish, sausage, tenders , Spam or chilled foods (e.g., ice cream , yogurt, kefir, sour cream or butter replicas).

~~[0272] The consumables of the present invention can compete with, supplement or replace animal based foods. For instance the consumables can be meat replicas made entirely from plant sources.~~ The consumables can be a meat replica. The consumables can be made to mimic the cut or appearance of meat ~~as it is currently sold~~. For instance, a consumable may be visually similar to or indistinguishable from ground beef or a particular cut of beef In an example embodiment, the replicas are combined in a manner that approximates the physical organization of natural ground meat (e.g., ground beef, ground chicken, or ground turkey). In other embodiments, the replicas are combined in a manner that approximates different cuts of beef, such as, e.g., rib-eye, filet mignon, London broil, among others. Alternatively, the consumables can be a made with a unique look or appearance. For instance, the consumable could contain patterns ~~or~~ (e.g., lettering or pictures) that ~~is based upon~~ are formed from the structure of the consumable. In some instances, the consumables look like traditional ~~meat~~ food products after they are prepared. For example, a consumable may be produced which is larger than a traditional cut of beef but which, after the consumable is sliced and cooked, appears the same as a

traditional cooked meet. In some embodiments the consumable may resemble a traditional ~~meat~~ food product shape in two dimensions, but not

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in a third. For example the consumable may resemble a cut of meat in two dimensions (for example when viewed from the top), but may be much longer (or ~~thinker~~thicker) than the traditional cut. ~~So in some embodiments a~~ In this example the composition ~~that~~ can be cut repeatedly into traditionally meat shaped products ~~is provided~~.

~~[0273] The consumable may be made entirely from plant based sources. In some instances the consumable can be made from organic sources. The consumables may also be made from a combination of plant based sources and animal based sources. For instance, the consumable may be a ground beef product supplemented with plant based products of the invention.~~

~~[0274]~~ The consumables can be made from local products. For instance the consumables can be made from plants grown within a certain radius of the eventual consumer. That radius could be 1, 10, 100, or 1000 miles for example. ~~So~~Thus, in some embodiments, the invention provides ~~a~~ method for producing a ~~meat replica~~ consumable which does not contain products which have been shipped over 1, 10, 100, or 1000 miles ~~prior to producing the meat replica~~.

~~[0275]~~ The present invention provides methods for producing consistent properties from the consumables when they are produced from various sources. ~~So, for~~ For example, a plant based meat replica produced from local plants in Iowa, USA, will have substantially similar taste, odor, and texture as a plant based meat replica produced from local plants in Lorraine, France. This consistency allows for methods for advertising locally grown foods with consistent properties. The consistency can arise from the concentration or purification of similar components at different locations. These components can be combined in predetermined ratios to insure consistency. In some embodiments, a high degree of characteristic consistency is possible using components (e.g. isolated or concentrated proteins and fats) which come from the same plant species. In some embodiments, a high degree of characteristic consistency is possible using components (e.g., isolated or concentrated proteins and fats) which come from the different plant species. In some embodiments, the same proteins can be isolated from different plant species (i.e. homologous proteins). In some embodiments, the invention provides for a method comprising isolating similar plant constituents from plant sources in different ~~locations~~locations, assembling in both locations compositions provided herein, and selling the compositions, wherein the compositions assembled and sold at different the geographic locations have consistent physical and chemical properties. In some embodiments, the isolated constituents are from different plant populations in different locations. In some

embodiments one or more of the isolated constituents are shipped to the separate geographic locations.

~~[0276]~~ The consumables may require fewer resources to produce than consumables produced from domesticated animals. Accordingly, the present invention provides for meat replicates which require less water or energy to produce than meat. For example a consumable described herein can require less than about 10, 50, 100, 200, 300, 500, or 1000 gallons of water per pound of consumable. For comparison producing beef can require over 2000 gallons of water per pound of meat.

~~[0277]~~ The consumable may require less land ~~are~~ area to produce than a meat product with similar protein content. For example ~~the~~, a consumable described herein may require 30% or less of the land area required to produce a meat product with similar protein content.

~~[0278]~~ The consumable may have health benefits compared to an animal product it replaces in the diet. For example it may have less cholesterol or lower levels of saturated fats than comparable meat products. The American Heart Association and the National Cholesterol Education Program recommends limiting cholesterol intake from food to 300 mg per day, which is equivalent to the consumption of 12 ounces of beef or two egg yolks. Consumables described herein that are indistinguishable to animal products such as ground beef, and having a reduced cholesterol content or no cholesterol, can help maintain a low cholesterol diet. In another example, a consumable described herein may contain no cholesterol, or higher levels of poly-unsaturated fatty acids compared to the animal product it replaces.

~~[0279]~~ The consumable may have animal welfare benefits compared to an animal product it replaces in the diet. For instance~~,~~, it may be produced without requiring confinement, forced feeding, premature weaning, disruption of maternal-offspring interactions, or slaughter of animals for their meat.~~[0280]~~

The consumable may have a smaller "carbon footprint" than the meat products they replace. For example the consumable may result in net greenhouse gas emissions of 1%, 5%, 10%, 25%, 50% or 75% of the greenhouse gas emissions attributable to the animal product it replaces. By way of example, according to the Environmental Working Group (2011) "meat eaters guide to Climate Change and Health," the production of beef causes the emission of 27 kg equivalents of carbon dioxide per kilogram of beef that is

consumed, and the production of lamb causes the emission of 39 kg equivalents of carbon dioxide per kilogram of beef that is consumed.

[0281] The consumable described herein may provide alternatives to animal products or combinations of animal products whose consumption is forbidden by religious beliefs. For example, the consumable may be a kosher replica pork chop.

[0282] The consumable can also be shipped in components and produced or assembled at a different location. When available, local components can be used for production of the consumable. These The local components can be supplemented with components which that are not locally available. This allows for methods of producing consumables, for instance meat replicates, using less energy in shipment than is required for meat. For example, local water can be used in combination with a kit which provides other components of the consumable. Using local water will reduce reduces shipping weight, thereby reducing cost and environmental impact.

[0283] The consumables described herein can be produced or assembled wholly or in part in areas where animal farming is not practical or is not allowed. The consumable can be produced or assembled within an urban environment. For example, a kit may be provided to a user to enable the user to produce the consumable. The user could use local water or use plants from a rooftop garden, for instance in Shanghai. In another example, the consumables could be produced aboard a space craft, space station, or lunar base. Accordingly, the present invention provides methods and systems for the production of meat replicas for use in space travel or for training for the same. For instance the present invention could be used in earth based training for space travel. The consumables could also be produced on an island or upon a manmade platform at sea where the keeping of livestock is difficult or prohibited.

II. Properties of the Consumable

[0284] The consumables are, in some embodiments, described herein are typically designed to replicate the experience of eating a food product, e.g., meat. The look, texture, and taste of the consumable can be such that it is similar to, or indistinguishable from meat., a food product, e.g., meat. The consumable can also be produced to have the desirable characteristics of

food products without incorporating other undesirable characteristics. For example, a consumable can be a replica steak that does not have gristle or other components not typically consumed in a predicate food product.

The invention ~~therefore~~ provides, in certain embodiments, methods for determining the suitability for a consumable to qualify as a replica of a food product, for example, by determining whether an animal or human can distinguish the consumable from ~~meat.~~ ~~[0285] One method to determine whether the consumable is comparable to meat~~ a predicate food product, e.g., a particular meat. One method to determine whether the consumable is comparable to a food product (e.g. meat) is to a) define the properties of meat and b) determine whether the consumable has similar properties.

Properties ~~of meat~~ that can be tested or used to compare or describe a food product or the consumable include mechanical properties such as hardness, cohesiveness, brittleness, chewiness, gumminess, viscosity, elasticity, and adhesiveness. Properties of ~~meat~~ food products that can be tested also include geometric properties such as particle size and shape, and particle shape and orientation. The three dimensional organization of particles may also be tested. Additional properties can include moisture content and fat content. These properties can be described using terms such as "soft," "firm" or "hard" describe hardness; "crumbly," "crunchy," "brittle," "chewy," "tender," "tough," "short," "mealy," "pasty," or "gummy," to describe cohesiveness; "thin" or "viscous" to describe viscosity; "plastic" or "elastic" to describe elasticity; "sticky," "tacky" or "gooey" to describe adhesiveness; "gritty," "grainy" or "course" to describe particle shape and size; "fibrous," "cellular" or "crystalline" to describe particle shape and orientation, "dry," "moist," "wet," or "watery" to describe moisture content; or "oily" or "greasy" to describe fat content. ~~So~~Accordingly, in one embodiment, a group of people can be asked to rate a certain ~~meat~~food product, for instance ground beef, according to properties which describe the food product. A consumable described herein can be rated by the same people to determine equivalence.

Flavor of the food product can also be assessed. Flavors can be rated according to similarity to food products, e.g., "eggy," "fishy," "buttery," "chocolaty," "fruity", "peppery," "baconlike," "creamy," "milky," "or "beefy." Flavors can be rated according to the seven basic tastes, i.e., sweet, sour, bitter, salty, umami (savory), pungent (or

~~meat~~piquant), and metallic. Flavors can be described according to the similarity to an experience caused by a chemical, e.g., diacetyl (buttery), 3-hydroxy-2 butanone (buttery), nona-2E-enal (fatty), 1-octene-3-ol (mushroom), hexanoic acid (sweaty), 4-hydroxy-5-methyl furanone (HMF, meaty), pyrazines (nutty), bis(2-methyl-3-furyl) disulfide (roast meat), decanone (musty/fruity), isoamyl acetate (banana), benzaldehyde (bitter almond), cinnamic aldehyde (cinnamon), ethyl propionate (fruity), methyl anthranilate (grape), limonene (orange), ethyl decadienoate (pear), allyl hexanoate (pineapple), ethyl maltol (sugar, cotton candy), ethylvanillin (vanilla), butanoic acid (rancid), 12-methyltridecanal (beefy), or methyl salicylate (wintergreen). These ratings can be used as an indication of the properties of the ~~meat~~food product. The consumables of the present invention can then be compared to the ~~meat~~ food product to determine how similar the consumable is to the ~~meat~~food product. In some instances the properties of the consumables are then altered to make the consumable more similar to the ~~meat~~food product. ~~So~~Accordingly, in some embodiments, the consumable is rated similar to ~~meat~~ a food product according to human evaluation. In some embodiments the consumable is indistinguishable from real meat to a human.

The consumables can be made to eliminate properties associated with the source of the components of the consumables. For example a consumable can be made from components obtained from beans but can be made to lack a "beany" flavor or texture. One way this can be achieved is by breaking down the component source materials into isolated and purified components and not using components that cause undesired characteristic properties of the source. In addition, as described herein, off-flavors or aromas (e.g., undesirable flavors or aromas) in isolated and/or purified components can be minimized by deodorizing with activated charcoal or by removing enzymes such as lipoxygenases (LOX), which can be present in trace amounts and which can convert unsaturated triacylglycerides (such as linoleic acid or linolenic acid) into smaller and more volatile molecules. LOX are naturally present in legumes such as peas, soybeans, and peanuts, as well as rice, potatoes, and olives. When legume flours are fractionated into separate protein fractions, LOX can act as undesirable "time-bombs" that can cause undesirable flavors or aromas on aging or storage. As shown in Example 34, compositions containing plant proteins (e.g., from ground plant seeds) can be subjected to

purification to remove LOX using, for example, an affinity resin that binds to LOX and removes it from the protein sample. The affinity resin can be linoleic acid, linolenic acid, stearic acid, oleic acid, propyl gallate, or epigalloccatechin gallate attached to a solid support such as a bead or resin. See, e.g., W02013138793. In addition, depending on the protein component, certain combinations of antioxidants and/or LOX inhibitors can be used as effective agents to minimize off-flavor or off-odor generation in protein solutions, especially in the presence of fats and oils. Such compounds can include, for example, one or more of f3-carotene, a-tocopherol, caffeic acid, propyl gallate, or epigallocatechin gallate. These may be included during purification of the proteins or during subsequent food processing steps to mitigate generation of off-flavors or off-odors in protein-based foods.

~~[0286]~~ In some ~~embodiments~~compositions, subjects asked to identify the consumable identify it as a form of ~~meat. In some embodiments one property of the compositions of the invention is that an animal, for example a human, will identify the composition as a meat. In some embodiments the human identifies the composition of the invention~~ a food product, or as a particular food product, e.g., a subject will identify the consumable as meat. For example, in some compositions a human will identify the consumable as having properties equivalent to meat. In some embodiments one or more properties of ~~meat~~ the consumable are equivalent to the corresponding properties of meat according to a human's perception. Such properties include the properties that can be tested. In some embodiments a human identifies a consumable of the present invention as more meat like than any meat substitutes found in the art.

~~[0287] In embodiments an experiment~~ Experiments can demonstrate that a consumable ~~s~~ is acceptable to consumers. A panel can be used to screen a variety of consumables described herein. A number of human panelists can ~~tested~~ test multiple consumable samples, namely, natural meats vs. the consumable compositions described herein, or a meat substitute vs. a consumable composition described herein. Variables such as fat content can be standardized., for example to 20% fat using lean and fat meat mixes. Fat content can be determined using the Babcock for meat method (S. S. Nielson, Introduction to the Chemical Analysis of Foods (Jones & Bartlett Publishers, Boston,

1994)). Mixtures of ground beef and consumables of the invention prepared according to the procedure described herein can be formulated.

EXHIBIT 1E

[0288] Panelists can be served samples (e.g., in booths), under red lights or under white light, in an open consumer panel. Samples can be assigned random three-digit numbers and rotated in ballot position to prevent bias. Panelists can be asked to evaluate samples for tenderness, juiciness, texture, flavor, and overall acceptability using a hedonic scale from 1 = dislike 1=dislike extremely, to 9 = like 9=like extremely, with a median of 5 = neither 5=neither like nor, dislike. Panelists can be encouraged to rinse their mouths with water between samples, and given opportunity to comment on each sample.

[0289] The results of this experiment can indicate significant differences ($p<0.05$) or similarities between the traditional meats and the compositions of the invention.

[0290] These results will can demonstrate that the compositions of the invention described herein are judged as acceptably equivalent to real meat products. Additionally, these results can demonstrate that compositions of the invention described herein are preferred by panelist over other commercially available meat substitutes. So Thus, in some embodiments the present invention provides for consumables that are significantly similar to traditional meats and are more meat like than previously known meat alternatives.

[0291] Consumables of the invention can also have similar physical characteristics as food products, e.g., traditional meat. In one embodiment, the force required to pierce a 1 inch thick structure (e.g., a patty) made of a consumable of the invention with a fixed diameter steel rod is not significantly different than the force required to pierce a 1 inch thick similar meat food product structure (e.g., a ground beef patty) with a similar fixed diameter steel rod. Accordingly, the invention provides for consumables with similar physical strength characteristics to meat. In another embodiment, the force required to tear a sample of the invention with a cross-sectional area of 100 mm^2 is not significantly different than the force required to tear a sample of animal tissue (muscle, fat or connective tissue) with a cross-sectional area 100 mm^2 measured the same way. Force can be measured using, for example, TA.XT Plus Texture Analyzer (Textrue Technologies Corp.). Accordingly, the invention provides for consumables with similar physical strength characteristics to meat.

Consumables described herein can have a similar cook loss characteristic as a food product, e.g., meat. For example a consumable can have a similar fat and protein

content to ground beef and have the same reduction in size when cooked as real ground beef. Similarities in size loss profiles can be achieved for various compositions of consumables described herein matched to various meats. The cook loss characteristics of the consumable also can be engineered to be superior to food products. For example a consumable can be produced that has less loss during cooking but achieves similar tastes and texture qualities as the cooked products. One way this is achieved is by altering the proportions of lipids based on melting temperatures in the consumable composition. Another way this is achieved is by altering the protein composition of the consumable by controlling the concentration of protein or by the mechanism by which the tissue replica is formed.

~~[0292] In some embodiments composition of the invention have a similar cook loss characteristic as meat. In one embodiment a consumable of the invention with a similar fat and protein content as ground beef has the same reduction in size when cooked as real ground beef. Similar similarities in size loss profiles can be achieved for various compositions of consumables described herein matched to various meats.~~ ~~[0293] In some embodiments~~ In some embodiments, the consumable is compared to ~~real~~ an animal based food product (e.g., meat~~-~~) based upon olfactometer readings. In various embodiments the olfactometer can be used to assess odor concentration and odor thresholds, or odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of appreciation, or relative intensity of odors. In some embodiments~~-~~, the olfactometer allows the training and automatic evaluation of expert panels. So in some embodiments the consumable is a product that causes similar or identical olfactometer readings. In some embodiments the ~~similarity is sufficient to be beyond~~ differences are sufficiently small to be below the detection threshold of human perception.

~~[0294] Gas chromatography-mass~~ Gas chromatography—mass spectrometry (GCMS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to separate and identify different substances within a test sample. GCMS can, in some embodiments, be used to evaluate the properties of a consumable. For example volatile chemicals can be isolated from the head space around meat. These chemicals can be identified using GCMS. A profile of the volatile chemicals in the ~~head space~~ headspace around meat is thereby created. In some instances each peak of the GCMS can be further evaluated. For instance, a human could rate the experience of smelling the chemical

responsible for a certain peak. This information could be used to further refine the profile. GCMS could then be used to evaluate the properties of the consumable. The GCMS profile ~~could~~ can be used to refine the consumable.

EXHIBIT 1E

~~[0295]~~ Characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions molecules including amino acids, fats and sugars which are found in plants as well as meat. Therefore~~-~~, in some embodiments~~-~~, the consumable is tested for similarity to meat during or after cooking. In some embodiments human ratings, human evaluation, olfactometer readings, or GCMS measurements, or combinations thereof, are used to create an olfactory map of cooked meat. Similarly, an olfactory map of the consumable, for instance a meat replica, can be created. These maps can be compared to assess how similar the cooked consumable ~~it so~~ is to meat. In some embodiments the olfactory map of the consumable during or after cooking is similar to or indistinguishable from that of cooked or cooking meat. In some embodiments the similarity is sufficient to be beyond the detection threshold of human perception. The consumable may be created so its characteristics are similar to a food product after cooking, but the uncooked consumable may be have properties that are different from the predicate food product prior to cooking.

Shelf life is the length of time that consumables are given before they are considered unsuitable for sale, use, or consumption. Generally, it is important to maintain a meat product at about 2°C as the shelf life decreases with exposure to higher temperatures.

The shelf life of meat is determined through research into the meat products' sensory cues over time (odor, visual appearance of the package, color, taste and texture), and through laboratory analysis under controlled conditions to determine how long a product remains safe, wholesome and enjoyable. Ground beef is being used as an example, but similar conditions would apply to steaks, chops and roasts from other meat types. Beef in its natural state is dark bluish-purple. However, oxygen can permeate into the meat and cause a chemical reaction with the myoglobin in meat, leading to a red color. Ongoing exposure to oxygen causes oxidation of myoglobin and causes red meat to become brown and develop "off' flavors. To control this oxidation, there has been significant research into different methods of storing and displaying meat products to increase the shelf life of meat products. These include the use of vacuum packing,

modified atmosphere packing (high oxygen), modified atmosphere packaging (low oxygen with carbon monoxide), and/or High Pressure Pasteurization (HPP).

The main determinant of the color of meat is the concentration of iron carrying proteins in the meat. In the skeletal muscle component of meat products, one of the main iron-carrying proteins is myoglobin. It is estimated that the white meat of chicken has under 0.05% myoglobin; pork and veal have 0.1-0.3% myoglobin; young beef has 0.4-1.0% myoglobin; and old beef has 1.5-2.0% myoglobin. Normally, myoglobin in meat exists in three states: Oxymyoglobin (Fe^{2}) (oxygenated = bright red); myoglobin (Fe^{2}) (non-oxygenated = purplish/magenta); and metmyoglobin (Fe^{3} ') (oxidized = brown). The transition of oxymyoglobin to metmyoglobin in the presence of oxygen is thought to be the cause of the color change of ground meat from red to brown. Meat shelf life extenders have been developed to extend the lifetime of the red color of meat products including but not limited to carbon monoxide, nitrites, sodium metabisulfite, Bombal, vitamin E, rosemary extract, green tea extract, catechins and other anti-oxidants.

However an intrinsically more stable heme protein such a hemoglobin isolated from *Aquifex aeolicus* (SEQ ID NO:3) or *Methylacidiphilum infernorum* (SEQ ID NO: 2) will oxidize more slowly than a mesophilic hemoglobin such as myoglobin. The heme proteins described herein (see, e.g., FIG. 1) also may have the lifetime of the reduced heme-Fe^{2} ' state extended by meat shelf life extenders such as carbon monoxide and sodium nitrite. Heme proteins may be selected for the desired color retention properties. For example for low temperature *sous-vide* cooking, a relatively unstable heme protein such as one from *Hordeum vulgare* may provide a brown product that appears cooked under conditions wherein myoglobin would retain its red, uncooked appearance. In some embodiments the heme protein may be selected to have increased stability where for example the meat replica may retain an attractive medium rare appearance despite being thoroughly cooked for food safety.

The main determinant of rancidity and production of off flavors or off odors is the oxidation of components of the consumable, including but not limited to, the fats. For example, oxidation of unsaturated fatty acids is a known cause of rancid odors. In some embodiments, meat replicas have extended shelf life because the makeup of the chemical

properties of the meat replica are controlled such that the taste, texture, smell, and chemical properties do not react with oxygen to create off flavors or off odors. In some embodiments the meat replicas are less sensitive to oxidation due to the presence of higher degree of unsaturated fatty acids than present in beef In some embodiments the meat replica contains no unsaturated fatty acids. In other embodiments the meat replica contains higher levels of anti-oxidants such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases than are present in meat. In other embodiments, off flavor or off odor generating components such as lipoxygenase are not present.

In some embodiments, a consumable described herein shows increased stability under commercial packaging conditions. In some embodiments, the improved shelf life is improved by using components with increased oxidative stability such as lipids with reduced levels of unsaturated fatty acids, and/or by using a more stable heme protein such a hemoglobin isolated from *Aquifex aeolicus* (SEQ ID NO:3) or *Methylacidiphilum infernorum* (SEQ ID NO: 2). In some embodiments, the improved shelf life is due to the combination of components used in the consumable. In some embodiments, the consumable is designed specifically for the desired packaging method.

III. Composition of the Consumables

~~[0296] In one aspect, the invention provides a meat substitute product (alternatively referred to herein as "consumable") that is substantially or entirely composed of ingredients derived from non-animal sources, yet recapitulates key features associated with the cooking and consumption of an equivalent meat product derived from animals. The equivalent meat product can be a white meat or a dark meat. The equivalent meat product can be derived from any animal. Non-limiting examples of animals used to derive the equivalent meat product include farmed animals such as, e.g., cattle, sheep, pig, chicken, turkey, goose, duck, horse, dog or game animals (whether wild or farmed) such as, e.g., rabbit, deer, bison, buffalo, boar, snake, pheasant, quail, bear, elk, antelope, pigeon, dove, grouse, fox, wild pig, goat, kangaroo, emu, alligator, crocodile, turtle, groundhog, marmot, possum, partridge, squirrel, raccoon, whale, seal, ostrich, capybara, nutria, guinea pig, rat, mice, vole, any variety of insect or other arthropod, seafood such as, e.g., fish, crab, lobster, oyster, muscle, scallop, abalone, squid, octopus, sea urchin, tuni cate and others. Many meat products are typically derived from skeletal muscle of an animal but it is understood that meat can also come from other muscles or organs of the animal. In some embodiments, the equivalent meat product is a cut of meat derived from skeletal muscle. In other embodiments, the equivalent meat product is an organ such as, e.g., a kidney, heart, liver, gallbladder, intestine, stomach, bone marrow, brain, thymus, lung, tongue. Accordingly, in some embodiments the compositions of the present invention are consumables similar to skeletal muscle or organs.~~

~~[0297] In some aspects, the present invention provides meat substitute products comprising one or more of a first composition comprising a muscle tissue replica, a second composition comprising an adipose tissue replica, and/or a third composition comprising a connective tissue replica, wherein the one or more compositions are combined in a manner that recapitulates the physical organization of meat. In other aspects, the present invention provides compositions for a muscle tissue replica (herein referred to as "muscle replica"), an adipose tissue replica (herein referred to as "fat replica"), and a connective tissue replica (herein referred to as "connective tissue replica"). In some embodiments, the compositions and meat substitute products are principally or entirely composed of ingredients derived from non-animal sources. In alternative embodiments, the muscle, fat, and/or connective tissue replica, or the meat substitute products comprising one or more of said replicas, are partially derived from animal sources but supplemented with ingredients derived from non-animal sources. In yet other alternative embodiments, the invention provides meat products substantially derived from animal sources~~

but which are supplemented with one or more of a muscle tissue replica, a fat replica, and/or a connective tissue replica, wherein said replicas are derived substantially or entirely from non-animal sources. A non-limiting example of such a meat product is an ultra-lean ground beef product supplemented with a non-animal derived fat replica which improves texture and mouthfeel while preserving the health benefits of a consumable low in animal fat. Such alternative embodiments result in products with properties that more closely recapitulate key features associated with preparing and consuming meat but which are less costly and associated with a lesser environmental impact, less animal welfare impact, or improved health benefits for the consumer.

[0298] The physical organization of the meat substitute product can be manipulated by controlling the localization, organization, assembly, or orientation of the muscle, fat, and/or connective tissue replicas described herein. In some embodiments the product is designed in such a way that the replicas described herein are associated with one another as in meat. In some embodiments the consumable is designed so that after cooking the replicas described herein are associated with one another as in cooked meat. In some embodiments, one or more of the muscle, fat, and/or connective tissue replicas are combined in a manner that recapitulate the physical organization of different cuts or preparations of meat. In an example embodiment, the replicas are combined in a manner that approximates the physical organization of natural ground meat. In other embodiments, the replicas are combined in a manner that approximates different cuts of beef, such as, e.g., ribeye, filet mignon, London broil, among others.

Proteins and Protein Sources

[0299] In some embodiments, any of the meat substitute products, muscle tissue replica, fat replica, or connective tissue replica, comprise one or more isolated, purified proteins. In some embodiments, the meat substitute products are comprised of one or more of a muscle replica, a fat replica, and/or connective tissue replica which comprise one or more isolated, purified proteins. In other embodiments, the muscle replica, fat replica, and/or connective tissue replica comprises one or more isolated, purified proteins. In some embodiments, about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component is comprised of one or more isolated, purified proteins. For the purposes of this document, "purified protein" will refer A consumable described herein includes one or more isolated and purified proteins. "Isolated and purified protein" refers to a preparation in which the cumulative abundance by mass of protein components other than the specified protein, which can be a single monomeric or multimeric protein species, is reduced by a factor of 2 or more, 3 or more, 5 or more, 10 or more, 20 or more, 50 or more, 100 or more or 1000 or more relative to the source material from which the specified protein was isolated. [For clarity, the 0300] In some embodiments, the one or more isolated, purified proteins are derived from non-animal sources. Non-limiting examples of non-animal sources include plants, funghi, bacteria, archaea, genetically modified organisms such as genetically modified bacteria or yeast, chemical or in vitro synthesis. In particular embodiments, the one or more isolated, purified proteins are derived from plant sources.isolated and purified protein is described as isolated and purified relative to its starting material (e.g., plants or other non-animal sources). In some embodiments, the term "isolated and purified" can indicate that the preparation of the protein is at least 60% pure, e.g., greater than 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% pure. The fact that a consumable may comprise materials in addition to the isolated and purified protein

does not change the isolated and purified nature of the protein as this definition typically applies to the protein before addition to the composition.

In some embodiments, the one or more isolated and purified proteins accounts for at least 1%, at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, or at least 50% of the protein content of the consumable by weight. In some embodiments, each of the one or more isolated proteins is isolated and purified separately.

A consumable described herein may be substantially or entirely composed of ingredients derived from non-animal sources, e.g., plant, fungal, or microbial based sources. The plant sources can be organically grown sources. Proteins can be extracted from the source material (e.g., extracted from animal tissue, or plant, fungal, algal, or bacterial biomass, or from the culture supernatant for secreted proteins) or from a combination of source materials (e.g., multiple plant species). The consumables also can be made from a combination of plant based and animal based sources. For instance, the consumable may be a ground beef product supplemented with plant based products of the invention.

A. SOURCES OF COMPONENTS OF THE CONSUMABLE

As described above, isolated and purified proteins can be derived from non-animal sources such as plants, algae, fungi (e.g., yeast or filamentous fungi), bacteria, or Archaea. In some embodiments, the isolated and purified proteins can be obtained from genetically modified organisms such as genetically modified bacteria or yeast. In some embodiments, the isolated and purified proteins are chemically synthesized or obtained via *in vitro* synthesis.

In some embodiments, the one or more isolated and purified proteins are derived from plant sources. The isolated and purified proteins can be isolated from a single plant source or, alternatively, multiple plant sources can serve as the starting material for the isolation and purification of proteins. As described herein, isolated and purified plant proteins are soluble in solution. The solution can comprise EDTA (0 — 0.1M), NaC1 (01M), KC1 (0-1M), $NaSO_4$ (0 — 0.2M), potassium phosphate (0-1M), sodium citrate (01M), sodium carbonate (0-1M), sucrose (0-50%), Urea (0-2M) or any combination

thereof The solution can have a pH of 3 to 11. In some embodiments, plant proteins can have a solubility in a solution of >25 g/L (e.g., at least 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, or 225 g/L) at a temperature between about 2°C and about 32°C (e.g., between 3°C and 8°C, 10°C and 25°C, or 18°C and 25°C), wherein the solution has a pH between 3 and 8 (e.g., pH of 3-6, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, or 8), and has a sodium chloride content of 0 to 300 mM (e.g., 50, 100, 150, 200, 250, or 300 mM). In some embodiments, the isolated and purified proteins are soluble in solution at greater than 10, 15, 20, 25, 50, 100, 150, 200, or 250g/L.

One of skill in the art will understand that proteins that can be isolated from any organism in the plant kingdom may be used to produce the consumables described herein. Non-limiting examples of plant sources include ~~grains~~ grain crops such as, e.g., ~~corn,~~ maize, oats, rice, wheat, barley, rye, millet, sorghum, buckwheat, amaranth, quinoa, triticale (a wheat rye hybrid), teff, ~~oilseeds~~ *(Eragrostis tel);* oilseed crops including cottonseed, sunflower seed, safflower seed, ~~rapeseed,~~ *Crambe, Camelina,* mustard, rapeseed *(Brassica napus); Acacia,* or plants from the legume family, such as, e.g., clover, *Stylosanthes, Sesbania,* vetch *(Vicia), Arachis, Indigofera, Leucaena, Cyamopsis,* peas such as cowpeas, english peas, yellow peas, or green peas, or beans such as, e.g., soybeans, fava beans, lima beans, kidney beans, garbanzo beans, mung beans, pinto beans, lentils, lupins, mesquite, carob, soy, and peanuts *(Arachis hypogaea);* leafy greens such as, e.g., lettuce, spinach, kale, collard greens, turnip greens, chard, mustard greens, dandelion greens, broccoli, or cabbage~~,~~; or green matter not ordinarily consumed by humans, including biomass crops~~, including~~ such as switchgrass *(Panicum virgatum), Miscanthus, Arundo donax,* ~~miscanthus~~energy cane, ~~sorghum~~*Sorghum,* or other grasses, alfalfa, corn stover, kelp or other seaweeds, green matter ordinarily discarded from harvested plants, sugar cane leaves, leaves of trees, root crops such as cassava, sweet potato, potato, carrots, beets, ~~turnips,~~ or turnips; or coconut.

Protein can be isolated from any portion of the plant, including the roots, stems, leaves, flowers, or seeds. For example~~plants from the legume family, such as, e.g., clover, peas such as cowpeas, english peas, yellow peas, green peas, beans such as, e.g., soybeans, fava beans, lima beans, kidney beans, garbanzo beans, mung beans, pinto beans, lentils, lupins, mesquite, carob, soy, and peanuts, vetch (vicia), stylo (sty- losanthes), arachis, indigofera, acacia, leucaena, cyamopsis, and sesbania. One of skill in the art will understand that proteins that can be isolated~~

~~from any organism in the plant kingdom may be used in the present invention.~~, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) can be isolated from, for example, alfalfa, carrot tops, corn stover, sugar cane leaves, soybean leaves, switchgrass, *Miscanthus,* energy cane, *Arundo donax,* seaweed, kelp, algae or mustard greens.

EXHIBIT 1E

[0301] Proteins that are abundant in plants can be isolated in large quantities from one or more source plants and thus are an economical choice for use in any of the compositions provided herein (e.g., the muscle, fat, or connective tissue replicas, or meat substitute products or others). Accordingly, in some embodiments, the one or more isolated and purified proteins comprises comprise an abundant protein found in high levels in a plant and capable of being isolated and purified in large quantities. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, or 70% of the total protein content of the source plant material. In some embodiments, the abundant protein comprises about 0.5-10%, about 5-40%, about 10-50%, about 20-60%, or about 30-70% of the total protein content of the source plant material. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the total weight of the dry matter of the source plant material. In some embodiments, the abundant protein comprises about 0.5-5%, about 1-10110%, about 5-20%, about 10-30%, about 15-40%, or about 20-50% of the total weight of the dry matter of the source plant material.

[0302] In particular embodiments, the The one or more isolated and purified proteins comprises can comprise an abundant protein that is found in high levels in the leaves of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% of the total protein content of the leaves of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%, about 20%-60%, or about 30-70% of the total protein content of the leaves of the source plant. In particular some embodiments, the one or more isolated proteins comprise ribulose-1,5-bisphosphate car-boxylase-oxygenase (rubisco activase). Rubisco RuBisCo, which is a particularly useful protein for meat replicas because of its high solubility and an amino acid composition with that is close to the optimum proportions of essential amino acids for human nutrition. In particular embodiments, the one or more isolated proteins comprise ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco RuBisCo activase). In particular some embodiments, the one or more isolated and purified proteins comprise a vegetative storage protein (VSP).

~~[0303] In some embodiments, the~~ The one or more isolated proteins ~~includes~~ can comprise an abundant protein that is found in high levels in the seeds of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or 90% or more of the total protein content of the seeds of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%, about 20%60%, or about 30-70% or >70% of the total protein content of the seeds of the source plant. Non-limiting examples of proteins found in high levels in the seeds of plants ~~are~~ include seed storage proteins, e.g., albumins, glycinins, conglycinins, legumins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, ~~glu tenin~~ glutenin, hordein, ~~prolamin~~prolamine, phaseolin (protein), proteinoplast, secalin, triticeae gluten, or zein, ~~any seed storage protein~~or oil body proteins such as oleosins, ~~ole osins,~~ caloleosins, or steroleosins ~~or other oil body proteins [0304].~~

The one or more isolated and purified proteins can include highly soluble proteins such as dehydrins, hydrophilins, natively unfolded proteins (also referred to as intrinsically disordered proteins), or other proteins of the late-embryogenesis abundant (LEA) family. LEA proteins have been found in animals, plants and microorganisms and are thought to act as osmoprotectants and stress response proteins. See, e.g., Battaglia, *et al., Plant Physiol.,* 148:6-24 (2008). Such proteins also are heat stable. Such LEA protiens can have a solubility in a solution of at least 1 g/L (e.g., 2, 4, 6, 8, 10, 15, 20, 25, 50, 100, 150, 200, or 250g/L) at a temperature of between 90°C and 110°C (e.g., between 95°C and 105°C, 95°C, or 100°C), wherein the solution has a pH between 5 and 8 (e.g., pH of 5, 5.5., 6, 6.5, 7, 7.5, or 8) and has a sodium chloride content of 0 to 300 mM (e.g., 50, 100, 150, 200, 250, or 300 mM). In some cases, the LEA proteins may be isolated by heating a protein extract to 90°C-110°C (e.g., 95°C or 100°) and, after centrifugation or filtration of insoluble material, concentrating the LEA protein fraction by, for example, ultrafiltration. In some cases, isoionic pH precipitation, trichloroacetic acid precipitation, and/or ammonium sulfate precipitation steps can be done before or after the heating step to additionally remove non-LEA proteins. Heating the solution to 90°C-110°C denatures most proteins, allows the majority of the proteins to be removed from solution.

B. PROTEINS

Without being bound by theory, it is believed that by isolating and purifying non-animal proteins (e.g., plant proteins), consumables can be made with greater consistency and greater control over the properties of the consumable. In some embodiments, about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component of the consumable is comprised of one or more isolated and purified proteins. The isolated and purified protein may be greater than 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% pure.

The isolated and purified proteins can be isolated from one or more other components of a non-animal source. For example, a protein fraction may be isolated from an isolate of a plant. The isolated proteins may in some cases be purified, wherein a certain kind of protein is separated from other components found in the non-animal source. Proteins can be separated on the basis of their molecular weight, for example, by size exclusion chromatography, ultrafiltration through membranes, or density centrifugation. In some embodiments, the proteins can be separated based on their surface charge, for example, by isoelectric precipitation, anion exchange chromatography, or cation exchange chromatography. Proteins also can be separated on the basis of their solubility, for example, by ammonium sulfate precipitation, isoelectric precipitation, surfactants, detergents or solvent extraction. Proteins also can be separated by their affinity to another molecule, using, for example, hydrophobic interaction chromatography, reactive dyes, or hydroxyapatite. Affinity chromatography also can include using antibodies having specific binding affinity for the protein of interest, nickel NTA for His-tagged recombinant proteins, lectins to bind to sugar moieties on a glycoprotein, or other molecules which specifically binds the protein of interest.

Isolating proteins allows for the elimination of unwanted material. In some embodiments, an isolated and purified protein is a protein that has been substantially separated from unwanted material (e.g., nucleic acids such as RNA and DNA, lipid membranes, phospholipids, fats, oils, carbohydrates such as starch, cellulose, and

glucans, phenolic compounds, polyphenolic compounds, aromatic compounds, or pigments) in the seeds, leaves, stems, or other portion of the plant.

The isolated and purified proteins also can be recombinantly produced using polypeptide expression techniques (e.g., heterologous expression techniques using bacterial cells, insect cells, fungal cells such as yeast cells, plant cells, or mammalian cells). In some cases, standard polypeptide synthesis techniques (e.g., liquid-phase polypeptide synthesis techniques or solid-phase polypeptide synthesis techniques) can be used to produce proteins synthetically. In some cases, cell-free translation techniques can be used to produce proteins synthetically.

The protein or proteins incorporated into the consumable can serve a nutritional function. In some instance, the protein also serves to alter the properties of the consumable, e.g., the flavor, color, odor, and/or texture of the consumable. For example, a meat substitute product can comprise a protein indicator that indicates cooking progression from a raw state to a cooked state, wherein the meat substitute product is derived from non-animal sources.

Examples of proteins that can be isolated and purified, and used in the consumables described herein include ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, proteases, lipases, amylases, glycoproteins, lectins, mucins, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, histones, ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo), ribulose-1,5-bisphosphate carboxylase oxygenase activase (RuBisCo activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, collagens, zein, kafirin, avenin, dehydrins, hydrophilins, late embyogenesis abundant proteins, natively unfolded proteins, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin, globulin, pea globulins, and pea albumins.

In some embodiments, ~~the one or more isolated proteins includes proteins that interact~~ an isolated and purified protein can be a protein that interacts with lipids and ~~help~~ helps to stabilize lipids in a structure, a protein that binds lipids and helps crosslink lipid structures, or a protein that binds lipids and helps crosslink lipid structures and non-lipid interacting proteins. Without wishing to be bound by a particular theory, using such proteins in a consumable described herein may improve the integration of lipids and/or fat replicas with other components of the meat substitute product, resulting in improved mouthfeel and texture of the final product. A non-limiting example of a lipid-interacting plant protein ~~is~~ includes proteins in the oleosin family ~~of proteins~~. Oleosins are lipid-interacting proteins that are found in oil bodies of plants. Other non-limiting examples of plant proteins that can interact with lipids and stabilize emulsions include seed storage proteins from Great Northern ~~Beans~~beans, albumins from peas, globulins from peas, 8S globulins from ~~Moong~~ moong bean, 8S globulins from Kidney bean, prolamin and lipid transfer proteins.

~~Muscle Replicas~~

In some embodiments, one or more of the isolated and purified proteins can be an iron-carrying protein such as a heme-containing protein. As used herein, the term "heme containing protein" can be used interchangeably with "heme containing polypeptide" or "heme protein" or "heme polypeptide" and includes any polypeptide that can covalently or noncovalently bind a heme moiety. In some embodiments, the heme-containing polypeptide is a globin and can include a globin fold, which comprises a series of seven to nine alpha helices. Globin type proteins can be of any class (e.g., class I, class II, or class III), and in some embodiments, can transport or store oxygen. For example, a heme-containing protein can be a non-symbiotic type of hemoglobin or a leghemoglobin. A heme-containing polypeptide can be a monomer, i.e., a single polypeptide chain, or can be a dimer, a trimer, tetramer, and/or higher order oligomers. The life-time of the oxygenated Fe^{II} state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption.

The life-time of the unoxygenated $Fe^{e'}$ state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or

more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption

Non-limiting examples of heme-containing polypeptides can include an androglobin, a cytoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a leghemoglobin, a flavohemoglobin, Hell's gate globin I, a myoglobin, an erythrocruorin, a beta hemoglobin, an alpha hemoglobin, a protoglobin, a cyanoglobin, a cytoglobin, a histoglobin, a neuroglobins, a chlorocruorin, a truncated hemoglobin (e.g., HbN or Hb0), a truncated 2/2 globin, a hemoglobin 3 (e.g., G1b3), a cytochrome, or a peroxidase.

Heme-containing proteins that can be used in the consumables described herein can be from mammals (e.g., farms animals such as cows, goats, sheep, horses, pigs, ox, or rabbits), birds, plants, algae, fungi (e.g., yeast or filamentous fungi), ciliates, or bacteria. For example, a heme-containing protein can be from a mammal such as a farm animal (e.g., a cow, goat, sheep, pig, ox, or rabbit) or a bird such as a turkey or chicken. Heme-containing proteins can be from a plant such as *Nicotiana tabacum* or *Nicotiana sylvestris* (tobacco); *Zea mays* (corn), *Arabidopsis thaliana,* a legume such as *Glycine max* (soybean), *Cicer arietinum* (garbanzo or chick pea), *Pisum sativum* (pea) varieties such as garden peas or sugar snap peas, *Phaseolus vulgaris* varieties of common beans such as green beans, black beans, navy beans, northern beans, or pinto beans, *Vigna unguiculata* varieties (cow peas), *Vigna radiata* (Mung beans), *Lupinus albus* (lupin), or *Medicago sativa* (alfalfa); *Brassica napus* (canola); *Triticum sps.* (wheat, including wheat berries, and spelt); *Gossypium hirsutum* (cotton); *Oryza sativa* (rice); *Zizania sps.* (wild rice); *Helianthus annuus* (sunflower); *Beta vulgaris* (sugarbeet); *Pennisetum glaucum* (pearl millet); *Chenopodium sp.* (quinoa); *Sesamum sp.* (sesame); *Linum usitatissimum* (flax); or *Hordeum vulgare* (barley). Heme-containing proteins can be isolated from fungi such as *Saccharomyces cerevisiae, Pichia pastoris , Magnaporthe oryzae, Fusarium graminearum,* or *Fusarium oxysporum.* Heme-containing proteins can be isolated from bacteria such as *Escherichia col i, Bacillus subtilis, Bacillus megaterium, Synechocistis sp., Aquifex aeolicus, Methylacidiphilum infernorum,* or thermophilic bacteria (e.g, that grow at temperatures greater than 45°C) such as *Thermophilus* . Heme-containing proteins can be isolated from algae such as *Chlamydomonas eugametos* . Heme-

containing proteins can be isolated from protozoans such as *Paramecium caudatum* or *Tetrahymena pyriformis.* In some embodiments, the bacterial hemoglobins are selected from the group consisting of *Aquifex aeolicus, Thermobifida fusca, Methylacidiphilum infernorum* (Hells Gate), *Synechocystis SP,* or *Bacillus subtilis.* The sequences and structure of numerous heme-containing proteins are known. See for example, Reedy, *et al., Nucleic Acids Research,* 2008, Vol. 36, Database issue D307—D313 and the Heme Protein Database available on the world wide web at littp://herneprotein.info/hemeatn.

For example, a non-symbiotic hemoglobin can be from a plant selected from the group consisting of soybean, sprouted soybean, alfalfa, golden flax, black bean, black eyed pea, northern, garbanzo, moong bean, cowpeas, pinto beans, pod peas, dried peas, quinoa, sesame, sunflower, wheat berries, spelt, barley, wild rice, or rice.

Any of the heme-containing proteins described herein that can be used for producing consumables can have at least 70% (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%) sequence identity to the amino acid sequence of the corresponding wild-type heme-containing protein or fragments thereof that contain a heme-binding motif. For example, a heme-containing protein can have at least 70% sequence identity to an amino acid sequence set forth in FIG. 1, including a non-symbiotic hemoglobin such as that from *Vigna radiata* (SEQ ID NO:1), *Hordeum vulgare* (SEQ ID NO:5), *Zea mays* (SEQ ID NO:13), *Oryza sativa subsp. japonica (rice)* (SEQ ID NO:14), or *Arabidopsis thaliana* (SEQ ID NO:15), a Hell's gate globin I such as that from *Methylacidiphilum infernorum* (SEQ ID NO:2), a flavohemoprotein such as that from *Aquifex aeolicus* (SEQ ID NO:3), a leghemoglobin such as that from *Glycine max* (SEQ ID NO:4), *Pisum sativum* (SEQ ID NO:16), or *Vigna unguiculata* (SEQ ID NO:17), a heme-dependent peroxidase such as from *Magnaporthe oryzae,* (SEQ ID NO:6) or *Fusarium oxysporum* (SEQ ID NO:7), a cytochrome c peroxidase from *Fusarium graminearum* (SEQ ID NO:8), a truncated hemoglobin from *Chlamydomonas moewusii* (SEQ ID NO:9), *Tetrahymena pyriformis* (SEQ ID NO:10, group I truncated), *Paramecium caudatum* (SEQ ID NO:11, group I truncated), a hemoglobin from *Aspergillus niger* (SEQ ID NO:12), or a mammalian myoglobin protein such as the *Bos taurus* (SEQ ID NO:18) myoglobin, *Sus scrofa* (SEQ ID NO:19) myoglobin, or *Equus*

caballus (SEQ ID NO:20) myoglobin, a heme-protein from *Nicotiana benthamiana* (SEQ ID NO:21), *Bacillus subtilis* (SEQ ID NO:22), *Corynebacterium glutamicum* (SEQ ID NO:23), Synechocystis PCC6803 (SEQ ID NO:24), Synechococcus sp. PCC 7335 (SEQ ID NO:25), *Nostoc commune* (SEQ ID NO:26), or *Bacillus megaterium* (SEQ ID NO:27). See FIG. 1.

The percent identity between two amino acid sequences can be determined as follows. First, the amino acid sequences are aligned using the BLAST 2 Sequences (B12seq) program from the stand-alone version of BLASTZ containing BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from Fish & Richardson's web site (e.g., www.fr.com/blast/) or the U.S. government's National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov). Instructions explaining how to use the Bl2seq program can be found in the readme file accompanying BLASTZ. Bl2seq performs a comparison between two amino acid sequences using the BLASTP algorithm. To compare two amino acid sequences, the options of Bl2seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seql.txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following
command can be used to generate an output file containing a comparison between two amino acid sequences: C:\B12seq —i c:\seql.txt —j c:\seq2.txt —p blastp —o c:\output.txt. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology, then the designated output file will not present aligned sequences. Similar procedures can be following for nucleic acid sequences except that blastn is used.

Once aligned, the number of matches is determined by counting the number of positions where an identical amino acid residue is presented in both sequences. The percent identity is determined by dividing the number of matches by the length of the full-length polypeptide amino acid sequence followed by multiplying the resulting value by 100. It is noted that the percent identity value is rounded to the nearest tenth. For example, 78.11, 78.12, 78.13, and 78.14 is rounded down to 78.1, while 78.15, 78.16,

78.17, 78.18, and 78.19 is rounded up to 78.2. It also is noted that the length value will always be an integer.

It will be appreciated that a number of nucleic acids can encode a polypeptide having a particular amino acid sequence. The degeneracy of the genetic code is well known to the art; i.e., for many amino acids, there is more than one nucleotide triplet that serves as the codon for the amino acid. For example, codons in the coding sequence for a given enzyme can be modified such that optimal expression in a particular species (e.g., bacteria or fungus) is obtained, using appropriate codon bias tables for that species.

Heme-containing proteins can be extracted from the source material (e.g., extracted from animal tissue, or plant, fungal, algal, or bacterial biomass, or from the culture supernatant for secreted proteins) or from a combination of source materials (e.g., multiple plant species). Leghemoglobin is readily available as an unused by-product of commodity legume crops (e.g., soybean, alfalfa, or pea). The amount of leghemoglobin in the roots of these crops in the United States exceeds the myoglobin content of all the red meat consumed in the United States.

In some embodiments, extracts of heme-containing proteins include one or more non-heme-containing proteins from the source material (e.g., other animal, plant, fungal, algal, or bacterial proteins) or from a combination of source materials (e.g., different animal, plant, fungi, algae, or bacteria).

In some embodiments, heme-containing proteins are isolated and purified from other components of the source material (e.g., other animal, plant, fungal, algal, or bacterial proteins) using techniques described above. As used herein, the term "isolated and purified" indicates that the preparation of heme-containing protein is at least 60% pure, e.g., greater than 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% pure.

Heme-containing proteins also can be recombinantly produced using polypeptide expression techniques (e.g., heterologous expression techniques using bacterial cells, insect cells, algal cells, fungal cells such as yeast cells, plant cells, or mammalian cells). For example, the heme-containing protein can be expressed in *E. coli* cells. The heme-containing proteins can be tagged with a heterologous amino acid sequence such as FLAG, polyhistidine (e.g., hexahistidine, HIS tag), hemagluttinin (HA), glutathione-S-

transferase (GST), or maltose-binding protein (MBP) to aid in purifying the protein. In some embodiments, a recombinant heme containing protein including a HIS-tag and a protease (e.g., TEV) site to allow cleavage of the HIS-tag, can be expressed in *E.coli* and purified using His-tag affinity chromatography (Talon resin, CloneTech). In some cases, standard polypeptide synthesis techniques (e.g., liquid-phase polypeptide synthesis techniques or solid-phase polypeptide synthesis techniques) can be used to produce heme-containing proteins synthetically. In some cases, cell-free translation techniques can be used to produce heme-containing proteins synthetically..

In some embodiments, the isolated and purified protein is substantially in its native fold and water soluble. In some embodiments, the isolated and purified protein is more than 50, 60, 70, 80, or 90% in its native fold. In some embodiments, the isolated and purified protein is more than 50, 60, 70, 80, or 90% water soluble.

The proteins used in the consumable can be altered (e.g., hydrolyzed, cleaved, crosslinked, denatured, polymerized, extruded, electrospun, spray dried or lyophilized, or derivatized or chemically modified). For example, the proteins can be modified by covalent attaching sugars, lipids, cofactors, peptides, or other chemical groups including phosphate, acetate, methyl, and other natural or unnatural molecule. For example, the peptide backbones of the proteins can be cleaved by exposure to acid or proteases or other means. For example, the proteins can be denatured, i.e., their secondary, tertiary, or quaternary structure can be altered, by exposure to heat or cold, changes in pH, exposure to denaturing agents such as detergents, urea, or other chaotropic agents, or mechanical stress including shear. The alignment of proteins in a solution, colloid, or a solid assembly can be controlled to affect the mechanical properties including tensile strength, elasticity, deformability, hardness, or hydrophobicity.

Proteins also can be assembled into fibers that can form a matrix for a structure for the compositions. A 3-dimensional matrix of protein fibers can, for example, contain chemicals that promote formation of inter-molecular disulfide cross-links (mixed glutathione, dithiothreitol (DTT), beta-mercaptoethanol (BME)). In some embodiments, the chemicals are proteins (thioredoxin, glutaredoxin). In some embodiments, the proteins are enzymes (disulfide isomerase). In some embodiments, fibers are crosslinked

by chemical cross-linkers with two reactive groups selected from the group consisting of N-hydroxysuccinimide (NHS) esters, imidoesters, aryl fluorides, aldehydes, maleimides, pyridyldithiols, haloacetyls, aryl azides, diazirines, carbodiimides, hydrazides and isocyanates.

In some embodiments, coacervates comprising one or more plant proteins can be formed and used, for example, as binding agents in meat or other replicas. Coacervation is the process during which a homogeneous solution of charged polymers undergoes a phase separation to result in a polymer-rich dense phase (the `coacervate') and a solvent-rich phase (supernatant). Protein-polysaccharide coacervates have been used in the development of biomaterials. See, for example, Boral and Bohidar (2010) *Journal of Physical Chemistry B.* Vol 114 (37): 12027-35; and Liu *et al.,* (2010) *Journal of Agricultural and Food Chemistry,* Vol 58:552-556. Formation of such coacervates is driven by associative interactions between oppositely charged polymers. However, as described herein, coacervates can be formed using proteins (e.g., plant proteins comprise one or more pea proteins, chickpea proteins, lentil proteins, lupine proteins, other legume proteins, or mixtures thereof). In general, a coacervate can be formed by acidifying a low ionic strength solution (e.g., a buffered solution at or below100 mM sodium chloride) comprising one or more isolated and purified plant proteins such as pea legumins or vicilins (e.g., a vicilin fraction comprising convicilins), a combination of both vicilins and legumins, or unfractionated pea proteins to a pH of 3.5 to 5.5. (e.g., pH 4 to 5). Under these conditions, the proteins separate out of solution and the mixture can be centrifuged to cleanly separate out the coacervate. This coavervate, unlike a precipitate, is a viscous material that can be stretched by pulling and that melts on heating. The process can be carried out in the presence of oils (up to 70%, e.g., palm or other oil), to form a creamy material. By varying the composition of the solution (ratio of vicilin: legumin, type and amount of oil used), the binding properties of the coacervate can be tuned as desired. In some embodiments, one or more gums (e.g., acacia gum or xanthan gum) can be used to form a coacervate. Coacervates can be used as binding agents in beef patty replicas to bind and hold together the adipose-, muscle- and connective tissue replicas.

Binding materials with different adhesive and cooking characteristics can be prepared by combining wheat gluten (0-20%) and pea protein fractions (0-50%) in the presence of a plasticizer such as glycerol (0-30%) or polyethylene glycol. Leghemoglobin or other heme-containing protein can be added to the mixture if necessary. Upon mixing to remove any clumps, the material may be incorporated into beef patty replicas.

In some embodiments, proteins can be subjected to freeze alignment to texturize the proteins without extrusion. The method involves slow freezing of protein comprising materials to allow for formation of ice crystals. When cooled from one side, ice crystals form preferentially in a direction perpendicular to the cooled side. After freezing, the ice can be removed from the material in a freeze-dryer, leaving behind material with several layers. The structure can then be stabilized by heating under pressurized, moist conditions to produce a material that can be used in meat replicas. Freeze-alignment of soy proteins has been described by Lugay and Kim (1981) (see Freeze alignment: A novel method for protein texturization. Page 177-187, Chapter 8 in: D.W. Stanley, E.D. Murray and D.H. Lees eds. 1981. Utilization of Protein Resources. Westport, CT: Food & Nutrition Press, Inc). The freeze-aligned proteins can be subjected to further processing (by soaking in solutions comprising beef flavors and/or leghemoglobin) and used in combination with adipose- and connective-tissue replicas to form beef replicas. The replicas may also be used as structures around which cold-set gels (comprising, for example, pea proteins and myoglobin) or crosslinked gels (comprising, for example, pea proteins and leghemoglobin) can be formed prior to their combination with adipose- and connective-tissues.

C. LIPIDS

Consumables described herein can include a lipid component. Lipids can be isolated and/or purified and can be in the form of triglycerides, monoglycerides, diglycerides, free fatty acids, sphingosides, glycolipids, phospholipids, or oils, or assemblies of such lipids (e.g., membranes, lecithin, lysolecithin, or fat droplets containing a small amount of lipid in a bulk water phase). In some embodiments, the lipid sources are oils obtained from non-animal sources (e.g., oils obtained from plants,

algae, fungi such as yeast or filamentous fungi, seaweed, bacteria, or Archae), including genetically engineered bacteria, algae, archaea or fungi. Non-limiting examples of plant oils include corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, palm oil, palm kernel oil, coconut oil, babassu oil, Shea butter, mango butter, cocoa butter, wheat germ oil, or rice bran oil; or margarine. The oils can be hydrogenated (e.g., a hydrogenated vegetable oil) or non-hydrogenated.

In some embodiments, the lipid can be triglycerides, monoglycerides, diglycerides, free fatty acids, sphingosides, glycolipids, lecithin, lysolecithin, phospholipids such as phosphatidic acids, lysophosphatidic acids, phosphatidyl cholines, phosphatidyl inositols, phosphatidyl ethanolamines, or phosphatidyl serines; sphingolipids such as sphingomyelins or ceramides; sterols such as stigmasterol, sitosterol, campesterol, brassicasterol, sitostanol, campestanol, ergosterol, zymosterol, fecosterol, dinosterol, lanosterol, cholesterol, or episterol; lipid amides, such as N-palmitoyl proline, N-stearoyl glycine, N-palmitoyl glycine, N-arachidonoyl glycine, N-palmitoyl taurine, N-arachidonoyl histidine, or anandamide; free fatty acids such as palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid (C18:2), eicosanoic acid (C22:0), arachidonic acid (C20:4), eicosapentanoic acid (C20:5), docosapentaenoic acid (C22:5), docosahexanoic acid (C22:6), erucic acid (C22:1), conjugated linoleic acid, linolenic acid (C18:3), oleic acid (C18:1), elaidic acid (trans isomer of oleic acid), trans-vaccenic acid (C18:1 *trans 11),* or conjugated oleic acid; or esters of such fatty acids, including monoacylglyceride esters, diacylglyceride esters, and triacylglyceride esters of such fatty acids.

The lipids can comprise phospholipids, lipid amides, sterols or neutral lipids. The phospholipids can comprise a plurality of amphipathic molecules comprising fatty acids (e.g., see above), glycerol and polar groups. In some embodiments, the polar groups are, for example, choline, ethanolamine, serine, phosphate, glycerol-3-phosphate, inositol or inositol phosphates. In some embodiments, the lipids are, for example, sphingolipids,

ceramides, sphingomyelins, cerebrosides, gangliosides, ether lipids, plasmalogens or pegylated lipids.

In some embodiments, the lipids used in the consumable are the cream fraction created from seeds, nuts, and legumes, including but not limited to sunflower seeds, safflower seeds, sesame seeds, rape seeds, almonds, macadamia, grapefruit, lemon, orange, watermelon, pumpkin, cocoa, coconut, mango, butternut squash, cashews, brazilnuts, chestnuts, hazelnuts, peanuts, pecans, walnuts and pistachios. As used herein, the term "cream fraction" can refer to an isolated emulsion comprising lipids, proteins and water.

To obtain a cream fraction from seeds, nuts, or legumes, one or more of the following steps can be performed. Seeds, nuts or legumes can be blended from 1 minute up to 30 minutes. For example, the seeds, nuts, or legumes can be blended by increasing the speed gradually to maximum speed over 4 minutes, then blending at maximum speed for 1 minute. The seeds, nuts or legumes can be blended in water or solutions that contain all or some of the following: EDTA (0 — 0.1M), NaC1 (0-1M), KC1 (0-1M), $NaSO_4$ (0 —0.2M), potassium phosphate (0-1M), sodium citrate (0-1M), sodium carbonate (0-1M), and/or sucrose (0-50%), from pH of 3 to 11 to obtain a slurry. The slurry can be heated to 20°C to 50°C and centrifuged to obtain the cream fraction (the top layer, also referred to as the "cream"). Further purification of the cream fraction may be achieved by washing the cream fraction with 0.1M to 2M urea solution before re-isolating the cream fraction by centrifugation. The residual liquid (referred to as the "skim" layer) that is a solution comprising proteins in water can also be used.

The "cream" can be used as is, or subjected to further purification steps. For example, washing and heating can remove color and flavor molecules (e.g., unwanted molecules), or unwanted grainy particles to improve the mouth feel and creaminess. In particular, washing with a high pH buffer (pH >9) can remove bitter tasting compounds and improve mouth feel, washing with urea can remove storage proteins, washing below pH 9, followed by washing with a pH above pH 9 can remove unwanted color molecules, and/or washing with salts can decrease taste compounds. Heating can increase the removal of grainy particles, color and flavor compounds. For example, the cream

fraction can be heated from 0-24 hours, at temperatures ranging from 25°C to 80°C. In some embodiments, the resulting creamy fraction comprises seed storage proteins. In some embodiments, the seed storage proteins are substantially removed from the resulting creamy fraction.

D. FIBER

Fiber can be isolated and/or purified for inclusion in the consumables described herein. Fiber can refer to non-starch polysaccharides such as arabinoxylans, cellulose, and other plant components such as resistant starch, resistant dextrins, inulin, lignin, waxes, chitins, pectins, beta-glucans, and oligosaccharides from any plant source.

Fibers can refer to extruded and solution spun proteins as described herein.

E. SUGARS

In some embodiments, the consumable also can comprise sugars. For example the consumable can comprise: monosaccharides, including but not limited to glucose (dextrose), fructose (levulose), galactose, mannose, arabinose, xylose (D- or L-xylose), and ribose, disaccharides including but not limited to sucrose, lactose, melibiose, trehalose, cellobiose, or maltose, sugar alcohols such as arabitol, mannitol, dulcitol, or sorbitol, sugar acids such as galacturonate, glucuronate, or gluconate, oligosaccharides and polysaccharides such as glucans, starches such as corn starch, potato starch, pectins such as apple pectin or orange pectin, raffinose, stachyose, or dextrans; plant cell wall degradation products such as salicin, and/or sugar derivatives such as N-acetylglucosamine.

F. GEL FORMATION

The components of the composition can be formed into a gel. In some embodiments, gels comprise protein, where the protein is derived from non-animal source (e.g., a plant source or other non-animal source such as a genetically modified yeast or bacteria). Gels can be formed using a variety of methods. The protein

concentration, enzyme concentration, pH, and/or process temperature will affect the rate of gel formation and quality of the final tissue replica.

Gels can be stabilized entirely by physical cross-links between the components. In some embodiments, gels can be produced by heat/cool cycles, in which case the gel is stabilized by physical interactions (entanglements, hydrophobic interactions) between protein molecules. For example, a gel can be formed by heating a protein solution to a temperature of at least 40°C, 45°C, 50°C, 60°C, 70°C, 80°C, 90°C, or 100°C and then cooling to room temperature, or to a temperature below 40°C.

In some embodiments, a gel can be formed by subjecting the composition containing the protein and any other components (e.g., a lipid) to high pressure processing.

In some embodiments, gels can be produced by adjusting the pH of the solution. For example, the pH of a concentrated protein solution can be adjusted to near the isolectric pH of the main protein component by adding hydrochloric acid or other acid, or sodium hydroxide or other base.

In some embodiments, gels can be produced by soaking protein powders in solutions. For example, protein powder can be soaked with at least 1%, 5%, 10%, 20% (wt/v) or more of a concentrated sodium hydroxide solution. In other examples, protein powder can be soaked in mixed water/ethanol solutions.

In some embodiments, a cold set gel is formed to avoid denaturing or the breakdown of any heat-labile components (e.g., oxidizing the iron in a heme moiety or generating undesirable flavors). See, Ju and Kilara A. (1998)J. *Food Science,* Vol 63(2): 288-292; and Maltais *et al.,* (2005) *J. Food Science,* Vol 70 (1): C67-C73) for general methodologies for forming cold set gels. In general, cold set gels are formed by first heat denaturing a protein solution below its minimum gelling concentration (dependent on pH and type of protein, typically <8% (w/v) at pH 6-9 for globular plant proteins such as pea proteins). The protein solution can heated to a temperature above the denaturation temperature of the protein under conditions where it does not precipitate out of solution (e.g., 0-500 mM sodium chloride, pH 6-9). The solution can be cooled back to room temperature or below, and any heat-labile components (e.g., heme-containing proteins

and/or oils) can be mixed in when the solution is sufficiently cool, but before gelling.. Gelation can be induced by adding sodium chloride or calcium chloride (e.g., 5 to 100 mM), and the solution can be incubated at or below room temperature to allow for gel formation (typically minutes-hours). The resulting gel can be used as-is in meat replicas or processed further (e.g., stabilized) before incorporation in meat replicas.

In some embodiments, gels can comprise or be produced (e.g., stabilized by) at least in part by a cross-linking enzyme. The cross-linking enzyme can be, for example, a transglutaminase, a tyrosinase, a lipoxygenase, a protein disulfide reductase, a protein disulfide isomerase, a sulfhydryl oxidase, a peroxidase, a hexose oxidase, a lysyl oxidase, or an amine oxidase.

In some cases, gels can comprise chemicals that promote formation of inter-molecular disulfide cross-links between the proteins. In some embodiments, the chemicals are proteins (e.g., thioredoxin, glutaredoxin). In some embodiments, the proteins are enzymes (disulfide isomerase).

Gels can be stabilized by chemical crosslinking by chemical cross-linkers with two reactive groups selected from the group consisting of N-hydroxysuccinimide (NHS) esters, imidoesters, aryl fluorides, aldehydes, maleimides, pyridyldithiols, haloacetyls, aryl azides, diazirines, carbodiimides, hydrazides and isocyanates.

In some embodiments, gels can be stabilized by the addition of starches and gums.

In some embodiments, more than one of these approaches are used in combination. For example, a transglutaminase cross-linked gel can be further stabilized by a heat/cool treatment.

G. MUSCLE REPLICAS

~~[0305]~~ A large number of meat products comprise a high proportion of skeletal muscle. Accordingly, the present invention provides a composition ~~derived from non-animal sources which replicates or approximates key features of animal skeletal muscle. In another aspect, the present invention provides a meat substitute product that comprises a composition~~ , which can be derived from non-animal sources which replicates or approximates key features of animal skeletal muscle. A

composition derived from non-animal sources, which replicates or approximates animal skeletal muscle can be used as a component of a consumable, for

example, a meat replica. Such a composition will be labeled herein as "muscle replica~~".~~." In some embodiments, the muscle replica and/or meat substitute product comprising the muscle replica are partially derived from animal sources. In some embodiments, the muscle replica and/or meat substitute product comprising the muscle replica are entirely derived from non-animal sources.

~~[0306]~~ The muscle tissue replica can comprise a protein content, wherein the protein content comprises one or more isolated and purified proteins, wherein the muscle tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source.

Many meat products comprise a high proportion of striated skeletal muscle in which individual muscle fibers are organized mainly in an ~~isotropic~~ anisotropic fashion. Accordingly, in some embodiments~~-~~, the muscle replica comprises fibers that are to some extent organized ~~isotropically~~anisotropically. ~~In some embodiments the~~ The fibers can comprise a protein component. In some embodiments, the fibers comprise about 1% (wt/wt), about 2%, about 5%, about 10%, about 15%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99% (wt/wt) or more of a protein component. ~~[0307] In some embodiments, the protein component comprises one or more isolated, purified proteins. For example the one or more isolated, purified protein can comprise the~~

The connective tissue component of skeletal muscle substantially contributes to texture, mouth feel and cooking behavior of meat products. Connective tissue is composed of protein (collagen, elastin) fibers in the range of 0.1 — 20 microns. In some embodiments, a mixture of fibers of diameters of <1-10 microns, and 10-300 microns is produced to replicate the fiber composition of animal connective tissue. In some embodiments, the 3-dimensional matrix of fibers is stabilized by protein crosslinks to replicate the tensile strength of animal connective tissue. In some embodiments, the 3-dimensional matrix of fibers contains an isolated, purified cross-linking enzyme. The cross-linking enzyme can be, for example, a transglutaminase, a tyrosinase, a lipoxygenase, a protein disulfide reductase, a protein disulfide isomerase, a sulfhydryl oxidase, a peroxidase, a hexose oxidase, a lysyl oxidase, or an amine oxidase.

Some proteins (e.g., 8S globulin from Moong bean seeds, or the albumin or globulin fraction of pea seeds~~. These proteins provide examples of proteins with~~) have favorable properties for

constructing meat replicas because of their ability to form gels with textures similar to animal muscle or ~~fat tissue. Examples and embodiments of the one or more isolated, purified proteins are described~~adipose

EXHIBIT 1E

~~herein. The list of potential candidates here is essentially open and may include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microorganisms, or synthesized in vitro~~tissue. See also the proteins identified in Section III A and B. The proteins may be artificially designed to emulate physical properties of animal muscle tissue.~~The proteins may be artificially designed to emulate physical properties of animal muscle tissue.~~

In some embodiments, one or more isolated~~,~~ and purified proteins accounts for about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component by weight of the meat replica. In some embodiments, one or more isolated and purified proteins accounts for about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein content of a consumable.

~~[0308]~~ Skeletal ~~muscle~~ muscles of animals such as beef cattle typically contain substantial quantities of glycogen, which can comprise on the order of 1% of the mass of the muscle tissue at the time of slaughter. After slaughter, a fraction of this glycogen continues to be metabolized~~,~~, yielding products including lactic acid, which contributes to lowering the pH of the muscle tissue, a desirable quality in meat. Glycogen is a branched polymer of glucose linked together by alpha (1->4) glycosidic bonds in linear chains, with branch points comprising alpha (1->6) glycosidic bonds. Starches from plants, particularly amylopectins are also branched polymers of glucose linked together by alpha (1->4) glycosidic bonds in linear chains, with branch points comprising alpha (1->6) glycosidic bonds and can therefore be used as an analog of glycogen in constructing meat replicas. Thus~~,~~, in some embodiments, the muscle or meat replica includes a starch or pectin.

~~[0309]~~ Additional components of animal muscle tissue include sodium, potassium, calcium, magnesium, and other metal ions, lactic acid~~, other organic acids, free amino acids, peptides, nucleotides and sulfur compounds. Thus in some embodiments, the muscle replica can include sodium, potassium, calcium, magnesium, other metal ions, lactic acid,~~ and other organic acids, free amino acids, peptides, nucleotides, and sulfur compounds. Thus, in some embodiments, a muscle replica can include sodium, potassium, calcium, magnesium, other metal ions such as iron, zinc,

copper, nickel, lithium, or selenium, lactic acid, and other organic acids such as fatty acids, free amino acids, peptides, nucleotides and sulfur compounds glutathione, beta mercaptoethanol, or dithiothreitol. In some embodiments-, the concentration of sodium, potassium, calcium, magnesium, other metal ions, lactic acid, other organic acids, free amino acids, peptides, nucleotides and/or sulfur compounds in

the muscle replica or consumable are within 10% of the concentrations found in a muscle or meat being replicated.

~~[0310] In another aspect, the~~ The invention also provides methods for making a muscle replica. In some embodiments, the method includes forming the composition ~~is formed~~ into asymmetric fibers prior to incorporation into the consumable. In some embodiments~~-~~, these fibers replicate muscle fibers. In some embodiments the fibers are spun fibers. In other embodiments the fibers are extruded fibers. Accordingly, the present invention provides for methods for producing asymmetric or spun protein fibers. In some embodiments, the fibers are formed by extrusion of the protein component through an extruder. Methods of extrusion are well known in the art, and are described~~-~~, for example, in U.S. ~~Pat.~~ Patent No. 6,379,738, U.S. ~~Pat.~~ Patent No. 3,693,533, ~~US20120093994~~and U.S. Patent Publication No. 20120093994, which are herein incorporated by reference. These methods can be applied to making the compositions provided herein.

Extrusion can be conducted using, for example, a Leistritz Nano-16 twin-screw co-rotating extruder (American Leistritz Extruder Corp. USA, Sommerville, NJ). Active cooling of the barrel section can be used to limit denaturation of proteins. Active cooling of the die section can be used to limit expansion of the extruded product and excessive moisture loss. Protein feed and liquid are added separately: protein is fed by a volumetric plunger feeder or a continuous auger-type feeder, and liquid can be added into the barrel through a high pressure liquid injection system. Die nozzles with various inner diameters and channel length can be used for precise control of extrudiate pressure, cooling rate and product expansion. In some examples, extrusion parameters were: screw speed 100-200 rpm, die diameter 3 mm, die length 15 cm, product temperature at the end of the die of 50°C, feed rate of 2 g/min, and water-flow rate of 3 g/min. Product temperature at the die during extrusion is measured by a thermocouple.

Spun fibers can be produced by preparing a high viscosity protein "dope" by adding sodium hydroxide to concentrated protein solutions or to precipitated proteins, and forcing the solution with a plunger-type device (in some examples, a syringe with a syringe pump) through a small steel capillary (in some examples, 27 gauge hypodermic needle) into a coagulating bath. In some examples, the bath is filled with a concentrated

acid solution (e.g. 3 M hydrochloric acid). In some examples, the bath is filled with a buffer solution at a pH approximately equal to the isoionic point of the protein. Coagulating protein solution jet forms a fiber that collects at the bottom of the bath.

Bundles of spun fibers can be produced by forcing protein "dope" through spinnerets with many small holes. In some examples, spinnerets are stainless steel plates with approximately 25,000 holes per cm^2, with diameter of each hole approximately 200 microns. In some embodiments muscle tissue replica is produced by immersing the 3-dimensional matrix of fibers (connective tissue replica) in solutions of proteins and creating protein gels incorporating 3-dimensional matrix of fibers.

H. FAT REPLICAS

~~[0311] In some embodiments extrusion can be conducted using an MPF19 twin screw extruder (APV Baker, Grand Rapids, Mich.) with a cooling die. The cooling die can cool the extrudate prior to return of the extrudate to atmospheric pressure, thus substantially inhibiting expansion or puffing of the final product. In the MPF19 apparatus, dry feed and liquid can be added separately and mixed in the barrel. Extrusion parameters can be, for example: screw speed of 200 rpm, product temperature at the die of 150 C., feed rate of 23 g/min, and water flow rate of 11 g/min. Product temperature can be measured during extrusion by a thermocouple at the end of the extrusion barrel. Observations can be made on color, opacity, structure, and texture for each collected sample. Collected samples can be optionally dried at room temperature overnight, then ground to a fine powder (~60 mesh) using a Braun food grinder. The pH of samples can be measured in duplicate using 10% (w/v) slurries of powdered sample in distilled water.~~

~~[0312] Fat Replica~~

~~[0313]~~ Animal fat is important for the experience of eating cooked meat and are important for some of the nutritional value of meat. Accordingly, the present invention provides compositions derived from non-animal sources, which recapitulate key features of animal fat, including the texture and/or flavor, by using components that mimic the chemical composition and physical properties of, for example, ground beef. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources ~~which recapitulates key features of animal fat. In another aspect~~, ~~the present invention provides a meat substitute product that comprises a composition derived from non-animal sources~~ which recapitulates animal fat. Such a composition will be labeled herein as an "adipose replica" or a "fat replica~~".~~." In some embodiments, the ~~fat~~ adipose replica and/or meat substitute product comprising the ~~fat~~ adipose replica are partially derived from animal sources. The consumable can also include adipose replicas that recapitulate key features of non-animal fats, including texture, flavor, firmness, percent fat release, and/or temperature of fat release. The fat content of the consumable can be at least 1%,

5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, 80%, 90%, or 95% fat.

Ground beef is typically prepared by mixing lean beef with adipose (fat) that is cut from steaks, with the adipose tissue added to 16-30% (Cox 1993). Without adipose, meat passed through a grinder is tough, crumbly, and dries out quickly. Fat is added to lean beef so that fat released during cooking provides a liquid surface to aid in cooking, and to generate key beef flavors, which largely are products of the fatty acids.

Engineering an adipose tissue replica that plays the same key roles in texture and flavor of the plant-based ground beef is an important driver for texture and flavor.

The adipose tissue replicas described here have a great health benefit over the beef adipose tissue as the fatty acid composition can be controlled such that the amount of saturated fats can be decreased. Additionally, the plant-based adipose replicas are cholesterol free. The plant-based adipose replicas can contain a lower percent total fat, and still have the same amount of fat being released or retained for the desired cooking properties, flavor, and texture.

As described herein, adipose replicas comprising emulsions of plant-derived lipids and one or more isolated and purified proteins can be produced in which the composition (e.g., fatty acid composition), cooking characteristics (e.g., fat release temperature or percent fat release), and physical properties (e.g., firmness) can be controlled, allowing the plant based composition to mimic animal based adipose. The adipose tissue replica includes (1) a vegetable oil containing triacylglycerides of fatty acids; (2) one or more isolated and purified proteins from non-animal sources (e.g., a plant protein); and (3) a phospholipid such as lecithin. The proteins can be plant or microbial proteins as described above (e.g., RuBisCo, an oleosin, an albumin, a globulin, or other seed storage protein). See, also the proteins described in Sections III A and B. The vegetable oils can be any of the oils described herein. See, e.g., Section III C.

The fat replica can be a gelled emulsion. In some embodiments, the gel is a soft, elastic gel comprising proteins and optionally carbohydrates. The gelled emulsion can comprise a protein solution comprising multiple proteins, e.g., 1-5 or 1-3 isolated and purified proteins, wherein the protein solution accounts for 1-30% of the volume of the emulsion. The gelled emulsion can comprise a fat droplet, wherein the fat droplet accounts for 70-99% of the volume of the emulsion. The gelled emulsion can comprise an isolated, purified cross-linking enzyme, wherein the cross-linking enzyme accounts for 0.0005% to 0.5% of the emulsion weight by volume, 0.5-2.5% of the emulsion weight by volume, or 0.001% or lower of the emulsion weight by volume. The emulsion of fat droplets in the protein solution can be stabilized by forming the emulsion into a gel by the cross-linking enzyme, e.g., a transglutaminase, by gelling proteins via heating and

cooling protein solutions, by forming a cold-set gel, by formation of a coacervate, or by combinations of these techniques as described for coacervates in section C and gel formation in section F.

In some embodiments, the fat replica comprises cross-linking enzymes that catalyze reactions leading to covalent crosslinks between proteins. Cross-linking enzymes can be used to create or stabilize the desired structure and texture of the adipose tissue replica, to mimic the desired texture of an equivalent desired animal fat. In some embodiments, the cross-linking enzymes are isolated and purified from a non-animal source, examples and embodiments of which are described herein. In some embodiments, the fat replica comprises at least 0.0001%, at least 0.001%, at least 0.01%, at least 0.1%, or at leastl% (wt/vol) of a cross-linking enzyme. The cross-linking enzyme can be selected from, for example, transglutaminases, tyrosinases, lipoxygenases, protein disulfide reductases, protein disulfide isomerases, sulfhydryl oxidases, peroxidases, hexose oxidases, lysyl oxidases, and amine oxidases. In some embodiments, the cross-linking enzyme is transglutaminase, a lysyl oxidase (e.g., a *Pichia pastoris* lysyl oxidase), or other amine oxidase.

~~[0314] In some embodiments the meat substitute product has a fat component. In some embodiments the fat content of the consumable is 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, or 60% fat. In some embodiments, the fat replica comprises a gel with droplets of fat suspended therein. In some embodiments, the gel is a soft, elastic gel comprising proteins and optionally carbohydrates. In particular embodiments, the proteins used in the gel are plant or microbial proteins. In some embodiments, the proteins used in the fat replica might include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microorganisms, or synthesized in vitro. The proteins may be artificially designed to emulate physical properties of animal fat. The proteins may be artificially designed to emulate physical properties of animal fat.~~

~~[0315] The fat droplets used in some embodiments of the present invention can be from a variety of sources. In some embodiments, the sources are non animal sources. In particular embodiments, the sources are plant sources. Non limiting examples of oils include corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, Shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, trig lycerides, monoglycerides, diglycerides, sphingosides, gly colipids, lecithin, lysolecithin, phosphatidic acids, lysophos phatidic acids, oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosa pentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pel argonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosa pentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid.~~

~~[0316]~~ The fat replica can comprise a gel with droplets of fat suspended therein. The fat droplets used in some embodiments of the present invention can be from a variety of

sources. In some embodiments, the sources are non-animal sources (e.g., plant sources). See, e.g., the examples provided in Section III C. In some embodiments, the fat droplets are derived from animal products (e.g., butter, cream, lard, and/or suet). In some embodiments, fat droplets are derived from pulp or seed oil. In other embodiments, the source may be ~~yeast~~ algae, yeasts, oleaginous yeasts such as *Yarrowia lipolytica,* or mold. For instance, in one embodiment ~~the fat droplets comprise~~ , triglycerides derived from *Mortierella* ~~*isabel lina*~~*isabellina* can be used. In some embodiments, the fat droplets contain synthetic or partially synthetic lipids

In some embodiments, the fat droplets are stabilized by addition of surfactants, including but not limited to phospholipids, lecithins, and lipid membranes. The lipid membranes may be derived from algae, fungi or plants. In some embodiments the surfactants comprise less than 5% of the fat replica. The fat droplets can in some examples range from 100 nm to 150 gm in diameter. The diameter of these stabilized

droplets may be obtained by homogenization, high-pressure homogenization, extrusion or sonication.

~~[0317] In some embodiments~~ In some embodiments, plant oils are modified to resemble animal fats. The plant oils can be modified with flavoring or other agents such as heme proteins, amino acids, organic acids, lipids, alcohols, aldehydes, ketones, lactones, furans, sugars, or other flavor precursor, to recapitulate the taste and smell of meat during and after cooking. Accordingly, some aspects of the invention involve methods for testing the qualitative similarity between the cooking properties of animal fat and the cooking properties of plant oils in the consumable.

~~[0318] In some embodiments, the fat replica comprises a protein component comprising one or more isolated, purified proteins. The purified proteins contribute to the taste and texture of the meat replica. In some embodiments purified proteins can stabilize emulsified fats. In some embodiments the purified proteins can form gels upon denaturation or enzymatic crosslinking, which replicate the appearance and texture of animal fat. Examples and embodiments of the one or more isolated, purified proteins are described herein. In particular embodiments, the one or more isolated proteins comprise a protein isolated from the legume family of plants. Non limiting examples of legume plants are described herein, although variations with other legumes are possible. In some embodiments, the legume plant is a pea plant. In some embodiments the isolated purified proteins stabilize emulsions. In some embodiments the isolated purified proteins form gels upon crosslinking or enzymatic crosslinking. In some embodiments, the isolated, purified proteins comprise seed storage proteins. In some embodiments, the isolated, purified proteins comprise albumin. In some embodiments, the isolated, purified proteins comprise globulin. In a particular embodiment, the isolated, purified protein is a purified pea albumin protein. In another particular embodiment, the isolated, purified protein is a purified pea globulin protein. In another particular embodiment the isolate purified protein is a Moong bean 8S globulin. In another particular embodiment, the isolated, purified protein is an oleosin. In another particular embodiment, the isolated, purified protein is a caloleosin. In another particular embodiment, the isolated, purified protein is Rubisco. In some embodiments, the protein component comprises about 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or more of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises about 0.1-5%, about 0.5-10%, about 1-20%, about 5-30%, about 10-50%, about 20-70%, or about 30-90% or more of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises a solution containing one or more isolated, purified proteins.~~

~~[0319] In some embodiments, the fat replica comprises cross-linking enzymes that catalyze reactions leading to covalent cro s slinks between proteins. Cross-linking enzymes can be used to create or stabilize the desired structure and texture of the adipose tissue replica, to mimic the desired texture of an equivalent desired animal fat. Non-limiting examples of cross-linking enzymes include, e.g., trans-glutaminase, lysyl oxidases, or other amine oxidases (e.g. *Pichia pastoris* lysyl oxidase). In some embodiments, the cross-linking enzymes are isolated and purified from a non-animal source, examples and embodiments of which are described herein. In some embodiments, the fat replica comprises at least 0.0001%, or at least 0.001%, or at least 0.01%, or at least 0.1%, or at least 1% (wt/vol) of a cross-linking enzyme. In particular embodiments, the cross-linking enzyme is transglutaminase.~~

In some embodiments, additional polysaccharides can be added to a fat replica, including flax seed polysaccharides and xanthan gum.

The creation of a plant-based adipose replica requires stabilization of the oil in water emulsions. Typically animal adipose tissue contains —95% fat, and is stabilized by the phospholipid bilayer and associated proteins. Adipose replicas described herein can be created with up to 95% fat in some instances, with 80% fat under many conditions, or with

lower amounts of fat (e.g., 50% or less) while mimicking the properties of animal fat. Achieving a high percent fat is controlled by the stabilization of the emulsion.

The composition (e.g., fatty acid composition), cooking characteristics (e.g., fat release temperature or percent fat release), and physical properties (e.g., firmness) can be manipulated by controlling the type and amount of fat, the amount of protein, the type and amount of lecithin, the presence of additives, and the method of gelling.

In some embodiments, the protein component comprises about 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, or 20%, 25%, or more of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises about 0.1-5% or about 0.5-10% or more of the fat replica by dry weight or total weight. In some embodiments, the protein component is 0.5 to 3.5% or 1 to 3% of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises a solution containing one or more isolated, purified proteins. The type of protein can affect the stability of the emulsion, RuBisCo and pea albumins allow for fat replicas to made a greater than 90%

fat. Addition of polysaccharides including flax seed and xanthan gum aid in emulsifying the mixture, allowing for an increase in fat content.

The type and amount of fat can be controlled by choosing the source of the fat and its lipid composition. In general, oils with higher amounts of saturated fatty acids are better able to be emulsified at lower protein concentrations, while oils with more unsaturated fatty acids require higher protein concentrations to be emulsified. Protein is required to stabilize the emulsion, and an increase in protein content increases the stability. If the amount of protein added is too little to emulsify the amount of fat, the mixture will separate into layers.

Lecithin also is a modulator of emulsion, and can either stabilize or disrupt the emulation depending on the amount of protein present and type of oil used. For example, lecithin may disrupt the protein/fat matrix to make a less stable emulsion, but can be added at low levels to modulate other physical properties. Emulsions made from oils with higher amounts of unsaturated fats can be de-stabilized by a high amount of lecithin (1%), such that the emulsion does not solidify. Emulsions made from oils with higher amounts of saturated fats can solidify at high amounts of lecithin (1%), but are very soft.

As described herein adipose replicas can be prepared that can range from very soft to very firm. The composition and amount of the fat controls the firmness of the replica. Firmer oils, which contain more long-chained saturated fats, make firmer gels. Oils that produce softer gels typically contain more unsaturated fatty acids or short-chained saturated fatty acids. In general, the firmness of the gel increases as total percent fat increases, as long as the emulsion is held and does not separate. The amount of protein also contributes to the firmness of the replica. In general, an increase in protein concentration increases replica firmness. The amount of lecithin is a modulator of replica firmness. Higher amounts of lecithin (1%) are much softer than lower amounts of lecithin (0.05%) when gels are formed with high percent protein (3%). When protein is decreased (1.8%), all gels are softer, and there is little difference in firmness between low level of lecithin (0.05%) and high (1%) if emulsion is held.

Addition of polysaccharides to replicas including but not limited to xanthan gum and flax seed paste can increase the firmness of adipose-replica gels.

When an adipose replica is cooked, fat leaks from the structured replica as it is cooked. Often there is fat that remains in the cooked product; it is important to achieve the balance between fat released to aid in cooking and fat retained for texture and taste. The percent fat released (per total fat) can be determined by measuring the amount of fat released upon cooking to completion. Percent fat released is reported as the weight of fat released per the total fat of the replica. For example, the percent fat release of an adipose tissue replica described herein can be 0 to 10%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, 50% to 60%, 60% to 70%, 70% to 80%, 80% to 90%, or 90% to 100% upon cooking. Adipose replicas typically release 0- 90% fat under standard cooking conditions. In comparison, beef adipose tissue typically releases 40-55% fat under equivalent conditions.

While vegetable oils have set melting temperatures, the range of temperatures over which adipose replicas can be made to release fat is wide. Fat release temperature is the temperature at which fat is visibly released from the replica at the cooking surface. As described herein, the fat release temperature of the adipose replica can be tailored based on the type and amount of fat, the amount of protein, the type and amount of lecithin, the presence of additives, the method of emulsification, and the method of gelling. The resulting adipose replicas can have a fat release temperature of between 23°C to 33°C, 34°C to 44°C, 45°C to 55°C, 56°C to 66°C, 67°C to 77°C, 78°C to 88°C, 89°C to 99°C, 100°C to 110°C, 111°C to 121°C, 122°C to 132°C, 133°C to 143°C, 144°C to 154°C, 155°C to 165°C, 166°C to 167°C, 168°C to 169°C, 170°C to 180°C, 181°C to 191°C, 192°C to 202°C, 203°C to 213°C, 214°C to 224°C, 225°C to 235°C, 236°C to 246°C, 247°C to 257°C, 258°C to 268°C, 269°C to 279°C, 280°C to 290°C, or 291°C to 301°C. Beef fat was measured to release fat at 100-150°C.

Emulsification also is a factor in controlling the temperature of fat release: Once fats are incorporated into a replica with protein, or protein and lecithin, the temperature at which the fat is released increases significantly above the temperature at which the fat alone melts.

Fatty acid composition also is a factor in the fat release temperature and percent fat release. Vegetable oils that contain a higher proportion of unsaturated fatty acids have

low melting temperatures and many are liquid at room temperature. Vegetable oils that contain a higher proportion of saturated fatty acids have a higher melting temperature, and are solid at room temperature. Replicas with a greater amount of unsaturated fats have a higher temperature of fat leakage than the same replica made with more saturated fatty acids. Gels made from 75% oils with higher amounts of unsaturated fatty acids, a high protein content (3%), and minimal lecithin content (0.05%), where the mixture was emulsified by a hand-held homogenizer and gelled using the heat-cool method, can be heated to 200°C with little or no fat release. Replicas containing oils with more long-chained saturated fats typically have more fat release at high protein content, but release less percent total fat compared to replicas containing oils with more short chained fats and at low percent protein. Gels made from oils with a higher proportion of short-chained saturated fatty acids, high protein content (3%), and minimal lecithin content (0.05%), can be heated to 200°C with little fat release.

The percent of fat release as an adipose replica is cooked is also is a function of the amount of protein and the amount of lecithin. Typically, the adipose replica contains 1-3% protein by mass. Increasing protein content leads to increasing temperatures of fat release, and reduces the fraction of fat released. Increasing the lecithin content to 1% can decrease the fat release temperature to 60-115°C, and increase the fraction of fat released (e.g., 25-30%). The source or composition of the lecithin used can modulate the amount of fat release and the temperature threshold for fat release. Without being bound to a particular mechanism, it is thought that lecithin destabilizes the emulsion by disrupting the protein-protein interactions. In one embodiment, at a high protein concentration of 3%, increasing the lecithin content to 1% decreased the fat release temperature to 5560°C, and increased the percent fat leaked to 60-65%.

The method of making the emulsion also is a factor in determining the amount of fat release. Emulsification forms a homogenous mixture of the fat held in a matrix of proteins and lecithin. Methods of emulsification can include high-pressure homogenization, sonication, or hand homogenization. The alternative methods result in characteristic differences in the size of the oil droplets in the emulsion, which influences the stability of the resulting emulsions and the maximum fat concentration at which

stable emulsions can be formed.

The method of gelling the replica also is a factor in determining the amount of fat release. While adipose replicas can be formed without forming gels, gelation results in a firmer and more stable emulsion. Methods of gelling are described above, and can include, for example, the addition of a crosslinking enzyme such as a transglutaminase (TG), or subjecting the emulsion to a heat/cool cycle. For example, either treatment with TG or the heat/cool method can convert an emulsion, as described above, into a gel. Furthermore, gelled emulsions formed by crosslinking catalyzed by TG typically release fat at a temperature higher than that at which emulsions gelled by the heat/cool technique do so. Gels formed by crosslinking with a TG also typically release less fat than do gels formed by the heat-cool technique.

In some embodiments, a fat replica can be made with a protein content <1.5% and a minimal lecithin content (0.05%) and have a fat release temperature of 45-65°C, and a high amount of fat released (e.g., 70-90%). These gels are at the higher end of percent fat released.

In some embodiments, a fat replica can be made with a lower protein content (<1.5%) and a high lecithin (>1%), and have a lower fat release temperature (e.g., 3050°C, e.g., 30 to 45°C), and with an intermediate percent fat leaked (45-65%). Thus, in gels formed from oils with short-chained fatty acids or long-chained fatty acids at low protein concentrations, lecithin may play a role in stabilizing the emulsion.

In some embodiments, >2% rubisco or pea albumin can be used to produce adipose replicas with greater than 70% fat. In some embodiments, gels formed with > 3% isolated and purified protein can result in adipose replicas with greater than 70% fat.

In some embodiments, adipose replicas made from oils with a higher proportion of long-chained saturated fatty acids, a protein content of 3%, and a minimal lecithin content (0.05%), can release fat at a temperature similar to that at which beef fat does so (50-100°C), and can release a low to intermediate level of fat (15-45%).

In some embodiments, an adipose replica with a higher protein concentration (> 3%), and a lecithin content > 1% can have a fat release temperature of 50-70°C, and a higher amount of fat release (50-80%). At high protein and low lecithin concentration,

gels with higher saturated fatty acids typically leak about 10% more fat than do corresponding gels formed with unsaturated fats.

In some embodiments, protease treatment of the protein constituents before gel formation can lead to an increase in fat release.

In some embodiments, a adipose tissue replica matrix stabilized by crosslinking enzymes releases more fat than a adipose tissue replica matrix stabilized by heat/cool protein denaturation. In one embodiment, an adipose tissue matrix comprised of moong bean 8S protein and canola oil, or an equal mixture of coconut, cocoa, olive, and palm oils, retains more mass when formed upon heat/cool denaturation than when formed by cross-linking with an enzyme. In one embodiment, an adipose tissue matrix formed by heat/cool denaturation of a preformed protein-oil emulsion containing Rubisco and cocoa butter, has a higher melting temperature than an adipose replica of similar composition stabilized by a cross-linking enzyme.

In some embodiments, adipose tissue replicas constructed from 1.4% wt/v moong bean 8S protein with 90% v/v canola oil and 0.45% wt/v soybean lecithin, can be homogenized in the presence of variable concentrations of sunflower oleosins. Concentration of oleosins can be varied from 1:10 to $1:10^6$ molar ratio of oleosin:triglyceride. An increase in mass retention after cooking is observed as the concentration of oleosins in the adipose tissue replica increase.

Firmness of an adipose tissue replica constructed as stabilized protein-fat emulsion can be modified by varying the concentration of the protein within adiopose tissue replica matrix. For example, a series of adipose tissue replicas formed with varying concentrations of Rubisco with 70-80% v/v sunflower oil, varied in firmness. Adipose tissue replicas with 0% and 0.18% (wt/vol) Rubisco were very soft, whereas replicas formed with 1.6% (wt/vol) Rubisco were soft, and replicas formed with 1.9% (wt/vol) Rubisco were medium in firmness.

In one embodiment, the firmness of adipose replica formed by stabilizing protein oil emulsion can be modified by varying amount of protein in the adipose replica. In one embodiment, adipose tissue replicas made from Rubisco and 70% sunflower oil are softer

at lower concentrations, such as 1%, of RuBisCo than at higher concentrations of Rubisco, such as 3%, in adipose tissue replicas.

[0320] In another aspect, the invention provides methods for making a fat replica. In some embodiments, the fat droplets are suspended in a gel. In some embodiments the present invention provides for methods for producing droplets of fat suspended in the gel. The fat can The fat can be isolated and homogenized. For example an organic solvent mixture can be used to help mix a lipid. The solvent can then be removed solubilize a lipid in a gel and then removed to provide the final gel. At this point, the lipid can be frozen, lyophilized, or stored. So in some aspects one aspect, the invention provides for a method for isolating and storing a lipid, which has been selected to have characteristics similar to animal fat. The lipid film or cake can then be hydrated. The hydration can utilize agitation or temperature changes. The hydration can occur in a precursor solution to a gel. After hydration the lipid suspension can be sonicated, homogenized, high-pressure homogenized or extruded to further alter the properties of the lipid in the solution.

[0321] In some embodiments, the fat replica is assembled to approximate the organization of adipose tissue in meat. In some embodiments some or all of the components of the fat replica are suspended in a gel. In various (e.g., proteinaceous gel). In other embodiments, the gel can be a proteinaceous gel, a hydrogel, an organogel, or a xerogel. In some embodiments, the gel can be thickened to a desired consistency using an agent based on polysaccharides or proteins. For example fecula, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, alginin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, cellulose, methylcel lulose methylcellulose, hydroxymethylcellulose, acadia gum, konjac, starch, pectin, amylopectin or proteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, of fungi can be used alone or in combination to thicken the gel, forming an architecture or structure for the consumable.

In some embodiments, the tensile strength of the fat replica mimics the tensile strength of adipose tissue. The tensile strength of the gelled emulsions can be increased by incorporation of fibers. The fibers may be derived from non-animal sources

including, but not limited to, watermelon, jack fruit, squash, coconut, green hair algae, corn and/or cotton. In some embodiments, the fibers are derived from self-polymerization of proteins, e.g., oleosins and prolamine. In some embodiments the fibers

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are derived from electrospun or extruded proteins. The fibers may form a three dimensional mesh or strands wherein each fiber may be less than 1 mm in diameter.

The adipose replica can be an emulsion comprising a solution of one or more proteins and one or more fats suspended therein as droplets. Slowly adding the oil phase to the aqueous phase can provide a more robust emulsion and prevents occasional failures to emulsify. Adding lecithin can, in some circumstances, destabilize a protein-stabilized emulsion, allowing for increased fat leakage when the replica is cooked. In some embodiments, the emulsion is stabilized by one or more cross-linking enzymes into a gel. In some embodiments, the emulsion is stabilized by a matrix formed by proteins induced to gel by a heat-cool technique or a cold-set-gel technique. Heating a protein-stabilized emulsion can heat denature the proteins leading to an increase in the firmness of the adipose replica. Heating to a sufficient temperature also can reduce the viability of natural microflora by at least 100x. In some embodiments, the emulsion is stabilized by gelled protein matrix formed by a combination of one or more protein cross-linking enzymes and a heat/cool technique or a cold-set gel technique. After the emulsion has sufficiently cooled, but before gelling is complete, one or more optional ingredients can be added, such as a heme-containing protein (e.g., up to about 0.4% such as 0.15, 0.2., 0.25, 0.3, or 0.4%) to give the adipose a more natural looking pink color and/or one of more flavor compounds such as amino acids, sugars, thiamine, or phospholipids to provide an improved flavor to the final product.

~~[0322] In particular embodiments, the fat replica is an emulsion comprising a solution of one or more proteins and one or more fats suspended therein as droplets. In some embodiments, the emulsion is stabilized by one or more cross-linking enzymes into a gel. In more particular embodiments, the one or more proteins in solution are isolated, purified proteins. In yet more particular embodiments, the isolated, purified proteins comprise a purified pea albumin enriched fraction. In other more particular embodiments, the isolated, purified proteins comprise~~ The one or more proteins in solution can comprise isolated and purified proteins, e.g. a purified pea albumin enriched fraction, a purified pea globulin enriched fraction~~. In other more particular embodiments~~, ~~the isolated, purified proteins comprise~~ a purified Moong bean 8S globulin enriched fraction~~. In yet more particular embodiments~~, ~~the isolated, purified proteins comprise~~ and/or a Rubisco enriched fraction. In other ~~particular embodiments, the one or more fats are derived from plant-based oils. In more particular embodiments, the one or more fats are derived from one or more of: corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil,~~

~~safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, Shea butter,~~embodiments, the one or more fats are derived from plant-derived oils (a rice bran oil or canola oil). See, e.g., Section III C. In some cases the composition comprises a cross-linking enzyme such as a transglutaminase, lysyl oxidase, or other amine oxidase. Thus, in some embodiments, an adipose tissue replica can be made by isolating and purifying one or more proteins; preparing a solution comprising one or more proteins; emulsifying one or more fats in the solution; and stabilizing the solution into a gelled emulsification with one or more cross-linking reagents.

In some embodiments, the fat replica is a high fat emulsion comprising a protein solution of purified pea albumin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel.

In some embodiments, the fat replica is a high fat emulsion comprising a protein solution of isolated moong bean 8S globulin emulsified with 40-80% rice bran oil or 4080% canola oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel.

The fat can be isolated from plant tissues and emulsified. The emulsification can utilize high-speed blending, homogenization, high pressure homogenization, sonication, shearing, agitation or temperature changes. The lipid suspension can be sonicated or extruded to further alter the properties of the lipid in the solution. At this point, in some embodiments other components of the consumable are added to the solution followed by a gelling agent. In some embodiments crosslinking agents (e.g. transglutaminase or lysyl oxidase) are added to bind the components of the consumable. In other embodiments the gelling agent is added and the lipid/gel suspension is later combined with additional components of the consumable.

CONTROL OF MELTING POINT BY CONTROL OF FAT COMPOSITION

The process of cooking meat is integral to the experience of using and enjoying meat. One important property of meat is that as the meat is heated, fats are released from the meat, which lubricates the cooking surface and increases heat transfer and is a component of the visual, aural and olfactory experience of cooking meat. The amount of fat that is released rather than retained during cooking varies with cooking temperature and contributes to the visual, aural and olfactory experience of cooking meat.

The composition and ratio of fatty acids in triglycerides and phospholipids, along with the ratio of phospholipid headgroups, contribute to the generation of distinct flavor profiles of cooked meat. For example, increased levels of phosphatidylcholine and phosphatidylethanolamine in fat provide a more intense beef flavor. As discussed above, the flavor of meat replicas can be modified by varying the ratios and type of different oils, and phospholipids that comprise the meat replica. For example, the flavor of the cooked meat replica can be controlled by varying the amount of phospholipids, sterols

and lipids (e.g., 0.2-1% wt/wt). In one embodiment, the flavor of the cooked meat replica can be controlled by varying the ratio of different phospholipid headgroups.

In some embodiments, the phospholipids comprise a plurality of amphipathic molecules comprising fatty acids, glycerol and polar groups. See, e.g., Section III C for examples of fatty acids, phospholipids, polar groups, and sterols associated with phospholipids. See also section III C for examples of useful plant oils.

In different cuts of meat, the fat has different properties, ranging from the structurally important nature of fat in bacon to the soft melting behavior of the marbling fat in Wagyu beef.

By controlling the melting point of adipose tissue replicas in the consumables, it is possible to replicate the cooking experience of different meat types. For example, adipose tissue replicas created from fats with a melting point of 23°C to 27°C can have melting points analogous to adipose tissue from wagyu beef; adipose tissue replicas created from fats with a melting point of 35°C to 40°C can have melting points analogous to adipose tissue from regular ground beef; and adipose tissue replicas created from fats with a melting point of 36°C to 45°C can have melting points analogous to adipose tissue from bacon. Adipose tissue replicas can be created and incorporated into consumables such that a ratio of fat which is released and the ratio of fat which is retained by adipose tissue replica during cooking is similar to the fat properties of meat, e.g. from ground beef.

In some embodiments, the fat release temperature of a fat replicas can be controlled by mixing different ratios of vegetable oils containing triacylglycerides and phospholipids (e.g., lecithin). The melting point of fats is governed by the chemical composition of fatty acids. In general, fats comprising saturated fatty acids (e.g., C10:0, C12:0, C14:0, C16:0, C18:0, C20:0, C22:0) are solid at refrigeration temperatures (e.g., about 1°C to about 5°C and at room temperature (e.g., about 20°C to 25°C). By controlling the fat release temperature upon cooking in a adipose tissue replica, the firmness of a adipose tissue replica during refrigeration (e.g., about 1.5°C to about 4°C) and at ambient temperature (e.g., about 20°C to 25°C) can be controlled. Fats comprising monounsaturated fatty acids (e.g., C16:1 or C18:1) are generally solid at refrigeration

temperatures and liquid at room temperatures. Fats comprising polyunsaturated fatty acids (e.g., C18:2, C18:3, C20:5, or C22:6) are generally liquid at refrigeration temperatures and at room temperatures. For example, virgin coconut oil melts at about 24°C while hydrogenated coconut oil melts at 36-40°C.

For example, adipose tissue replicas containing triglycerides and phospholipids that are liquid at room temperature (about 20°C to 25°C) will be softer than adipose tissue replicas containing triglycerides and phospholipids that are solid at refrigeration temperatures.

Adipose tissue replicas can contain oils from a single or multiple sources that are liquid at both refrigeration and ambient room temperatures (e.g., canola oil, sunflower oil, and/or hazelnut oil). In one embodiment, a adipose tissue replica contains oils from single or multiple sources that are solid at refrigeration temperature, but liquid at room temperature (e.g., olive oil, palm oil, and/or rice brain oil). In one embodiment, a adipose tissue replica contains oils from single or multiple sources that are solid at room temperature but liquid at mouth-temperatures (about 37°C) (e.g., palm kernel oil, coconut oil, and/or cocoa butter. In one embodiment, a adipose tissue replica contains oils from single or multiple sources that are solid at mouth temperature (about 37°C) (e.g., oil from mango butter).

In one embodiment, a adipose tissue replica includes triglycerides and phospholipids with a high ratio of saturated fatty acids, and is firmer than a adipose tissue replica containing a higher ratio of monounsaturated and polyunsaturated triglycerides and lipids. For example, a adipose tissue replica containing sunflower oil is softer than a adipose tissue replica containing cocoa butter. Adipose tissue replicas can be formed with 0%, 0.18%, 1.6%, or 2.4% wt/v Rubisco with 70%, 80%, or 90% v/v sunflower or cocoa butter. Each adipose tissue replica that contained cocoa butter was firmer than the replicas that were formed with sunflower oil.

In one embodiment, a adipose tissue replica made as a stable emulsion of moong bean 8S protein with sunflower oil is softer than a adipose tissue replica made as a stable emulsion of moong bean 8S protein and cocoa butter. Adipose tissue replicas formed with 2%, 1%, or 0.5% wt/v moong bean 8S protein with 70%, 80%, or 90% v/v

sunflower or cocoa butters. Each adipose tissue replica that contained cocoa butter was firmer than the replicas that were formed with sunflower oil.

In one embodiment, a adipose tissue replica made as a stable emulsion of moong bean 8S protein with canola oil is softer than a adipose tissue replica made as a stable emulsion of moong bean 8S protein with an equal mixture of coconut, cocoa, olive, and palm oils. Adipose tissue replicas can be formed with 1.4% wt/v moong bean 8S protein with 50%, 70%, or 90% v/v sunflower or a mixture of oils. Each adipose tissue replica that contained a mixture of oils was firmer than the replicas that were formed with sunflower oil.

In one embodiment, an adipose tissue replica made as a stable emulsion of soy proteins with sunflower oil is softer than a adipose tissue replica made as a stable emulsion of soy proteins and cocoa butter. Adipose tissue replicas were formed with 0.6%, 1.6%, or 2.6% wt/v Soy with 50%, 70%, 80%, or 90% v/v sunflower or mixture of oils. Each adipose tissue replica that contained a mixture of oils was firmer than the replicas that were formed with sunflower oil.

In some embodiments, the adipose tissue replicas comprising 0%, 0.18%, 1.6%, and 2.4% wt/v Rubisco with 70%, 80%, and 90% v/v cocoa butter are solid at room temperature but melt at about mouth temperature. In some embodiments, the adipose tissue replicas comprising 0.6%, 1.6%, and 2.6% wt/v soy with 50%, 70%, 80%, and 90% v/v cocoa butter are solid at room temperature but melt at about mouth temperature. In some embodiments, the adipose tissue replicas comprising 1.4% wt/v moong bean 8S protein with 50%, 70%, and 90% v/v of an equal mixture of coconut, cocoa, olive, and palm oil is solid at room temperature but melts at about mouth temperature. In one embodiment, the melting temperature of adipose tissue replicas will be similar to beef fat. In some embodiments the fat replicas comprise oils with a 1:1 ratio of saturated to unsaturated fatty acids. In some embodiments, the adipose tissue replica contains equal amounts of cocoa and mango butters. In some embodiments, the adipose tissue replica contains equal amounts of coconut oil, cocoa butter, olive oil and palm oil.

In one embodiment, a adipose tissue replica that comprises triglycerides and phospholipids will contain a ratio of fatty acids similar to that found in beef (C14:0 0-5%

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wt/wt, C16:0 0-25%, C18:0 0-20%, C18:1 0-60%, C18:2 0-25%, C18:3, 0-5%, C20:4 0-2%, and C20:6 0-2%). For example the adipose tissue replica may comprise equal proportions of olive oil, cocoa butter, coconut oil and mango butter. In another example, the adipose tissue replica may comprise equal proportions of olive oil and rice brain oil.

In one embodiment, the melting temperature of adipose tissue replicas will be similar to Wagyu beef fat. In some embodiments the fat replicas comprise oils with a 1:2 ratio of saturated to unsaturated fatty acids (e.g., for example 1 part coconut oil to 2 parts sunflower oil). In some embodiments, the adipose tissue replica contains equal amounts of olive oil, rice bran oil, cocoa butter and mango butter.

I. CONNECTIVE TISSUE REPLICA

~~mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phosphatidic acids, lysophosphatidic acids, oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, unde canoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pel argonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid. In yet even more particular embodiments, the one or more fats is a rice bran oil. In another particular embodiment, the one or more fats is a canola oil. In other particular embodiments, the cross linking enzyme is transglutaminase, lysyl oxidase, or other amine oxidase. In yet even more particular embodiments, the cross linking enzyme is transglutaminase. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of purified pea albumin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/ vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially purified moong bean 8S globulin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/ vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially purified moong bean 8S globulin emulsified with 40-80% canola oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of purified pea albumin emulsified with 40-80% rice bran oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially purified moong bean 8S globulin emulsified with 40-80% rice bran oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially purified moong bean 8S globulin emulsified with 40-80% canola oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel.~~

~~[0323] Connective Tissue Replica~~

~~[0324]~~ Animal connective tissue provides key textural features that are an important component of the experience of eating meat. Accordingly, the present invention provides a composition derived from non-animal sources which recapitulates key features of animal connective tissue. ~~In another aspect, the~~ The present invention additionally provides a meat substitute ~~prod uct~~ product that comprises a composition derived from non-animal

sources~~.~~ , which recapitulates important textural and visual features of animal connective tissue. Such ~~a composition~~ compositions will be labeled herein as "connective tissue ~~replica~~ replicas". In some embodiments, the connective tissue replica and/or meat substitute product comprising the connective tissue replica are partially derived from animal sources.

~~[0325]~~ Animal connective tissue can generally be divided into fascia-type and cartilage-type tissue. Fascia-type tissue is highly fibrous, resistant against extension (has high elastic modulus), and has a high protein content, a moderate water content (ca. 50%), and ~~low to none~~ low-to-no fat and polysaccharide content. Accordingly, the present invention provides a connective tissue replica that recapitulates key features of fascia type tissue. In some embodiments, the connective tissue replica comprises about 50% protein by total weight, about 50% by liquid weight, and has a low fat and polysaccharide component.

~~[0326] The protein content of most fascia-type connective tissue is comprised mainly of collagen. Collagen is characterized by a high fraction of proline and alanine, and also is assembled into characteristic elongated fibrils or rod-like, flexible structures. Prolamins are one family of proteins found in non-animal sources, such as plant sources. Prola-mins are highly abundant in plants and are similar in amino acid composition to collagen. Among proteins we tested for this purpose, prolamins were particularly favorable because of their low cost and their ability to readily form fibers or sheets when spun or extruded. Non limiting examples of prolamin family proteins include, e.g., zein (found in corn), these include hordein from barley, gliadin from wheat, seca lin, extensins from rye, kafirin from sorghum, avenin from oats. In fascia type connective tissue, the prolamin family of proteins, individually or combinations thereof, demonstrates suitability for the protein component because they are highly abundant, similar in global amino acid composition to collagen (high fraction of proline and alanine), and amenable to processing into films and fibers. In addition to zein (found in corn), these include hordein from barley, gliadin from wheat, secalin, extensins from rye, kafirin from sorghum, avenin from oats. Other proteins may be necessary to supplement prolamins in order to achieve targets specifications for physicochemical and nutritional properties. The list of potential candidates here is essentially open and may include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microorganisms, or synthesized in vitro. The proteins may be artifi-cially designed to emulate physical properties of animal connective tissue. animal-derived or recombinant collagen, extensins (hydroxyproline-rich glycoproteins abundant in cell walls e.g. *Arabidopsis thaliana,* monomers of which are "collagen-like" rod-like flexible molecules). The proteins may be artificially designed to emulate physical properties of animal connective tissue.~~

~~[0327] Methods for forming fascia-type connective tissue will be as those practiced in the art with a bias towards methods producing fibrous or fibrous-like structures by biological, chemical, or physical means, individually or in combination, serially or in parallel, before final forming. These methods may include extrusion or spinning.~~

~~[0328] Cartilage-type tissue is macroscopically homogenous, resistant against compression, has higher water content (up to 80%), lower protein (collagen) content, and higher polysaccharide (proteoglycans) contents (ca. 10% each).~~

~~[0329] Compositionally, cartilage-type connective tissue will be very similar to fascia-type tissue with the relative ratios of each adjusted to more closely mimic 'meat' connective tissue.~~

~~[0330] Methods for forming cartilage-type connective tissue will be similar to those for fascia-type connective tissue, but with a bias towards methods producing isotropically homogenous structures.~~

The fibrous nature of fascia type connective tissue is largely comprised of collagen fibers. Collagen fibers are observed to be cord or tape shaped species, 1-20 microns wide. These fibers consist of closely packed thin collagen fibrils 30 to 100

nanometers thick. These fibrils also associate into elastic and reticular fibrous networks with individual fibers that may be 200 nanometers thick.

In one embodiment, the fascia-type connective tissue replica consists of a fibrous or fibrous-like structure which can consist of proteins. In some embodiments, the protein content is derived from non-animal source (e.g., a plant source, algae, bacteria, or fungi, see e.g., Sections IIIA and B). In some embodiments, the isolated proteins account for 50%, 60%, 70%, 80%, or 90% or more of the protein content by weight. In some embodiments, multiple isolated proteins are isolated and purified separately and account for the total protein content.

~~[0331] The fat can be suspended in a gel. In some embodiments the present invention provides for methods for producing droplets of fat suspended in the proteinaceous gel. The fat can be isolated from plant tissues and emulsified. The emulsification can utilize high-speed blending, homogenization, agitation or temperature changes. The lipid suspension can be sonicated or extruded to further alter the properties of the lipid in the solution. At this point, in some embodiments other components of the consumable are added to the solution followed by a gelling agent. In some embodiments crosslinking agents (e.g. transglutaminase or lysyl oxidase) are added to bind the components of the consumable. In other embodiments the gelling agent is added and the lipid/gel suspension is later combined with additional components of the consumable.~~ In fascia-type connective tissue, the prolamin family of proteins, individually or combinations thereof, demonstrates suitability for the protein component because they are highly abundant, similar in global amino acid composition to collagen (high fraction of proline and alanine), and amenable to processing into films. In ~~addition to~~ some embodiments, the prolamin family proteins are selected from the group consisting of zein (found in corn), ~~these include~~ hordein from barley, gliadin from wheat, secalin, ~~extensions~~ extensins from rye, kafirin from sorghum, or avenin from oats. ~~Other proteins may be necessary~~ In some embodiments, the one or more isolated and purified proteins is zein. In some embodiments, other proteins can be used to supplement prolamine in order to achieve targets specifications for physicochemical and nutritional properties. ~~The list of potential candidates here is essentially open and may include~~ See, the list in Sections III A and B, including any major seed storage proteins, animal-derived or recombinant collagen, or extensins (hydroxyproline-rich glycoproteins abundant in cell walls e.g. *Arabidopsis thaliana,* monomers of which are "collagen-like" rod-like flexible molecules).

The proteins can be freeze-dried and milled and combined with one or more other ingredients (e.g., wheat gluten, fiber such as bamboo fiber, or soy protein isolate).

The fibrous or fibrous-like structures can be formed by extrusion. In some embodiments extrusion are conducted using Leistritz Nano-16 twin-screw co-rotating extruder (American Leistritz Extruder Corp. USA, Sommerville, NJ). Active heating and cooling of the barrel section is used to optimize the mechanical properties, extent of puffing and water content of fibers. For example, water content can be adjusted to about 50% to make a hard connective tissue replica. Protein feed and liquid are added

separately: protein is feed by a volumetric plunger feeder, and liquid is added into the barrel through a high pressure liquid injection system. In some examples, extrusion parameters were: screw speed 200 rpm, product temperature at the die of 120°C, feed rate of 2.3 g/min, and water-flow rate of 0.7 g/min. Product temperature at the die during extrusion is measured by a thermocouple.

The fibrous or fibrous-like structures can be formed by extrusion through filament and multi-filament dies to produce fibrous structures. In some embodiments, dies incorporating multiple different orifice sizes in the range 10-300 microns can be used to create mixed fibrous tissue replicas with precise control over dimensions and compositions of fibers. Fibers of different sizes can be incorporated into the compositions to control the properties of the compositions.

Electrospinning can be used to create fibers in the <1-10 micron range. In some embodiments, electrospinning is used to create fibers in the <1-10 micron diameter range. For example, a concentrated solution of moong bean globulin (140 mg/ml) containing 400 mM sodium chloride can be mixed with solution of poly(vinyl alcohol) or poly(ethylene oxide) (9% w/v) to obtain mixed solutions with 22.5 mg/ml moong bean globulin and 6.75% w/v of the respective polymer. The resulting solution is slowly (for example, at 3 µl/min) pumped, using a syringe pump, from a 5 ml syringe through a Teflon tube and a blunted 21 gauge needle. The needle is connected to a positive terminal of a high voltage supply (for example, Spellman CZE 30 kV) and fixed 20-30 cm from a collection electrode. Collection electrode is an aluminum drum (ca. 12 cm long, 5 cm in diameter) that is wrapped in aluminum foil. The drum is attached to a spindle that is rotated by an IKA RW20 motor at about 600 rpm. The spindle is connected to a ground terminal of the high voltage supply. Portein/polymer fiber accumulate on foil and, after electrospinning is completed, are removed from foil and added to tissue replicas.

The dimension and composition of the fibers produced by the methods of the invention has an effect upon the taste, texture, and mechanical properties of the tissue replicas. Tissues comprising between 1 and 50% of the fibers in the <1-10 micron range, and between 10 and 50% of fibers in the 10-300 micron range most closely approach animal connective tissues in terms of taste, mouthfeel and mechanical properties.

Cartilage-type tissue is macroscopically homogenous, resistant against compression, has higher water content (up to 80%), lower protein (collagen) content, and higher polysaccharide (proteoglycans) contents (ca. 10% each). Compositionally, cartilage-type connective tissue replicas are similar to fascia-type tissue replicas with the relative ratios of each adjusted to more closely mimic 'meat' connective tissue. During extrusion, water content can be adjusted to about 60% to make a soft connective tissue replica.

Methods for forming cartilage-type connective tissue are similar to those for fascia-type connective tissue, but methods that produce isotropic non-fibrous gels are preferred.

A connective tissue replica can be made by isolating and purifying one or more proteins; and precipitating the one or more proteins, wherein the precipitating results in the one or more proteins forming physical structures approximating the physical organization of connective tissue. The precipitating can comprise solubilizing the one or more proteins in a first solution; and extruding the first solution into a second solution, wherein the one or more proteins is insoluble in the second solution, wherein the extruding induces precipitation of the one or more proteins.

~~[0332]~~ In some embodiments some or all of the components of the consumable are suspended in a gel (e.g., a proteinacious gel). In various embodiments the gel can be a hydrogel, an organogel, or a xerogel~~,~~. The gel can be ~~made thick~~ thickened using an agent based on polysaccharides or proteins. For example fecula, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, ~~algi nin~~alginin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, cellulose, methylcellulose, hydroxymethylcellulose, acadia gum, ~~kon jac~~konjac, starch, pectin, amylopectin or proteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, of fungi can be used alone or in combination to thicken the gel, forming an architecture or structure for the consumable. Enzymes that catalyze reactions leading to covalent crosslinks between proteins can also be used alone or in combination to form an architecture or structure for the consumable. For example ~~transclutaminase~~transglutaminase, tyrosinases, lysyl oxidases, or other

amine oxidases (e.g. *Pichia pastoris* lysyl oxidase (PPLO)) can be used alone or in combination to form an architecture or structure for the consumable by crosslinking the component proteins. In some embodiments, multiple gels with different components are combined to form the consumable. For example a gel containing a ~~plant-based~~ plant-derived protein can be associated with a gel containing a ~~plant-based~~ plant-derived fat. In some embodiments fibers or ~~stings~~ strings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

~~[0333]~~ The compositions of the invention can be puffed or expanded by heating, such as frying, baking, microwave heating, heating in a forced air system, heating in an air tunnel, and the like, according to methods well known in the art.~~[0334]~~

In some embodiments multiple gels with different components are combined to form the consumable. For example a gel containing a ~~plant-based~~ plant-derived protein can be associated with a gel containing a ~~plant-based~~ plant-derived fat. In some embodiments fibers or strings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

~~[0335] In some embodiments the meat replica contains no animal products, less than 1% wheat gluten, no methylcellu-lose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.~~
~~[0336] In some embodiments the meat replica contains no animal products, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.~~
~~[0337] In some embodiments the meat replica contains no animal products, no soy protein isolate, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.~~
~~[0338] In some embodiments the meat replica contains no animal products, no soy protein concentrate, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.~~
~~[0339] In some embodiments the meat replica contains no animal products, no soy protein, no wheat gluten, no methyl cellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.~~
~~[0340] In some embodiments the meat replica contains no animal products, no tofu, no wheat gluten, no methylcellu-lose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.~~
~~[0341] In some embodiments the meat replica contains no animal products, no tofu, and no wheat gluten.~~
~~[0342] In some embodiments the meat replica contains no animal products, no soy protein, and no wheat gluten.~~
~~[0343] In some embodiments the meat replica contains no methylcellulose, no carrageenan, no caramel color, no Konjac flour, no gum Arabic, and no acacia gum.~~

J. OMISSIONS FROM THE COMPOSITIONS

Because the consumable can be put together from defined ingredients, which may themselves be isolated and purified, it is possible to produce consumables that do not contain certain components. This, in some cases, allows for the production of consumables that are lacking ingredients that may be not desirable to consumers (e.g., proteins that some humans are allergic to can be omitted or additives). In some embodiments, the

consumable contains no animal products. In some embodiments the consumable contains no or less than 1% wheat gluten. In some embodiments the consumable contains no methylcellulose. In some embodiments the consumable contains no carrageenan. In some embodiments the consumable contains no caramel color. In some embodiments the consumable contains no Konjac flour. In some embodiments the consumable contains no gum arabic (also known as acacia gum). In some embodiments the consumable contains no wheat gluten. In some embodiments the consumable contains no soy protein isolate. In some embodiments the consumable contains no tofu. In some

~~[0344] In some embodiments the meat replica contains no animal products and~~ embodiments the consumable contains less than 5% carbohydrates. In some embodiments the consumable contains less than 1% cellulose. In some embodiments the consumable contains less than 5% cellulose. In some embodiments the consumable contains less than 5% insoluble carbohydrates. In some embodiments the consumable contains less than 1% insoluble carbohydrates. In some embodiments the consumable contains no artificial color. In some embodiments the consumable contains no artificial flavorings.

In some embodiments the consumable contains one or more of the following characteristics: no animal products; no methylcellulose; no carrageenan; no Konjac flour; no gum arabic; less than 1% wheat gluten; no wheat gluten; no tofu; about 5% carbohydrates; less than 5% cellulose; less than 5% insoluble carbohydrates; less than 1% insoluble carbohydrates; no edible colorants such as caramel color, paprika, cinnamon, beet color, carrot oil, tomato lycopene extract, raspberry powder, carmine, cochineal extract, annatto, turmeric, saffron, F.D&C Red No.3, Yellow number 5, Yellow No 6., Green No.3, Blue No.2, Blue No. 1, Violet No. 1,FD&C Red No. 40 — Allura Red AC, and/or E129 (red shade); and/or no artificial flavorings. In some embodiments the consumable contains no soy protein isolate. In other embodiments, the consumable contains no soy protein or protein concentrate.

In some embodiments, the muscle tissue replica additionally contains less than 10%, less than 5%, less than 1%, or less than 0.1% wheat gluten. In some embodiments, the muscle tissue replica contains no wheat gluten.

IV. Combinations of the components

A. MEAT REPLICAS

A meat substitute product (alternatively a meat replica) can comprise compositions described herein. For example a meat replica can comprise a muscle replica; a fat tissue replica; and a connective tissue replica (or a sub-combination thereof). The muscle replica, adipose tissue replica, and/or connective tissue replica can be assembled in a manner that approximates the physical organization of meat. In some

embodiments, a binding agent such as a coacervate is used to help binding the replicas to each other.

~~[0345] In some embodiments the meat replica contains no animal products, no soy protein, no wheat gluten, no methyl cellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum and less than 5% carbohydrates.~~
~~[0346] In some embodiments the meat replica contains no animal products, and less than 1% cellulose.~~
~~[0347] In some embodiments the meat replica contains no animal products, and less than 5% insoluble carbohydrates. [0348] In some embodiments the meat replica contains no animal products, no soy protein, and less than 1% cellulose. [0349] In some embodiments the meat replica contains no animal products, no soy protein, and less than 5% insoluble carbohydrates.~~
~~[0350] In some embodiments the meat replica contains no animal products, no wheat gluten, and less than 1% cellulose. [0351] In some embodiments the meat replica contains no animal products, no wheat gluten, and less than 5% insoluble carbohydrates.~~

~~[0352]~~ The percentage of different components may also be controlled. For example non-animal-based substitutes for muscle, ~~fat~~ adipose tissue, connective tissue, and blood components can be combined in different ratios and physical organizations to best approximate the look and feel of meat. The various ~~can also~~ components can be arranged to insure consistency between bites of the consumable. The components can be arranged to insure that no waste is generated from the consumable. For example, while a traditional cut of meat may have portions that are not typically eaten, a meat replicate can improve upon meat by not including these inedible portions (e.g, bone, cartilage, connective tissue, or other materials commonly referred to as gristle). Such an improvement allows for all of the product made or shipped to be consumed, which cuts down on waste and shipping costs. Alternatively, a meat replica may include inedible portions to mimic the experience of meat consumption. Such portions can include bone, cartilage, connective tissue, or other materials commonly referred to as gristle, or materials included simulating these components. In some embodiments the consumable may contain simulated inedible portions of meat products which are designed to serve secondary functions. For example a simulated bone can be designed to disperse heat during cooking, making the cooking of the consumable faster or more uniform than meat. In other embodiments a simulated bone may also serve to keep the consumable at a constant temperature during shipping. In other embodiments, the simulated inedible portions may be biodegradable~~.~~ (e.g., a biodegradable plastic).

~~[0353]~~ In some embodiments ~~the~~, a meat substitute ~~compositions contains no animal protein, comprising~~ composition comprises between 10-30% protein, between 5-80% water, and between 5-70% fat, ~~comprising~~ wherein the composition includes one or more isolated and

purified proteins. ~~In particular~~ Such a meat substitute can include no animal protein. In some embodiments, the meat substitute compositions comprise a transglutaminase.

~~[0354]~~ In some embodiments, a meat substitute product includes a muscle replica, an adipose tissue replica, and connective tissue replica, where the muscle replica accounts for 40-90% of the product by weight, the adipose tissue replica accounts for 1-60% of the

product by weight, and the connective tissue replica accounts for 1-30% of the product by weight.

In some embodiments, the meat substitute product comprises 60-90% water; 530% protein content; and 1-20% of a fat; wherein the protein content comprises one or more isolated and purified plant proteins.

In some embodiments the consumable contains components to replicate the components of meat. The main component of meat is typically skeletal muscle. Skeletal muscle typically consists of roughly 75 percent water, 19 percent protein, 2.5 percent intramuscular fat, 1.2 percent carbohydrates and 2.3 percent other soluble non-protein substances. These include organic acids, sulfur compounds, nitrogenous compounds, such as amino acids and nucleotides, and inorganic substances such as minerals. Accordingly, some embodiments of the present invention provide for replicating approximations of this composition for the consumable. ~~F or~~ For example, in some embodiments~~,~~, the consumable is a plant-based meat replica ~~can comprise~~ comprising roughly 75% water, 19% protein, 2.5% fat, 1.2% carbohydrates; and 2.3 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 60-90% water, 10-30% protein, 1-20% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 60-90% water, 5-10% protein, 1-20% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 0-50% water, 5-30% protein, 20-80%% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances.

In some embodiments, ~~the~~ a meat replica contains between 0.01% and 5% by weight of a heme containing protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin. Some meat also contains myoglobin, a heme containing protein, which accounts for most of the red color and iron content of some ~~meat. In some embodiments, the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin~~meat. It is understood that these percentages can vary in meat and the meat replicas can be produced to approximate the

natural variation in meat. In embodiments that include a heme-containing protein and optional flavors, k-carrageenan can be used absorb some of

the liquid contributed from the flavor and heme solution so the ground tissue is not excessively wet. During addition of flavor heme mix solution and the k-carrageenan powder are distributed evenly over the tissue mixture to ensure homogeneity in the final ground product.

It will be appreciated that when proteins are supplied as a solution, water removal techniques such as freeze-drying or spray drying can optionally be used to concentrate the protein. The proteins may then be reconstituted in an amount of liquid that prevents the ground tissue from being too moist.

Additionally, in some instances, the present invention provides for improved meat replicas, which comprise these components in ~~typically~~ unnatural percentages. The concentration of heme containing protein is an important determinant of meat flavor and aroma. Thus for example a meat replica could have a higher heme protein content than typical beef. For example a meat replica can be produced with a higher than typical average fat content. The percentages of these components may also be altered to increase other desirable properties.

~~[0355]~~ In some instances a meat replica is designed so that, when cooked, the percentages of components are similar to cooked meat. So, in some embodiments, the uncooked consumable has different percentages of components than uncooked meat, but when cooked~~,~~ the consumable is similar to cooked meat. For example, a meat replica may be made with a higher than typical water content for raw meat, but when cooked in a microwave~~,~~ the resulting product has non-starch polysaccharides such as arabinoxylans, cellulose, and many other plant components such as resistant starch, resistant dextrins, inulin, lignin, waxes, chitins, pectins, beta-glucans, and oligosaccharide percentages of components similar to meat cooked over a fire.

~~[0356]~~ In some embodiments~~,~~ the consumable is a meat replica with a lower that typical water content for meat. In some embodiments the inventions provides for methods for hydrating a meat replica to cause the meat replica to have a water content similar to meat. For example a meat replica with a water content that would be low for meat, for example 1%, 10%, 20%, 30%, 40% or 50% water, ~~is~~ can be hydrated to roughly 75% water. Once hydrated, in some embodiments, the meat replica is then cooked for human consumption.

~~[0357]~~ The consumable can have a protein component. In some embodiments the protein content of the consumable is 10%, 20%, 30%, or 40%. In some embodiments the protein content of the consumable is similar to meat. In some embodiments the protein content in the consumable is greater than that of meat. In some embodiments the consumable has less protein than meat.

~~[0358]~~ The protein in the consumable can come from a variety or combination of sources. Non-animal sources can provide some or all of the protein in the consumable. Non-animal sources can include vegetables, non food biomass such as carrot tops and *Miscanthus,* seaweed, fruits, nuts, grains, algae, bacteria, or fungi. See, e.g, Sections III A and B. The protein can be isolated or concentrated from one or more of these sources. In some embodiments the consumable is a meat replica comprising protein only obtained from non-animal sources.

~~[0359]~~ In some embodiments protein is formed into asymmetric fibers for incorporation into the consumable. In some embodiments these fibers replicate muscle fibers. In some embodiments the protein are spun fibers. Accordingly, the present invention provides for methods for producing asymmetric or spun protein fibers. In some embodiments the consumable contains a protein or proteins that have all of the amino acids found in proteins that are essential for human nutrition. In some embodiments the proteins added to the consumable are supplemented with amino acids.

~~[0360] Indicators of Cooking Meat~~

The physical organization can be a determinant of the response of the meat substitute to cooking. For example, the flavor of meat is modified by the size of the particles. Ground meat that has been reduced to a paste provides different flavors than more crudely ground beef upon cooking. The ability to control the relative size and orientation of individual tissue replicas enables the flavor and aroma profile of consumables to be modified during cooking. For example, muscle tissue replicas and adipose tissue replicas provide different flavor profiles when cooked independently or when mixed. Further changes in the flavor profile are observed based on the method by which the different tissue replicas are mingled.

The physical organization of the meat substitute product can be manipulated by controlling the localization, organization, assembly, or orientation of the muscle, fat,

and/or connective tissue replicas described herein. In some embodiments the product is designed in such a way that the replicas described herein are associated with one another as in meat. In some embodiments the consumable is designed so that after cooking the replicas described herein are associated with one another as in cooked meat.

Characteristic flavor and fragrance components of meat are mostly produced during the cooking process by chemical reactions, the substrates for which are amino acids, fats and sugars which are found in plants as well as meat. Therefore in some embodiments the consumable is tested for similarity to meat during or after cooking. In some embodiments human ratings, human evaluation, olfactometer readings, or GCMS measurements, or combinations thereof, are used to create an olfactory map of cooked meat. Similarly, an olfactory map of the consumable, for instance a meat replica, can be created. These maps can be compared to assess how similar the cooked consumable it so meat. In some embodiments the olfactory map of the consumable during or after cooking is similar to or indistinguishable from that of cooked or cooking meat. In some embodiments the differences are sufficiently small as to be below the detection threshold of human perception.

In some embodiments the individual tissue replicas are assembled in layers, sheets, blocks and strings in defined positions and orientations.

In some embodiments, the replicas are combined in the process of passing through the plates of a meat grinder with the holes set at less than 1/2 inch (e.g., at $^{1}/_{4}$ inch). The grinder provides multiple functions of reducing the particle size, providing additional mixing or working, and forming the material into cylindrical portions like what is typically done for ground beef. During the assembly, grinding, and forming, it is important to keep the replica tissues cold (e.g., 4 — 15°C) to control microbial growth, limit flavor reactions, and also to maintain the adipose in a solid state so discrete pieces of adipose will be maintained through the grinding process.

Prior to grinding, the replica tissues are usually broken down in some manner to a defined particle size. For example, in some embodiments, the individual tissue replicas can be formed into small pieces less than 1 cm in diameter or less than 5 mm in diameter before combination with the other tissue replicas. Adipose tissue can be crumbled into

about 3 — 7 mm particles. This is important to both the appearance of the final material and the behavior of fat leakage during cooking. This size range allows for a natural appearance of adipose flecks in the final raw product. If the adipose tissues are too small (e.g., less than 2 mm), there will be an insufficient amount of fat leaked from the product when cooked.

Soft connective tissue replicas can be broken down into pieces of about 1 — 3 mm in length with ragged edges. If the pieces are too large (e.g., greater than about 4 mm), the texture of the final product can be too beady.

Sticky tissue or noodles tissue replicas, composed of amorphous or long noodle like tissue replica pieces, respectively, and raw tissue replicas can be manually broken down into pieces about 1 - 3 cm in diameter. Achieving particles in this size range allows for adequate mixing and suitable homogeneity in the final ground material.

In some embodiments, hard connective tissue replicas can be chopped to three levels, (e.g., coarse, intermediate, and fine). Chopping to three levels provides a greater amount of heterogeneity than a single step chopping process, and makes the mouthfeel of the final product more similar to ground beef.

In formulations containing gluten an additional function of the food grinder is to work the gluten and develop a gluten network of aligned gluten molecules. For gluten containing formulations it is important to minimize interaction of the adipose with the gluten network. This is done by prechilling the adipose replicas and ground tissue replicas prior to combining and also by minimizing the amount of manipulation after the adipose is added. Overworking of adipose replicas into the gluten will break down or "shorten" the gluten network.

Finally for gluten containing formulations the patties are allowed to rest at room temperature for 30 min or overnight at 4C prior to cooking. This allows time for the gluten network to relax, giving an overall better texture.

In some embodiments, the connective tissue replica is incorporated into the protein solution before formation of the muscle tissue replica.

In some embodiments, the connective tissue replica is directly incorporated into the emulsion before formation of the adipose tissue replica.

In some embodiments, the adipose tissue replica is added to the muscle tissue replica in strands and sheets to replicate the effect of "marbling," or streaky bacon.

Mixed meat tissue replicas can increase the sense of flavors, such flavors including, but not limited to, multiple aromatic compounds associated with fruity/grean bean/metallic, nutty/green, peanut butter/musty, raw potato/roasted/earthy, vinegary, spicy/caramel/almond, creamy, sweet, fruity/stale beer, musty/nutty/coumarin/licorice/walnut/bread, coconut/woody/sweet, penetrating/sickening, minty, or toasty caramel aromas.

In some embodiments the mixed meat replicas increase the presence of volatile odorants, such as 2-pentyl-furan; 4-methylthiazole; ethyl pyrazine,; 2,3-dimethylpyrazine, acetic acid; 5-methyl-2-furancarboxaldehyde; butyrolactone; 2,5-dimethy1-3-(3-methyl butyl) pyrazine; 2-cyclopentene-l-one, 2-hydroxy-3-methyl; 3-acetyl- l h-pyrolline; pantolactone; 1-methyl l (H)-pyrrole-2-2carboxaldehyde,; caprolactam; 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one. in some embodiments, the undesired flavors including but not limited to gasoline-like, petroleum, sour/putrid/fish-like, bland/woody/yogurt, fatty/honey/citrus, pungent/sweet/caramelic and nutty/burnt green aromas, form only in individual tissue replicas, but do not accumulate in mixed meat replicas. In some embodiments, the individual tissue replicas increase the presence of volatile odorants including but not limited to nonane, 2,6-dimethyl, 3-methyl 3-hexene,; pyridine; acetoin; octanal; 1-hydroxy-2-propanone; and/or ethenyl pyrazine. In some embodiments the levels to which all of the above compounds accumulate during cooking depend on the sizes of tissue replica units and how they are mixed (coarse, fine, or blended).

In some embodiments, the mixed meat tissue replicas increase the sense of flavors including but not limited to, multiple aromatic compounds associated with fruity/grean bean/metallic, nutty/green, peanut butter/musty, raw potato/roasted/earthy, vinegary, spicy/caramel/almond, creamy, sweet, fruity/stale beer, musty/nutty/coumarin/licorice/walnut/bread, coconut/woody/sweet, penetrating/sickening, minty, or toasty caramel aromas. In some embodiments, the mixed meat replicas increase the presence of volatile odorants including but not limited

to phenylacetaldehyde, 1-octen-3-one, 2-n-heptylfuran, 2-thiophenecarboxaldehyde, 3-thiophenecarboxaldehyde, butyrolactone, 2-undecenal, methyl-pyrazine, furfural, 2-decanone, pyrrole, 1-octen-3-ol, 2-acetylthiazole, (E)-2-octenal, decanal, benzaldehyde, (E)-2-nonenal, pyrazine, 1-hexanol, 1-heptanol, dimethyl trisulfide, 2-nonanone, 2-pentanone, 2-heptanone, 2,3-butanedione, heptanal, nonanal, 2-octanone, 1-octanol, 3-ethylcyclopentanone, 3-octen-2-one, (E,E)-2,4-heptadienal, (Z)-2-heptenal, 2-heptanone, 6-methyl-, (Z)-4-heptenal, (E,Z)-2,6-nonadienal, 3-methyl-2-butenal, 2-pentyl-furan, thiazole, (E, E)-2,4-decadienal,, hexanoic acid, 1-ethyl-5-methylcyclopentene, (E,E)-2,4-nonadienal, (Z)-2-decenal, dihydro-5-penty1-2(3H)-furanone, trans-3-nonen-2-one, (E,E)-3,5-octadien-2-one, (Z)-2-octen-l-ol, 5-ethyldihydro-2(3H)-furanone, 2-butenal, 1-penten-3-ol, (E)-2-hexenal, formic acid, heptyl ester, 2-pentyl-thiophene, (Z)-2-nonenal, 2-hexyl-thiophene, (E)-2-decenal,, 2-ethyl-5-methyl-pyrazine, 3-ethy1-2,5-dimethyl-pyrazine, 2-ethyl-l-hexanol, thiophene, 2-methyl-furan, pyridine, butanal, 2-ethyl-furan, 3-methyl-butanal, trichloromethane, 2-methyl-butanal, methacrolein, 2-methyl-propanal, propanal, acetaldehyde, 2-propyl-furan, dihydro-5-propy1-2(3H)- furanone, 1,3-hexadiene, 4-decyne, pentanal, 1-propanol, heptanoic acid, trimethyl-ethanethiol,, 1-butanol, 1-penten-3-one, dimethyl sulfide, 2-ethyl furan, 2-pentyl-thiophene, 2-propenal, 2-tridecen-l-ol, 4-octene, 2-methyl thiazole,, methyl-pyrazine, 2-butanone, 2-pentyl-furan, 2-methyl-propanal, butyrolactone,, 3-methyl-butanal, methyl-thiirane, 2-hexyl-furan, butanal,, 2-methyl-butanal, 2-methyl-furan, furan, octanal, 2-heptenal, 1-octene, formic acid heptyl ester, 3-pentyl-furan, and 4-penten-2-one. In some embodiments the levels to which all of the above compounds accumulate during cooking depend on the sizes of tissue units and how they are mixed (coarse, fine, or blended).

The production of volatile odorants can be enhanced when the adipose, muscle and connective tissue replicas are contacting one another. In some embodiments, the production of volatile odorants is enhanced when the adipose, muscle and connective tissues are intimately mixed with a mean size of the individual tissue replicas of 5 mm. In some embodiments, the production of volatile odorants is enhanced when the fat, muscle and connective tissues are intimately mixed with mean size of the individual tissue replicas of 2 mm. In some embodiments, said production of volatile odorants is enhanced

when the fat, muscle and connective tissue replicas are intimately mixed with mean size of the individual tissue replicas of 1 mm.

In some embodiments, the meat substitute is optimized for particular cooking methods (optimized for cooking in a microwave oven, or optimized for cooking in a stew).

In some embodiments, the meat substitute is optimized for dehydration.

In some embodiments, said meat substitute is optimized for fast rehydration upon exposure of the dehydrated meat replica to water.

In some embodiments, said meat substitute is optimized for use as emergency, camping or astronaut food.

The methods described herein can be used to provide a meat replica with defined cooking characteristics to allow the production of meat replicas that are optimized for particular cooking techniques. For example, stews require slow cooking to gelatinize the connective tissue in the meat, whereas meat replicas can be designed wherein the connective tissue replica is more easily gelatinized thus allowing stews to be prepared quickly.

B. INDICATORS OF COOKING MEAT

~~[0361]~~ The consumable can include compositions which can indicate that the consumable is cooking or has cooked. The release of odorants upon cooking is an important aspect of meat consumption. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking beef. In some embodiments, the consumable when cooked generates an aroma recognizable by humans as typical of cooking pork. ~~In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking bacon. In some embodiments~~ bacon, ~~the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking chicken. In some embodiments~~ chicken, ~~the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking lamb. In some embodiments~~ lamb, ~~the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking fish. In some embodiments~~ fish, ~~the consumable is a~~

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~~meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking~~ or turkey. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with an odorant that is released upon cooking~~. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with an odorant~~ or that is produced by chemical reactions that take place upon cooking. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing mixtures of proteins, peptides, amino acids, nucleotides, sugars and polysaccharides and fats in

combinations and spatial arrangements that enable these compounds to undergo chemical reactions during cooking to produce odorants and flavor-producing compounds.

In some embodiments~~,~~ the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a volatile or labile odorant that is released upon cooking.~~In some embodiments the consumable is a method for preparing a meat replica where meat replicas principally or entirely composed of ingredients derived from non-animal sources are heated to release a volatile or labile odorant.~~

In some embodiments, the indicator is a visual indicator that accurately mimics the color transition of a meat product during the cooking progression. The color transition can be, for example, from red to brown, from pink to white or tan, or from a translucent to opaque color during the cooking progression.

In some embodiments, the indicator is an olfactory indicator that indicates cooking progression. In one embodiment, the olfactory indicator is one or more volatile odorants released during cooking.

In some embodiments, the indicator comprises one or more isolated, purified iron-containing proteins. In some embodiments, the one or more isolated, purified iron-containing proteins (e.g., a heme-containing protein, see section III B) is in a reduced state before cooking. In some embodiments, the one or more isolated and purified iron carrying proteins in a reduced or oxidized state has a similar UV-VIS profile to a myoglobin protein derived from an animal source when in an equivalent reduced or oxidized state. The *Aquifex aeolicus* hemoglobin has a peak absorbance wavelength at 413 nm; the *Methylacidiphilum infernorum* hemoglobin has a peak absorbance wavelength at 412 nm; the *Glycine max* leghemoglobin has a peak absorbance wavelength at 415 nm; the *Hordeum vulgare* and *Vigna radiata* non-symbiotic hemoglobins each have a peak absorbance wavelength at 412 nm. The *Bos taurus* myoglobin has a peak absorbance wavelength at 415 nm.

In some embodiments, the difference between the peak absorbance wavelength of the one or more isolated and purified iron-containing proteins and the peak absorbance wavelength of myoglobin derived from an animal source is less than 5%.

[0362] Odorants released during cooking of meat are generated by reactions that can involve as reactants fats, protein, amino acids, peptides, nucleotides, organic acids, sulfur compounds, sugars and other carbohydrates. In some embodiments the odorants that

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combine during the cooking of meat are identified and located near one another in the consumable, such that upon cooking of the consumable the odorants combine. So, in some embodiments, the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions involving amino acids, fats and sugars found in plants as well as meat. So, in some embodiments, the characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions involving one or more amino acids, fats, peptides, nucleotides, organic acids, sulfur compounds, sugars and other carbohydrates found in plants as well as meat.

~~[0363]~~ Some reactions that generate odorants released during cooking of meat can be catalyzed by iron, in particular the heme iron of myoglobin. Thus in some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by iron. In some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by heme. In some embodiments, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by the heme iron in leghemoglobin. In some embodiments, some of the ~~charac~~ characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by the heme iron in a heme protein. For example, hemeproteins (e.g., from *Aquifex aeolicusm, Methylacidiphilum infernorum, Glycine max, Hordeum vulgare,* or *Vigna radiate)* provide a significantly different profile of volatile odorants when heated in the presence of cysteine and glucose than any subset of the three components when analysed by GC-MS. Volatile flavor components that are increased under these conditions include but are not limited to furan, acetone, thiazole, furfural, benzaldehyde, 2-pyridinecarboxaldehyde, 5-methyl-2-thiophenecarboxaldehyde, 3-methyl-2-thiophenecarboxaldehyde, 3-thiophenmethanol and decanol. Under these conditions, cysteine and glucose alone or in the presence of iron salts such as ferrous glucanate produced a sulfurous, odor but addition of heme proteins reduced the sulfurous odor and

replaced it with flavors including but not limited to chicken broth, burnt mushroom, molasses, and bread.

Additionally, a hemeprotein (e.g., from *Aquifex aeolicus, Methylacidiphilum infernorum, Glycine max, Hordeum vulgare,* or *Vigna radiata)* when heated in the presence of ground chicken increased specific volatile odorants that are elevated in beef compared to chicken when analyzed by GC-MS. Volatile flavor components that are increased under these conditions include but are not limited to propanal, butanal, 2-ethyl-furan, heptanal, octanal, trans-2-(2-pentenyl)furan, (Z)-2-heptenal (E)-2-octenal pyrrole, 2,4-dodecadienal, 1-octanal, or (Z)-2-decenal 2-undecenal.

C. COLOR INDICATORS

~~teristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by the heme iron in a heme protein.~~

~~[0364] Evidence that the presence of leghemoglobin contributes favorably to aroma of meat replicas: A muscle replica comprising pea flour, sunflower oil, and glucose was heated for 10 minutes at 140 C in the presence of either reduced leghemoglobin (LHb) or a mixture of iron (Fe3+), sodium and EDTA (EFS) in sealed containers carrying solid phase microextraction (SPME) fibers. These fibers contain poly dimethylsiloxane (PDMS) which adsorbs volatile compounds for analysis by GC-MS. Analysis of GC-MS data from multiple replicas reveal consistent differences between the LHb and EFS samples. Non-limiting examples of compounds found exclusively or more abundantly in the LHb samples are: 2-octanone, 2-methyl furan, which are often associated with the aroma of cooked meat, and many other unidentified compounds.~~

~~[0365] Color Indicators~~

~~[0366]~~ The color of meat is an important part the experience of cooking and eating meat. For instance, cuts of beef are of a characteristic red color in a raw state and gradually transition to a brown color during cooking. As another example, white meats such as chicken or pork have a characteristic pink color in their raw state and gradually transition to a white or brownish color during cooking. The amount of the color transition is used to indicate the cooking progression of beef and titrate the cooking time and temperature to produce the desired state of done-ness. In some aspects, the invention provides a non-meat based meat substitute product that provides a visual indicator of cooking progression. In some embodiments, the visual indicator is a color indicator that undergoes a color transition during cooking. In ~~particular~~ some embodiments, the color indicator recapitulates the color transition of a cut of meat as the meat progresses from a raw to a cooked state. In more ~~particular~~ embodiments, the color indicator colors the meat substitute product a red color before cooking to indicate a raw state and causes the meat substitute product to transition to a brown color during cooking progression. In other ~~particular~~ embodiments, the color indicator colors the meat substitute product a pink color

before cooking to indicate a raw state and causes the meat substitute product to transition to a white or brown color during cooking progression.

~~[0367]~~ The main determinant of the nutritional definition of the color of meat is the concentration of iron carrying proteins in the meat. In the skeletal muscle component of meat products, one of the main iron-carrying proteins is myoglobin. ~~It is estimated that~~ As described above, the myoglobin content of varies from under 0.05% in the white meat of chicken to 1.5-

~~has under 0.05%; pork and veal have 0.1-0.3%; young beef has 0.4-1.0%; and~~ 2.0% in old beef ~~has 1.5-2.0%.~~ So, in some embodiments, the consumable is a meat replica which comprises an iron-carrying protein (e.g., a heme-containing protein). In some embodiments, the meat replica comprises about 0.05%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, or more than about 2% of an iron-carrying protein (e.g., a heme-containing protein) by dry weight or total weight. In some cases, the iron carrying protein has been isolated and purified from a source. In other cases, the iron carrying protein has not been isolated and purified. In some cases, the source of the iron-carrying protein is an animal source, or a non-animal source such as a plant, fungus, or genetically modified ~~organ-isms~~ organisms such as, e.g., plant, algae, bacteria or ~~yeast~~fungus. In some cases, the iron-carrying protein is myoglobin. In some embodiments the consumable is a plant based meat replica that has animal myoglobin added. So, for example a replica of young beef can have about 0.4-1% myoglobin. In some ~~cases, the iron-carrying protein is leghemoglobin. In some~~ embodiments the consumable is a plant based meat replica that has ~~leghemo-globin~~ a leghemoglobin or a cytochrome added. So, for example~~.~~, a replica of young beef can have about 0.4-1% leghemoglobin~~. In some cases, the iron-carrying protein is a cytochrome. In some embodiments the consumable is a plant based meat replica that has a cyto-chrome added. So, for example a replica of young beef can have about 0.4-1% of a cytochrome.~~ or cytochrome.

~~[0368]~~ Another example of iron-carrying proteins is hemoglobin, the iron-containing oxygen-binding protein in the red blood cells of vertebrates. Hemoglobin is similar in color to myoglobin. In some embodiments the invention provides methods of saving and recycling blood from animal farming to supplement the color of a consumable. For example~~,~~ blood is saved from a slaughter house, and hemoglobin from the blood is used to enhance the color of a consumable. In some aspects the consumable is a plant-based meat replica containing hemoglobin.

~~[0369]~~ Additional iron containing proteins exist in nature. In some embodiments the consumable comprises an iron containing protein that is not myoglobin. In some embodiments the consumable does not contain myoglobin. In some embodiments the consumable does not contain hemoglobin. In some embodiments the consumable is a

meat replica that comprises an iron containing protein other than myoglobin or hemoglobin. See, for example, Section III B for examples of heme-containing proteins, as well as FIG. 3. For example, in some embodiments the consumable comprises a

[0370] Examples of iron-containing proteins include hemoprotein (e.g., a hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, a bacterial hemoglobinshemoglobin, a ciliate myoglobinsmyoglobin, flavohemoglobins. In various embodiments these iron-containing proteins are added to the consumable to alter the visual characteristics or iron content of the consumable. In some embodiments the consumable comprises a hemoprotein (e.g. hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins).or a flavohemoglobin).

[0371] Leghemoglobin, similar in structure and physical properties to myoglobin, is readily available as an unused by-product of commodity legume crops (ege.g., soybean, or pea). The leghemoglobin in the roots of these crops in the US exceeds the myoglobin content of all the red meat consumed in the US.

In some embodiments, the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, and containing a heme protein (e.g., a leghemoglobin or member of the globin protein family). For example, a meat replica can be principally or entirely composed of ingredients derived from non-animal sources, including a muscle tissue replica, an adipose tissue replica, a connective tissue replica, and leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a heme protein. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a member of the globin protein familya heme protein. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a high iron content from a heme protein. In some embodiments the iron content is similar to meat. In some embodiments the consumable has the distinctive red color of meat, such color provided by leghemoglobin.

[0372] Leghemoglobin is, in some embodiments, A heme protein (e.g., a heme-containing protein described in Section III B) can be used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin, which has migrated from the interior

of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.

~~[0373] A heme protein is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.~~

~~[0374] A heme protein from the group of: Hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.~~

~~Food Products Comprising Isolated, Purified Leghemoglobin~~

In some embodiments, the increased shelf life is provided by an extension of the lifetime of the desired red color of food products (e.g., a non-meat based meat substitute).

In one embodiment, this invention provides hemoproteins that provide a desired color to non-meat meat substitutes. In some embodiments, the hemoproteins are derived from a non-animal source such as a plant, fungus, or genetically modified organisms such

as, e.g., plant, algae, bacteria or fungus. See, e.g., section **III** B. In some embodiments the life time of the hemoproteins is extended by treatment with meat shelf life extenders.

In some embodiments, the meat shelf life extenders are selected from a groups consisting of carbon monoxide, nitrites, sodium metabisulfite, Bombal, rosemary extract, green tea extract, catechins and other anti-oxidants.

In one embodiment, this invention provides hemoproteins that provide a desired flavor profiles to food products (e.g., non-meat meat substitutes). In some embodiments, the ability of the hemoproteins to generate the desired flavor profile is similar to that of myoglobin

In some embodiments, the life time of the ability of the hemoproteins to generate the desired flavor profile is 10%, 20%, 30% 50%, or 100% or more greater than that of myoglobin.

D. Food products comprising isolated, purified heme proteins

In some embodiments, heme proteins described herein are added to meat or a consumable described herein to enhance the properties of the meat or consumable. For example, a heme protein containing solution can be injected into raw (e.g., raw white meat) or cooked meat to improve the organoleptic properties of the meat during cooking adding a "beefy" flavor (e.g., to white meats such as chicken).

~~[0375] In some embodiments leghemoglobin is added to meat to enhance the properties of meat. For example, a leghe-moglobin containing solution can be injected into raw or cooked meat.~~ In another example ~~a leghemoglobin~~, a heme protein solution ~~is~~ can be dripped over meat or a consumable of the invention to enhance appearance. In one embodiment~~.~~, advertising, photography, or videography of food products such as meat or a meat substitute ~~is~~ can be enhanced with ~~leghemoglobin~~a heme protein.

~~Sources of Leghemoglobin~~

In another embodiment, a heme protein is added to the consumable as an iron supplement.

In one application of the invention, hemeproteins may be used as food dyes. In one embodiment, the heme proteins may be used as a safe, digestible replacement for FD&C Red No. 40 — Allura Red AC, E129 (red shade) in a variety of applications. A non-

limiting list of such potential uses would include making pictures, especially in forms such as body-painting or as theatrical blood.

[0376] In some embodiments, the present invention provides methods for obtaining hemeproteins (e.g., leghemoglobin) from plants a plant. Leghemoglobin can be obtained from a variety of plants. Various legumes species and their varieties, for example, Soybean, Fava bean, Lima bean, Cowpeas, English peas, Yellow peas, Lupine, Kidney bean, Garbanzo beans, Peanut, Alfalfa, Vetch hay, Clover, Lespedeza and Pinto bean, (e.g., soybean, fava bean, lima bean, cowpeas, English peas, yellow peas, Lupine, kidney beans, garbanzo beans, peanuts, Alfalfa, Vetch hay, clover, Lespedeza, or pinto bean) contain nitrogen-fixing root nodules in which leghemoglobin has a key role in controlling oxygen concentrations (for example root nodules from a pea plant, FIG. 1). FIG. 2 shows 100 mls of leghemoglobin solution isolated from 30 grams of pea root nodules. Leghemoglobins from different species are homologs and have similar color properties (FIG. 3). In FIG. 3, panel A shows an SDS_PAGE gels of lysed root-nodules of three legume plant species (1) Fava bean (2) English Pea (3) Soybean. Arrows mark respective leghemoglobins. Note that leghemoglobin is the most abundant soluble protein in each lysate. Panel B shows the similarity of UV-VIS spectral profile of leghemoglobins from two different plant species (Fava -). In one embodiment leghemoglobin protein is purified from root nodules of legume plants (e.g., soybeans, favabeans, or peas) using ion-exchange chromatography. In an embodiment, leghemoglobin is purified from soybean, favabean or sweet pea root nodules.

bean and Soybean). We purified leghemoglobin from fava bean (green curve) and Soybean (red curve) root nodules using the protocol described elsewhere in the specification. UV-VIS spectra of both proteins shows that the heme iron is in the reduced (+2) state. Note that they are almost perfectly superimposed, consistent with their visually identical red color. The heme iron in the respective leghemoglobins was reduced to the +2 oxidation state by incubating Fava bean and Soybean leghemoglobin with 10 mM sodium hydrosulfite in 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride buffer. Sodium hydrosulfite was then removed from the leghemoglobin solution using gel exclusion chromatography. Inset shows a zoom in of UV-VIS spectra in 450 nm to 700 nm region. Some plant species express several leghemo globin isoforms (for example soybean has four leghemoglo bin isoforms). Minor variations in precise amino acid sequence can modify overall charge of the protein at a particular pH and can modify precise structural conformation of iron containing heme group in leghemoglobin. Differences in structural conformation of heme group of different leghemo globins can influence oxidation and reduction rates of the heme iron. These differences may contribute to color and flavor generation properties of different leghemoglobins.

[0377] Leghemoglobin has a virtually identical absorbance spectrum and visual appearance to myoglobin from animal muscle. FIG. 4 shows a comparison of reduced (heme iron 2+) and oxidized (heme iron 3+) soybean leghemoglobin (FIG. 4 panel A) and equine heart muscle myoglobin (FIG. 4 panel B) showing similarity of UV-VIS absorption profiles of two proteins. We purified soybean leghemoglobin from soybean root nodules using here described protocol. Purified equine myoglobin was purchased from SigmaAldrich. Soybean leghemoglobin (FIG. 4 panel A) and equine myoglobin (FIG. 4 panel B) were reduced with 1 mm sodium hydro-sulfite. Shown are UV-VIS absorption spectra of heme-Fe3+ (blue line) and heme-Fe2+ (red line) of soybean leghemoglo bin (FIG. 4 panel A) and equine myoglobin (FIG. 4 panel B). Insets show a zoom in of UV-VIS spectra in 450 nm to 700 nm region. (FIG. 4 panel C) Images of 10 ul liquid droplet of a 40 mg/ml solution of soybean leghemoglobin in the heme-Fe3+ state (left droplet) showing characteristic rusty red color and a 40 mg/ml solution of soybean leghemoglobin solution in the heme-Fe2+ state (right droplet) showing characteristic red color of and (right image) corresponding samples of equine myoglobin.

[0378] In other embodiments, leghemoglobin can be sourced from non-plant sources, such as from organisms such as bacteria or yeast which have been genetically modified to express high levels of leghemoglobin.

[0379] The oxidation state of the iron ion in leghemoglobin is important for its color. Leghemoglobin with the heme iron in the +2 oxidation state appears vivid red in color, while leghemoglobin with the heme iron in the +3 oxidation state appears brownish red. Thus, in using leghemoglobin as a source of red color in a meat replica, it is desirable to reduce the heme iron from the +3 state to the +2 state. Heme iron in leghemoglobin can be switched from oxidized (+3) state to reduced (+2) state with reducing

reagents. Examples of successful reduction of leghemoglobin heme iron with sodium hydrosulfite and titanium citrate are illustrated in FIG. 5. In FIG. 5 the UV-VIS spectrogram of purified soybean leghe-moglobin in which the heme iron is in the oxidized (+3) state is represented by the blue curves in each panel. The red curves in each panel represent the UV-VIS spectra of the same leghe-

moglobin species after reduction to the (+2) state (red lines) by addition of (Panel A) 1 mM sodium hydro-sulfite or (Panel B) 0.24% (wt/v) titanium citrate.

Plants can be grown using standard agricultural methods, with the exception that, in some instances, fertilizer is not applied and soil is enriched in natural nitrogen-fixing bacteria from the *Rhizobium* genus. Either whole roots or root nodules can be harvested and lysed, for example in 20mM potassium phosphate pH 7.4, 100mM potassium chloride and 5mM EDTA using grinder-blender. During this process, leghemoglobin is released into the buffer. Root-nodule lysate containing leghemoglobin can be cleared from cell debris by filtration through 5[Lm filter. In some embodiments, filtration is followed by centrifugation (7000g, 20min). Clarified lysate containing leghemoglobin is then filtered through 200nm filter and applied onto anion-exchange chromatography column (High Prep Q; High Prep DEAE, GE Healthtcare) on fast protein liquid chromatography machine (GE Healthcare). Leghemoglobin is

in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride buffer. The Insets show a zoom-in of UV-VIS spectra in 450-700 nm region. For this example, leghemoglobin was purified from soybean root nodules using 60/90% ammonium sulfate fractionation and exchanged into 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride buffer. Sodium hydrosulfite stock solution was prepared by dissolving 100 mM sodium hydro-sulfite in 1 mM sodium hydroxide in water. Titanium citrate stock solution was prepared from 20% (wt/v) Ti-chloride in hydrochloric acid by mixing it with 0.2M sodium citrate (1:10 v/v). pH was adjusted using sodium carbonate to pH 7.0.[0380] Leghemoglobin can be purified from legume root nodules, such as the root nodules of peas or soybeans (FIG. 1 shows Leghemoglobin isolated from pea root nodules). Root nodules from soy beans were thoroughly cleaned to remove soil and extraneous root tissues prior to root nodule lysis in 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mm EDTA and 1 mM ascorbic acid. Root nodules were lysed by grinding root-nodules using a Vitamix blender. For some samples Polyvinylpyrrolidone polymer was added at 30% wt/v to aid in removal of plant phenolic small molecules that mediate oxidation of leghemoglobin heme iron. Root nodule lysate was fractionated using ammonium sulfate in two steps, first ammonium sulfate was added to 60% wt/v. Pellet was discarded and supernatant brought to 90% wt/v. ammonium sulfate. Leghemoglobin was collected as a precipitated pellet in 90% ammonium sulfate fraction. Ammonium sulfate precipitated leghemoglobin was resuspended in 20 mM potassium phosphate, 1 mM EDTA, 50 mM sodium chloride and ammonium sulfate was removed using dialysis or size exclusion chromatography in the same buffer. In some instances this was the last purification step, while in other instances leghemoglobin was further purified using anion exchange chromatography (FFQ GE Healthcare), which was sometimes followed by size exclusion chromatography (Sephacryl S-100, GE Healthcare). Soybean leghemoglobin from 90% ammonium sulfate pellet was loaded on anion exchange columns (FFQ or DEAF, GE Healthcare) in different buffers (20 mM potassium phosphate pH 7.4, containing 0 to 100 mM sodium chloride, 20 mM Tris pH 8 containing 0 to 100 mM sodium chloride, 20 mM sodium borax pH 9.8, 20 mM sodium chloride, 20 mM sodium carbonate pH 9, 20 mM sodium chloride) and purified either in flow-through or using sodium chloride (0-1M salt gradient). An example of the leghemoglobin purification flow from soybean root nodules is represented in FIG. 6. The figure shows SDS-PAGE fractionation of different soybean leghemoglobin purification steps (Lane 1) Soybean root nodule lysate; (Lane 2) Soybean root nodule lysate purified by 60/90% (wt/v) ammonium sulfate fractionation. Shown is the protein content of 90% ammonium sulfate fractionated protein pellet resuspended in 20 mm potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mM EDTA buffer; Proteins from 90% ammonium sulfate pellet were further purified by anion exchange chromatography (FFQ GE Healthcare) in 20 mM potassium phosphate ph 7.4, 100 mM sodium chloride. Leghemoglobin collected in the flowthrough is shown in Lane 3. Anion exchange flowthrough was fractionated using size exclusion chromatography (Sephacryl S-100 GE Healthcare) and resulting leghemoglobin fraction is shown in Lane 4. Leghemoglobin content at different purification steps was determined by

determining the fraction of leghemoglobin band on SDS-PAGE gel in a respective sample using ImageDoc analysis software (BioRad). Purity (partial abundance) of leghemo-globin at respective steps in the purification steps was: lysate: 32.7% (lane

~~1), 60/90% (wt/v) ammonium sulfate fractionation 78% (lane 2), anion exchange chromatography — 83% (lane 3), and size-exclusion chromatography to — 95% (lane 4).~~

~~[0381] Leghemoglobin can also be produced by genetically engineering a bacterium or fungus to produce it. One illustrative example is shown in FIG. 7. FIG. 7 shows stained SDS-PAGE gel analysis of (A) soybean leghemoglobin expressed and purified using recombinant protein technology and (B) soybean leghemoglobin purified from soybean root nodules. (A) Recombinant Soybean leghemoglobin A carrying His-tag and TEV protease His-tag removal site was expressed in *E. coli* BL21 strain and purified using His-tag affinity chromatography (Talon resin, CloneTech). The left lane contains molecular weight standards, the right lane contains purified recombinant soybean leghemoglobin A (arrow). Expected molecular weight of the recombinant soybean leghemoglobin A is 17.1 kDa. (B) SDS-PAGE gel of purified Soybean leghemoglobin from root nodules. The left lane contains molecular weight standards, the right lane contains purified soybean leghemoglobin A (arrow). Mass spectrometry analysis of purified material determined that all four soybean leghemoglobin isoforms are present, and are full length (data not shown). Expected molecular weights (MW) of soybean leghemoglobin isoforms range from MW15.4 to 15.8 kDa.~~

collected in the flowthrough fraction and concentrated over 3kDa filtration membrane to a desired concentration. Purity (partial abundance) of purified leghemoglobin is analyzed by SDS-PAGE gel: in lysate leghemoglobin is present at 20-40%, while after anion-exchange purification it is present at 70-80%. In another embodiment, soybean leghemoglobin flowthrough from anion-exchange chromatography is applied onto size-exclusion chromatography (Sephacryl S-100 HR, GE Healthcare). Soybean leghemoglobin is eluted as two fractions corresponding to dimer and monomer species. Purity (partial abundance) of leghemoglobin was analyzed by SDS-PAGE and determined to be — 90-100%.

Proteins in legume root-nodule lysate can be transferred into 10 mM sodium carbonate pH 9.5, 50mM sodium chloride buffer, filtered through 200nm filter and applied onto anion-exchange chromatography column on fast protein liquid chromatography instrument (GE Healthcare). Leghemoglobin can be bound to anion-exchange chromatography matrix and eluted using sodium chloride gradient. Purity (partial abundance) of leghemoglobin can be analyzed by SDS-PAGE and determined to be — 60-80%.

Undesired small molecules from legume roots can be removed from purified leghemoglobin by passing leghemoglobin in solution over anion-exchange resin. These small molecules imbue varying shades of brown color to root-root nodule lysates, thus decreasing the color quality of leghemoglobin solution. In one embodiment, anion-exchange resin is FFQ, DEAE, Amberlite IRA900, Dowex 22, or Dowex 1x4. Leghemoglobin purified either by ammonium sulfate fractionation (60% wt/v and 90% wt/v ammonium sulfate) or by anion-exchange chromatography was buffer exchanged into 20mM potassium phosphate pH 7.4, 100mM sodium chloride and solution passed over one of the above mentioned anion-exchange resins. Flowthrough can be collected and its colored compared to the color of the solution before passage over anion-exchange resins. Color improvement to purified leghemoglobin solution as evaluated by visual inspection can be observed (from yellow/brown to more apparent red), however to different extent of removal of yellow-brown tinge.

Alternatively, the heme-containing protein can be recombinantly produced as described in section III B. For example, a non-symbiotic hemoglobin from moong bean can be recombinantly expressed in *E.coli* and purified using anion-exchange chromatography or cation-exchange chromatography. A cell lysate can be loaded over FF-Q resin on fast protein liquid chromatography instrument (GE Healthcare). Moong bean non-symbiotic hemoglobin eluted in the flowthrough fractions. Purity (partial abundance) of Moong bean non-symbiotic hemoglobin was analyzed by SDS-PAGE and determined to be as a fraction of total protein: 12 % in *E.coli* lysate, and 31% after purification on FFQ. UV-Vis analysis of purified protein showed spectra characteristic of heme bound protein.

Alternatively, the cell lysate can be loaded over a FF-S resin on a fast protein liquid chromatography instrument (GE Healthcare). Moong bean non-symbiotic hemoglobin can be bound to FF-S column and eluted using sodium chloride gradient (50mM- 1000mM). Purity (partial abundance) of Moong bean non-symbiotic hemoglobin can be analyzed by SDS-PAGE and determined to be: *E.coli* lysate 13 %, after purification on FFQ 35%. UV-Vis analysis of purified protein can show spectra characteristic of heme bound protein.

~~[0382] Leghemoglobin purified from soybean and fava root nodules, respectively was~~ In some embodiments, the heme proteins are utilized as ingredients in food products where the flavor of blood is desired. Heme-containing proteins of the invention were tasted by a panel of volunteers and in each case described as tasting like blood.

~~[0383] Leghemoglobin can be isolated from the roots nodules of legumes such as soy beans, fava beans, cow peas, lima beans, garbanzo beans, peas, lupine, lotus japonicum or other legumes. The root nodule (for example root nodules from a pea plant) is obtained and homogenized in an aqueous solution, soluble proteins including leghemoglobin are recovered after insoluble matter is removed by precipitation or filtration. Leghemoglobin can be purified by selective precipitation and/or chromatography and/or the use of molecules with specific affinity for leghemoglobin. (FIG. 1, showing 100 mls of solution of leghemoglobin isolated from 30 grams of pea root nodules).~~

~~[0384]~~ Heme proteins, for example leghemoglobin, can be combined with other plant based meat replica components. In some embodiments the heme proteins are captured in a gel which contains other components, for example lipids and or other proteins. In some aspects ~~a~~, multiple gels are combined with non-gel based heme proteins. In some embodiments~~,~~, the combination of the heme proteins and the other compounds of the consumable are done to insure that the heme proteins are able to diffuse through the consumable. In some embodiments the consumable is ~~ed in a heme protein containing solution, for instance a leghemoglobin solution. In some embodiments the consumable is~~ soaked in a heme-protein containing solution, for instance a leghemoglobin solution ~~for 1~~, ~~5, 10, 15, 20 or 30 hours. In some embodiments the consumable is soaked in a heme containing solution, for instance a leghemoglobin solution~~ e.g., for 1, 5, 10, 15, 30, or 45 minutes~~.~~ or for 1, 5, 10, 15, 20 or 30 hours.

~~[0385] FIG. 8 shows an example of 6 cubes of a commercial meat analog (Quorn chicken analog), about 1 cm on a side, 4 of which (Left and lower right) have been soaked in a solution~~

~~of about 10 mg/ml soybean leghemoglobin in 20 mM Potassium phosphate pH 7.4 and 100 mM NaCl; the remaining two (Upper right) were soaked in the same buffer without leghe-moglobin. Note the deep pink color of the leghemoglobin-infused cubes in contrast to the pale tan color of the un-infused cubes.~~

~~[0386] FIG. 9 shows the 4 leghemoglobin-infused cubes of Quorn chicken analog in the process of cooking in a pan at 350° C. The two lower cubes have been turned over to expose the grilled surface, which has turned brown. Note in the upper two cubes that the heated portion has turned grey-brown, while the cooler top surface retains its pink color. In some embodiments the consumable is injected with a heme containing solution, for instance a leghemoglobin solution, until the consumable is the color of uncooked meat.~~

~~[0387]~~ Given the usefulness of heme proteins for coloring consumables ~~it will be~~, it is useful to detect whether a product contains a particular heme protein. Accordingly the present invention includes in some embodiments methods to determine whether a product contains a heme protein. ~~Methods for detecting proteins are well known in the art.~~ For example~~,~~, an ELISA ~~or~~, a proximity-ligation assay ~~or luminex~~, a luminex assay~~,~~ or western blot analysis can be performed to determine whether leghemoglobin or other heme-containing protein is present in a food product such as meat or a meat replica. In one embodiment the detection methods are performed to determine whether meat has been altered with leghemoglobin or other heme-containing protein.

E. MAYONNAISE SPREAD REPLICA.

Mayonnaise is thick, creamy sauce. Traditional mayonnaise is a stable emulsion of oil and egg yolk. It is thought that lecithin and proteins from egg yolk stabilize the emulsion. Traditional commercial mayonnaise typically contains 70-80% (wt/wt) fat and 5% (wt/wt) of egg yolk. Lower fat commercial products can contain — 20% wt/wt fat. The consumable can comprise a composition that has comparable properties to mayonnaise.

In one embodiment, purified plant proteins can be used as substitute for egg proteins to make stable, creamy protein-fat emulsions whose visual and mouth-feel appearance resemble traditional mayonnaise. Fat (— 20- 80% wt/wt) can be from a single source or from multiple sources as described herein. Non-traditional mayonnaise products can be used for all the culinary applications that traditional mayonnaise is used for. In one embodiment, vinegar and/or lemon and/or lime juice are added as flavor additives. In one embodiment, the purified plant proteins are not soy proteins. In one embodiment, the flavor can be modified by addition of mustard, spices, herbs, and/or pickles.

A mayonnaise replica can comprise a mixture of non-animal proteins. In one embodiment, a mayonnaise replica is a mixture of 50% (wt/v) rice bran oil and 7% (wt/v) Moong bean 8S protein. In one embodiment, a mayonnaise replica is a mixture of 70% (wt/v) sunflower oil or cocoa butter, 2.4% (wt/v) RuBisCo,0.29% (wt/wt) soybean lecithin, and optionally 8 ILIM oleosin.

The mixture can be emulsified, and the stability of the emulsion can be controlled by modifying the size of the oil-water-protein particles by high pressure homogenization or sonication. Oil can be added as liquid. Protein can be added as solution in buffer. Soybean lecithin can be resuspended in water and sonicated prior to mixing with an oil and protein solution. A resulting oil, protein and lecithin solution can be homogenized, for example, first at 5000psi and then at 8000psi, or can be sonicated at 40% duty cycle for 2 minutes at maximum setting. Thickness, texture, creaminess and visual appearance of resulting products are similar to one of traditional mayonnaise. In some instances (e.g. using Moong bean 8S protein and rice bran oil), a product is a light off-white in color.

F. CREAM LIQUOR REPLICA

Traditionally, cream liquors contain dairy cream and liquor as their base. Examples of liquor include whiskey, Irish whiskey, Scotch whiskey, rum, vodka, grappa, or fermented fruits (e.g., cherry liquor), plum brandy, tequila, or herbal bitters. A cream liquour replica can be produced by substituting the dairy cream in cream liquor with a non-dairy cream fraction from plant sources. In one embodiment, dairy cream in a cream liquor can be substituted by a stable emulsion of plant fats and isolated or purified proteins of a consistency similar to dairy cream. In one embodiment, purified plant proteins and/or plant fats can be from single or multiple sources as described herein. For example, a cream liquor can include a sunflower cream fraction, RuBsiCo and whiskey, and one or more optional flavorings (e.g., vanilla, chocolate, and or coffee).

G. PROTEIN ENRICHED ALCOHOLIC BEVERAGE

Traditionally, alcoholic beverages contain negligible to low amounts of protein. Addition of plant proteins to various alcoholic beverages would positively modify their flavor, mouthfeel, physical state and increase their nutritional protein content. In addition, the presence of protein in various alcoholic beverages used in cocktails would positively modify the cocktail's flavor, mouthfeel, physical state and increase their nutritional protein content. Different classes of alcoholic beverages contain different amounts of alcohols. For example, wine coolers contain about 4-7% alcohol, beer contains about — 310% alcohol, wine contains about 8-14% v/v alcohol, dessert wines contain about 1720% alcohol, whiskey contains about — 40% alcohol, and vodka contains about 35-50% alcohol. In addition, some traditional alcoholic beverages include sugars (for example, Bacardi Razz at 10% wt/v).

Accordingly beverages containing alcohol can be supplemented by addition of purified plant proteins at for example 0.1-5% wt/v and optionally sugar (1-15% wt/v). The sugar can be, for example, cane sugar, brown sugar, sucrose, or glucose. For example, purified Rubisco at 180mg/m1 in 20mM K-phosphate pH 7.0, 150mM NaC1 can be added to a whiskey. Jameson whiskey supplemented with 5% wt/v Rubsico formed a soft gel, with a consistency similar to traditional Jello shots.

For example, Rubsico, moong bean 8S, and pa Globulin enriched alcoholic beverages were made by adding purified Rubsico, Moong bean 8S and pea globulin proteins at final protein concentrations of 0.5%, 1% and 5% wt/v, respectively, to Corona beer, Pinot grigio wine and Jameson whiskey. Zein was added at 0.5%, 1% and 5% wt/v to a 60% ethanol, 5% sucrose solution in water.

Pea proteins were extracted from pea flour by resuspending the flour in 5%, 20% or40% ethanol, 5% sucrose solutions in water, followed by incubation for 1 hr at room temperature. Any undissolved solids were removed by centrifugation at 5000g for 10min. The resultant supernatant solution was clear in appearance. The 5% ethanol solution was particularly useful.

A sensory panel evaluated all protein enriched alcoholic beverages as having aromas and flavors different from beverages not-enriched in protein. In some cases, generated aromas and flavors were judged as neutral, in some cases as more appealing and in some cases as less appealing than controls. In particular example, addition of both 0.5% wt/v and 1% wt/v of Moong bean 8S protein to Jameson whiskey softened the aroma and mouthfeel of Jameson. Addition of 0.5% wt/v of moong bean to Jameson whisky added a slightly creamy flavor to Jameson, with an aroma similar to a traditional White Russian cocktail. Addition of 5% wt/v zein to Jameson whiskey generated aromas and flavor characterized as moldy beans and raw potato.

In another example where Corona beer was enriched with 0.5% wt/v pea Globulin, the aroma changed to hoppy and resembled one of Indian Pale Ale, and the flavor changed to carrying pea notes. Addition of 0.5% wt/v and 5% wt/v of moong bean 8S protein changed the Corona aroma towards sweet peony flower with an intensified hop aroma. The flavor was neutral in the case of 0.5% wt/v moong bean 8S and carried planty-nutty notes in the case of 5% wt/v moong bean 8S.

In another example where Pinot Grigio wine was enriched with 1% wt/v moong bean 8S protein, additional aroma notes of sweet and citrus were detected, and the flavor changed to that of carrying notes of peanut-butter. Addition of 1% wt/v of Pea Globulins modified the aroma to that of strong moldy oak and wet leaves. Flavor was modified to

carry notes of mud. Addition of 5% wt/v of Rubisco generated aroma and flavor of wet hay.

A zein enriched 60% ethanol, 5% sucrose solution carrried burnt tortilla chip aroma notes compared to a corresponding solution without zein. There was no difference in flavor.

Pea proteins enriched 5%, 20% and 40% ethanol, 5% sucrose solutions all developed earthy aroma and flavor compared to protein free controls. In addition, flavor of peas was detected and bitter flavor increased at higher alcohol content.

H. CHOCOLATE SPREAD

Chocolate spread is a chocolate flavored spread whose traditional main ingredients are cocoa powder, dairy milk, plant oil, and sugar. Traditional chocolate spreads are either firm or soft solids at ambient room temperature and melt at temperatures below that of cocoa butter. The product can be used as a spread on bread, crepes, pancakes, icing for cakes and cookies, filling for chocolate confectionary, or nondairy chocolate cake filling.

In one embodiment, dairy milk and milk products such as ice cream, whey, cream, yoghurt, sour cream or butter fat are substituted by a non-dairy cream fraction made as described herein. In one embodiment, a non-dairy cream fraction comes from a single source or multiple sources described herein. In one embodiment, dairy milk and milk products are substituted by any non-dairy milk described herein. In one embodiment, dairy milk and milk products are substituted by purified plant proteins described herein. In one embodiment, dairy milk and milk products are substituted by soft solid stable emulsions made from single or multiple plant oil and single or multiple purified plant proteins.

I. OTHER APPLICATIONS:

In one embodiment, non-dairy plant cream fraction can be used as a substitute for dairy milk and dairy milk products to make non-dairy milk chocolate bars or non-dairy milk chocolate confectionary.

In one embodiment, non-dairy plant cream fraction and purified plant proteins can be used as substitute for dairy milk and dairy milk products to make non-dairy milk chocolate bars or a non-dairy milk chocolate confectionary.

In one embodiment, non-dairy plant cream fraction and plant proteins can be used to make chocolate mousse. Traditional main chocolate mousse ingredients are bittersweet or semisweet chocolate, dairy butter and eggs. In one embodiment, dairy butter can be substituted by a non-dairy plant cream fraction. In one embodiment, dairy butter and eggs can be substituted by a non-dairy plant cream fraction and a foam stabilizing plant seed storage proteins such as pea albumin.

In one embodiment, a vegan consumable such as pate analog can be made. Vegan pate analog can be made by finely chopping 10 g of fat replica and heating it on a frying pan with finely chopped shallots for 2-3 min. Muscle replica (20 g) made without connective tissue replica fibers can be chopped into $^{1}/_{2}$-inch cubes and browned in the fat and shallots mix for another 3-5 min. The mixture can be forced through a sieve until homogeneous. The pan, while still warm, can be rinsed with a tablespoon of madeira without allowing it to evaporate fully. The liquid from the pan is added to the homogenized mix, spices (salt, pepper) are added to taste, and the mix is forced through a sieve again. After chilling in a refrigerator (e.g., for 15 minutes), the pate is ready to be served.

In some embodiments, other fat-to-muscle replica ratios are used to create leaner or richer pates. For example, pate can contain 0.5-10%, about 5%-40%, about 10%-60%, or about 30-70%, or >70% of an adipose tissue replica.

In some embodiments, a muscle tissue replica with a higher iron content can be used for pate to make it a closer imitation of pig or bird liver pate. For example, muscle tissue replica can contain about 1%, about 1.5%, about 2%, or >2% of a heme protein.

In some embodiments, muscle tissue replica with lower iron content can be used for pate to make it closer imitation of bird meat or fish pate. For example, muscle tissue replica can contain about 1%, about 0.5%, about 0.2%, or <0.2% of a heme protein.

In one embodiment, a vegan consumable such as a blood sausage analog can be made. Vegan blood sausage is made from a blood analog created by mixing solutions of

heme protein and purified plant protein. For example, 35 ml of a mixed solution of leghemoglobin (120 mg/ml) and pea albumin (100 mg/ml), which approximates the composition of blood, can be carefully mixed with a slurry of corn flour in salt water (6:5 w/v flour to water ratio). A tablespoon of chopped onion can be fried with 10 g of chopped adipose tissue replica, mixed with a few raisins and cooled to room temperature before mixing with blood/flour mix. The mixture can be seasoned to taste (for example, using salt, pepper, parsley and/or cinnamon), loaded into vegetarian sausage casings, and poached in near-boiling water for about 45 min. After cooling, the sausage can be consumed as is or further cooked, for example smoked, crisped in an oven or roasted.

In some embodiments, muscle replica can be included in the recipe to imitate meat/blood sausages. In some embodiments, barley, buckwheat, oat, rice, rye, sorghum, wheat or other grains can be used in the blood sausage. In some embodiments, bread, chestnuts, potato, sweet potato, starch or other fillers can be added to, or substitute for, grains in blood sausage.

EXAMPLES

~~[0388] An exemplary muscle replica composition comprising one or more isolated, purified plant proteins is described herein.~~

EXAMPLE 1: PROTEIN ISOLATION.

All steps were carried out at 4°C or room temperature. Centrifugation steps were at 8000 g for 20 mins, 4°C or room temperature. The flour is suspended in a specific buffer, the suspension is centrifuged and the supernatant is microfiltered through a 0.2 micron PES membrane and then concentrated by ultrafiltration on a 3 kDa, 5 kDa, or 10 kDa molecular weight cutoff PES membrane on a Spectrum Labs KrosFlo hollow fiber tangential flow filtration system.

Once fractionated, all ammonium sulfate precipitate fractions of interest were stored at -20°C until further use. Prior to their use in experiments, the precipitates were resuspended in 10 volumes of 50 mM K Phosphate buffer, pH 7.4, + 0.5 M NaCl. The suspensions were centrifuged and the supernatants microfiltered through a 0.2 micron PES membrane and then concentrated by ultrafiltration on a 3 kDa, 5 kDa, or 10 kDa

molecular weight cutoff PES membrane on a Spectrum Labs KrosFlo hollow fiber tangential flow filtration system. Protein composition at individual fractionation steps

~~[0389] Protein Purification for Components of the Replica [0390] Moong bean seeds, Green Pea dry seed were purchased as milled flour and used for purification of respective seed storage proteins. Rubsico was purified from fresh alfalfa plant. Protein composition at individual fractionation steps~~ was monitored by SDS-PAGE and protein concentrations were measured by standard ~~UV-VIS and Pierce assay~~ UV-Vis methods.

~~[0391] Moong bean 8S globulins: Moong bean flour was resuspended in in 50 mM potassium phosphate buffer pH 7 and 0.5M NaC1 at 1:4 (wt/v) ratio, and mixture was incubated for 1 hr. Unsoluble material was separated by centrifugation and proteins in the supernatant were fractionated by addition of ammonium sulfate in 2 steps: 50% (wt/v) followed by 90% (wt/v). Protein precipitated in 90% fraction contained the moong bean 8S globulins and was stored at —20 C until further use.~~

~~[0392] Pea-albumins: Green pea dry seed flour was resuspended at 1:10 (wt/v) ratio in 50 mM sodium acetate buffer pH 5 and incubated for 1 hr. Unsoluble material was separated by centrifugation and proteins in the supernatant were fractionated by ammonium sulfate precipitation in two steps: 50% (wt/v) followed by 90% (wt/v). Ammonium sulfate solutions were stirred for 1 hour and ammonium sulfate precipitated proteins removed by centrifugation. Proteins of interest precipitated in 90% (wt/v) ammonium sulfate. Pellet was stored at —20 C until further use.~~

~~[0393] Pea-globulins: Green pea dry seed flour was resuspended at 1:10 (wt/v) ratio in 20 mM potassium phosphate buffer pH 8, 0.4M sodium chloride and stirred for 1 hr. After centrifugation, the supernatant was subjected to ammonium sulfate fractionation. First, supernatant was brought to 50%~~
~~(wt/v) ammonium sulfate, and precipitated proteins removed. Second, 50% (wt/v) ammonium sulfate supernatant was brought to 80% (wt/v) ammonium sulfate saturation. The 80% (wt/v) ammonium sulfate pelleted protein contained globulins of interest. Pellet was stored at —20° C. until further use.~~

(0 Pea-albumins: Dry green or yellow pea flour was used as a source of pea albumins. The flour was suspended in 10 volumes of 50 mM sodium acetate buffer pH 5 and stirred for 1 hr. Soluble protein was separated from un-extracted protein and pea seed debris by either centrifugation (8000 g, 20 minutes) or filtration through a 5 micron filter. Supernatant or filtrate, respectively, was collected. To this crude protein extract, solid ammonium sulfate was added to 50% wt/v saturation. The solution was stirred for 1 hour and then centrifuged. To the supernatant from this step, ammonium sulfate was added to bring to 90% wt/v saturation. The solution was stirred for 1 hour, and then centrifuged to collect the pea albumin proteins in the pellet. The pellet was stored at -20°C until further use. Protein was recovered from the pellet and prepared for use as described above, with the exception that final buffer can contain 0-500 mM sodium chloride.

In some embodiments, the flour was suspended in 10 volumes of 50 mM NaC1, pH 3.8 and stirred for 1 hour. Soluble protein was separated from un-extracted protein and pea seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 Kda cutoff PES membrane.

(ii) Pea-globulins: Dry green pea flour was used to extract pea globulin proteins. The flour was suspended in 10 volumes of 50 mM potassium phosphate buffer pH 8 and 0.4M sodium chloride and stirred for lhr. Soluble protein was separated from

pea seed debris by centrifugation. The supernatant was subjected to ammonium sulfate fractionation in two steps at 50% and 80% saturation. The 80% pellet containing globulins of interest was stored at -20°C until further use. Protein was recovered from the pellet and prepared for use as described above.

iii) Soybean 7S and 11S globulins: Globulins from soybean flour were isolated by first suspending lowfat/defatted soy flour in 4-15 volumes of 10 (or 20) mM potassium phosphate pH 7.4. The slurry was centrifuged at 8000 rcf for 20 mins or clarified by 5 micron filtration and the supernatant was collected. The crude protein extract contained both the 7S and 11S globulins. The solution then was 0.2 micron filtered and

concentrated using a 10 kDa molecular weight cutoff PES membrane on a Spectrum Labs KrosFlo hollow fiber tangential flow filtration system or by passing over anion-exchange resin prior to use in experiments. The 115 globulins were separated from the 7S proteins by isoelectric precipitation. The pH of the crude protein extract was adjusted to 6.4 with dilute HC1, stirred for 30 min-1 hr and then centrifuged to collect the 11S precipitate and 7S proteins in the supernatant. The 115 fraction was resuspended with 10mM Potassium phosphate pH 7.4 and the protein fractions were microfiltered and concentrated prior to use.

Soybean proteins also can be extracted by suspending the defatted soy flour in 4-15 volumes (e.g., 5 volumes) of 20 mM sodium carbonate, pH 9 (or water, pH adjusted to 9 after addition of the flour) or 20 mM potassium phosphate buffer pH 7.4 and 100 mM sodium chloride to decrease off-flavors in the purified protein. The slurry is stirred for one hour and centrifuged at 8000 xg for 20 minutes. The extracted proteins are ultrafiltered and then processed as above or alternatively, the supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(iv) Moong bean 8S globulins: Moong bean flour was used to extract 8S globulins by first suspending the flour in 4 volumes of 50 mM KPhosphate buffer pH 7 (+ 0.5M NaC1 for lab scale purifications). After centrifugation, proteins in the supernatant were fractionated by addition of ammonium sulfate in 2 steps at 50% and 90% saturation respectively. The precipitate from the 90% fraction contained the 8S globulins and was saved at -20°C until further use. Protein was recovered from the pellet and prepared for use as described above.

Moong bean globulins also can be extracted by suspending the flour in 4 volumes of 20 mM sodium carbonate buffer, pH 9 (or water adjusted to pH 9 after addition of the moong flour) to reduce off-flavors in the purified protein fractions. The slurry is centrifuged (or filtered) to remove solids, ultrafiltered and then processed as described above.

(v) Late embryogenesis abundant proteins: Flour (including but not limited to moong bean and soy flour) was suspended in 20 mM Tris—HC1, pH 8.0, 10 mM NaC1,

and stirred at room temperature for 1 hour then centrifuged. Acid (HC1 or acetic acid) was added to the supernatant to a 5% concentration (v/v), stirred at room temperature then centrifuged. The supernatant was heated to 95°C for 15 minutes, and then centrifuged. The supernatant was precipitated by adding Trichoroacetic acid to 25%, centrifuged, then washed with acetone. Heating and acid wash steps can be carried out in the reverse direction as well.

(vi) Pea-Prolamins: Dry green pea flour was suspended in 5x (w/v) 60% ethanol, stirred at room temperature for one hour, then centrifuged (7000g, 20min) and the supernatant collected. The ethanol in the supernatant was evaporated by heating the solution to 85°C and then cooling to room temperature. Ice-cold acetone was added (1: 4 v/v) to precipitate the proteins. The solution then was centrifuged (4000g, 20min), and protein recovered as the light-beige colored pellet.

(vii) Zein-Prolamins: Corn protein concentration or flour was suspended in 5x (w/v) 60% ethanol, stirred at room temperature for one hour, then centrifuged. Ethanol in supernatant was evaporated with heat, and then the solution is centrifuged, and the protein recovered as the pellet.

(viii) ~~[0394] RuBisCO:~~ RuBisCO was fractionated from alfalfa greens ~~(or other green plants eg soybean plants, spinach etc)~~ by first grinding leaves with 4 volumes of cold 50 mM ~~KPhos-phate~~ potassium phosphate buffer pH 7.4 buffer (~~with (in lab) or without (in field) 0.5M NaC1+2 mM DTT+1 mM~~ 0.5M NaC1+ 2mM DTT + 1mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (crude lysate) was used in further purification steps. Proteins in the crude lysate were fractionated by addition of ammonium sulfate to 30% (wt/v) saturation. The solution was stirred for ~~1 hr~~ lhr and then centrifuged. The pellet from this step was discarded and additional ammonium sulfate was added to the supernatant to 50 % (wt/v) ammonium sulfate saturation. The solution was centrifuged again after stirring for ~~1 hr~~ lhr. The pellet from this step contains RuBisCO, and was kept at ~~—20 C~~ -20°°C until used. Protein was recovered from the pellet and prepared for use as described above.

RuBisCO also can be purified by adjusting the crude lysate to 0.1M NaC1 and applying to an anion exchange resin. The weakly bound protein contaminants are washed with 50 mM KPhosphate buffer pH 7.4 buffer + 0.1M NaCl. RuBisCO was then eluted with high ionic strength buffer (0.5M NaC1).

RuBisCO solutions were decolorized (pH 7-9) by passing over columns packed with activated carbon. The colorants bound to the column while Rubisco was isolated in the filtrate.

RuBisCO solutions were also alternatively decolorized by incubating the solution with FPX66 (Dow Chemicals) resin packed in a column (or batch mode). The slurry is incubated for 30mins and then the liquid is separated from the resin. The colorants bind to the resin and RuBisCO was collected in the column flow-through.

In some embodiments, RuBisCO was isolated from spinach leaves by first grinding the leaves with 4 volumes of 20mM potassium Phosphate buffer pH 7.4 buffer + 150 mM NaC1+ 0.5 mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (crude lysate) was filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

In some embodiments, RuBisCO was extracted from alfalfa or wheatgrass juice powder by mixing the powder with 4 volumes of 20mM potassium Phosphate buffer pH 7.4 buffer + 150 mM NaC1 + 0.5 mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (crude lysate) was filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

~~by ammonium sulfate fractionation as described. Pellet was resuspended in 20 mM potassium phosphate pH pH 7.4 and 0.5M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16000 g, 10 min and protein concentrated to desired concentration. Pea globulins purified by ammonium sulfate fractionation as described. Protein pellet was resuspended in 20 mM potassium phosphate pH pH 7.4 and 0.4M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16000 g, 10 min and protein concentrated to desired concentration. Pea albumin purified by ammonium sulfate fractionation as described. Protein pellet was resuspended in 20 mM potassium phosphate pH pH 7.4 and 0.1M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16000 g, 10 min and protein concentrated to desired concentration.~~ **~~[0397]~~** ~~Constructing a Muscle Tissue Analog~~

~~[0398]~~ ~~Moong bean seed 8S protein was purified by ammonium sulfate fractionation as described above, For preparation of gels, 200 g of pellet was dissolved in 400 ml of dialysis buffer (20 mM potassium phosphate, 400 mM NaC1, pH 7.3) and the resulting solution dialyzed for 6 hours against 5l of dialysis buffer, replaced twice with fresh buffer. Protein solution was centrifuged at 12,000 g for 15 min to remove debris. Protein was concentrated by dialyzing for 36 hours against 5l of 30% w/w solution of PEG 8000 (polyethylene glycol, molecular weight 8000) in dialysis buffer. Final protein concentration was 150 mg/ml.~~

~~[0399]~~ ~~Leghemoglobin was purified from soybean root nodules. Legume root nodules were cleaned to remove soil and extraneous root tissues prior to root nodule lysis in 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mm EDTA and 1 mM ascorbic acid. Root nodules were lysed by grinding root nodules using juicer blender. Unsoluble material was separated by centrifugation. Root nodule lysate was fractionated using ammonium sulfate in two steps, first ammonium sulfate was added to 60% wt/v and solution incubated for 1 hr, 4° C. Pellet was discarded and supernatant brought to 90% wt/v ammonium sulfate and~~

~~incubated for 12 hr, 4° C. Leghemoglobin was collected as a precipitated pellet in 90% ammonium sulfate fraction and resuspended in 20 mM potassium phosphate, 1 mM EDTA, 100 mM sodium chloride. SDS-PAGE gel analysis determined that protein solution contains 70% leghemoglobin and 30% other root nodule proteins. Ammonium sulfate was removed using size-exclusion chromatography in the same buffer. Leghemoglobin was concentrated by dialyzing for 48 hr against 30% PEG 8000 (polyethylene glycol, molecular weight 8000) in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride. Total protein concentration was 57 mg/ml. UV-VIS spectra suggested that leghemoglobin was in heme-iron oxidized state. Thus,~~

leghemoglobin was incubated with 5 mM sodium hydro sulfite for 5 min and sodium hydro sulfite was removed using size-exclusion chromatography in 20 mM potassium phosphate, 100 mM sodium chloride buffer. Leghemoglobin was further concentrated to 35.4 mg/ml. UV-VIS spectra analysis confirmed that leghemoglobin is in heme iron reduced state.

[0400] Transglutaminase was obtained commercially from (Activa TI, Ajimoto). Stock solution (20% wt/v) was made in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride buffer.

[0401] To prepare "dark" muscle tissue analog (FIG. 10), 43 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 37 ml of leghemoglobin solution (46.5 mg/ml leghemoglobin and 20 mg/ml of other soybean root nodule protein) in 20 mM potassium phosphate, 100 mM NaC1, pH 7.3). 20 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly mixed, divided into two 50 ml Falcon tubes and incubated overnight at room temperature. Final protein concentrations were 65 mg/ml for moong bean protein, 18 mg/ml of leghemoglobin, 91 mg/ml total protein.

[0402] "White" muscle analog (FIG. 11) was prepared by mixing 43 ml moong bean protein solution (150 mg/ml) with 45 ml of 11.7 mg/ml solution of leghemoglobin and 0.8% (wt/v) of transglutaminase solution. Final protein concentrations were 63 mg/ml for moong bean protein, 5.2 mg/ml of leghemoglobin, 68 mg/ml total protein.

[0403] The "dark" muscle tissue analog formed an opaque gel of dark chocolate color, smooth uniform texture, with glistening surface, and a small amount (<1 ml) of dark red, venous blood colored liquid on top. The gel was freely standing, elastic but fragile, similar in appearance to thin Jell-O. The gel has a medium aroma with notes of beans and blood clearly discernible. The flavor is dominated by notes of beans and iron/blood, with weaker grassy and medicinal/chemical flavors. The taste is salty, with a long aftertaste of blood. **[0404]** The "white" muscle tissue analog was very similar, but with much lighter, cappuccino-like, color. It was also more fragile, 2-3-fold less strong against compression.

[0405] Fat Tissue Analog

[0406] Fat tissue analog using moong bean 8S globulin fraction was prepared as follows: 15 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 15 ml of rice bran oil. 6 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly emulsified using a homogenizer (VWR) at speed #2. Emulsion was aliquoted into 1.6 eppendorf tubes and incubated overnight at room temperature. After that, tubes were heated at 95° C. for 5 min in a heat block, and allowed to cool down to room temperature on a bench. Final concentrations were 75 mg/ml for moong bean protein, 50% w/w oil.

[0407] Fat tissue analog using pea globulin (100 mg/ml) was prepared by the same method. Additionally, fat tissue analog was prepared from pea globulin, and either rice bran or canola oil, in bulk by the same method, but without aliquoting emulsions into eppendorf tubes. Instead, emulsions in 50 ml Falcon tubes were rotated overnight on a nutator, and were subsequently incubated at 90° C. for 30 min.

[0408] Fat tissue analog based on moong beans (FIG. **12)** and prepared in eppendorf tubes formed an opaque gel of off-white color, smooth uniform texture, with no visible discernible liquid that was not incorporated into the gel. The gel was freely standing, elastic and springy. The gel has a slight, pleasant aroma and a mild and pleasant flavor. The taste is mildly salty.

[0409] Fat tissue analog based on pea globulin (FIG. **13)** and prepared in eppendorf tubes was very similar to moong bean-based fat analog, except that it gave up a little of oil upon compression. Fat tissue analog prepared in 50 ml Falcon tubes were similar in appearance, texture and aromas, but substantially softer (2-fold softer for canola oil, and 3-fold softer for rice bran oil, according to compressibility measurements).

[0410] Connective Tissue Analog

[0411] Connective tissue analog prototypes were developed using zein protein sourced from 100% yellow corn gluten meal, or from commercial sources, such as Amazein (Prairie Gold, Bloomington, Ill.). Zein proteins were solubi-lized in 70-90% ethanol with desired ratios at 1:3 to 1:5 (solids:solution). By precipitating zein proteins, for example by a change in pH, in a controlled manner, large zein structures result with physicochemical properties that can be manipulated as desired. For example, FIG. **14** shows connective tissue analog strands that were created using a 1:3 ratio in 70% ethanol, loaded into a syringe with a 23 gauge needle (ID 0.337 mm). The solution was slowly extruded from the bot

tom of a 5 inch high vessel into an excess of 5 M NaCl solution. The ethanol zein solution being less dense than the NaCl solution, floated upward, drawing out a fibrous stand of solidifying zein. The NaCl was constantly stirred as the strands began to develop to assist in the strand lengthening. The strands bunch together and become a hard, dense mass. **[0412]** Ground Beef Replica Prototypes Made from Gels of Plant Proteins and Plant Oils.

[0413] A ground beef prototype patty was made by combining 62% (wt/wt) muscle analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "white muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (FIG. **15** panel A). A ground beef prototype patty was made by combining 62% muscle analog (62% "dark muscle analog" and 38% "white muscle analog), 29% fat tissue analog (from moong bean seed 8S protein and rice bran oil), 5% connective tissue analog (FIG. **15** panel B). A ground beef prototype patty was made by combining 71% (wt/wt) muscle tissue analog (composed of 60% "white" muscle analog, 40% "dark" muscle analog), 23% fat tissue (from pea seed globulin proteins and canola oil) (FIG. **15** panel C). A ground beef prototype patty was made by combining 67% "White" muscle analog, with 28% fat tissue analog (from pea globulins and rice bran oil), (FIG. **15,** panel D)

[0414] In a further test, the effect of cooking the ground beef replica patties was evaluated by grilling on a 350° F. pan. A ground beef patty analog was made by combining 62% (wt/wt) muscle tissue analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (FIG. **16).** The panel on the left shows the patty before cooking and the panel on the right shows the same patty after cooking for about 2 minutes. Observers described the aroma of the cooking ground beef replica as distinctly "beefy".

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His Thr Arg Lys Asp Cys Leu Gly Lys
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1. 162. (canceled)

163. A meat substitute product, comprising:

a muscle replica;

a fat tissue replica; and

a connective tissue replica;

wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat.

164. The meat substitute product of claim **163,** comprising two or more of said muscle replica, fat tissue replica, and connective tissue replica.

165. The meat substitute product of claim **163,** wherein (i) said muscle replica accounts for 40-90% of said product by weight, (ii) said fat tissue replica accounts for 1-60% of said product by weight, and (iii) said connective tissue replica accounts for 1-30% of said product by weight.

[0395] Obtaining Plant Proteins.

[0396] Moong bean seed 8S protein was purified (ix) Leghemoglobin. Soy root nodules were suspended and lysed in 20 mM potassium phosphate pH 7.4, 100mM potassium chloride

and 5mM EDTA using grinder-blender. During this process leghemoglobin is released into the buffer. Root-nodule lysate containing leghemoglobin was cleared from cell debris by filtration through 5 micron filter. In some embodiments, filtration was followed by centrifugation (7000g, 20min). Clarified lysate containing leghemoglobin was then filtered through 0.2 micron filter and applied onto anion-exchange chromatography column (High Prep Q; High Prep DEAE, GE Healthtcare) on fast protein liquid chromatography instrument (GE Healthcare). Leghemoglobin was collected in flowthrough fraction and concentrated over 3kDa molecular weight cutoff PES membrane on a Spectrum Labs KrosFlo hollow fiber tangential flow filtration system to a desired concentration. Purity (partial abundance) of purified leghemoglobin was analyzed by SDS-PAGE gel: in lysate leghemoglobin is present at 20-40%, while after anion-exchange purification it is present at 70-80%. In

another embodiment, soybean leghemoglobin flow through from anion-exchange chromatography was applied onto size-exclusion chromatography (Sephacryl S-100 HR, GE Healthcare). Soybean leghemoglobin eluted as two fractions corresponding to dimer and monomer species. Purity (partial abundance) of leghemoglobin was analyzed by SDS-PAGE and determined to be — 90-100%. Analysis of UV-VIS spectra (250-700nm) revealed spectral signature consistent with heme loaded leghemoglobin.

(x) Non-symbiotic hemoglobin from moong bean was cloned into pJexpress401 vector (DNA2.0) and transformed into *E. coli* BL21. Cells were grown in LB media containing soytone instead of tryptone, kanamycin, 0.1mM ferric chloride and 10 µg/ml 5-aminolevulinic acid. Expression was induced by 0.2mM IPTG and cells grown at 30°C for 20hr. *E.coli* cells expressing moong bean non-symbiotic hemoglobin were collected and resuspended in 20mM MES buffer pH 6.5, 50mM NaCL, lmmM $MgC1_2$, 1mM $CaC1_2$. Add a bit of DNAaseI, and protease inhibitors. Cells were lysed by sonication. Lysate was cleared from cell debris by centrifugation at 16 000g for 20 min, followed by filtration over 200nm filter. Cell lysate was then loaded over FF-S resin on fast protein liquid chromatography instrument (GE Healthcare). Moong bean non-symbiotic hemoglobin bound to FF-S column and was eluted using sodium chloride gradient (50mM- 1000mM). Purity (partial abundance) of moong bean non-symbiotic hemoglobin was analyzed by SDS-PAGE and determined to be: *E.coli* lysate 13 %, after purification on FFQ 35%. UV-Vis analysis of purified protein showed spectra characteristic of heme bound protein.

(xi) Hemeproteins were synthesized with an N-terminal His6 epitope tag and a TEV cleavage site, cloned into pJexpress40l vector (DNA2.0), and transformed into *E. coli* BL21. Transformed cells were grown in LB media containing soytone instead of tryptone, kanamycin, 0.1 mM ferric chloride and 10 µg/ml 5-aminolevulinic acid. Expression was induced by 0.2mM IPTG and cells grown at 30°C for 20hr. *E.coli* cells expressing heme proteins were collected and resuspended in 50 mM potassium phosphate pH 8, 150 mM NaC1, 10 mM imidazole, 1 mM $MgC1_2$, 1 mM $CaC1_2$, DNAaseI, and protease inhibitors. Cells were lysed by sonication and clarified by centrifugation at 9000 x g. Lysate was incubated with NiNTA resin (MCLAB), washed with 5 column volumes

(CV) of 50 mM potassium phosphate pH 8, 150 mM NaC1, 10 mM imidazole, and eluted with 50 mM potassium phosphate pH 8, 150 mM NaC1, 500 mM imidazole. SDS-PAGE and UV-vis spectra confirmed expected molecular weights and complete heme-loading, respectively.

In some embodiments, transformed cells were grown in seed media comprised of 10g/L glucose monohydrate, 8g/L Monopotassium Phosphate, 2.5g/L Sensient Amberferm 6400, 2.5g/L Sensient Tastone 154, 20: Diammonium Phosphate, Irrit/L 'Trace Metals Mixture (Teknova 1000x Trace Metals Mixture Cat. ˉNo. T1001), ig/L Magnesium Sulfate, 0.25mL 0.1M solution Ferric Chloride, 0.5MLIL Sigma Anti-fbam 204, lrnL/L Kanarnycin Sulfate 1000x solution. 25OmL of media was used in a (4)1 IL baffeled shakeflasks, innoculated with O.25mL each from a single vial of glycerol stock culture. Shakeflasks were grown for 5.5 hours, with 250RPM agitation at 37°C. 40L of seed media was steam-sterlized in a 100L bioreactor, cooled to 37°C, pH-adjusted. to 7.0 and innoculated with 800mL of shakeflask culture once a shakeflask OD of 2.5 was achieved. Aeration to the bioreactor was supplied at 40L/m and agitation was 250RPM. After 2.2 hours of growth, an OD of 2.20 was reached and 22L of culture was transferred to the final $4M^3$ bioreactor. Jhe starting media for the final bioreactor comprised of the following components steamed-in-place: 1775L deionised water, 21.75.kg Monopotassium Phosphate, 2.175kg Diammonium Phosphate, 4.35kg Ammonium Ferric Citrate, 8.7kg Ammonium Sulfate, 10.875kg Sensient Amberferm 6400, 10.875kg Sensient Tastone 154. After 30 minutes of steaming, the media components were cooled to 37°C and post-sterilzation additions were made: 2.145L of 0.1M Ferric Chloride solution, 59.32kg 55%w/w Glucose Monohydrate, 3.9L of Trace Metals Mixture (Teknova 1000x Trace Metals Mixture Cat. No. T1001), 10.88L of 200g/L Diammonium Phosphate, 36.14L iMMagnesium Sulfate, and 2.1751, Sigma _Anti-foam 204, 2.1751, Kanamycin Sulfate 1000x solution. pH was controlled at 7.0 via the addition of 30% Ammonium Hydroxide. Aeration was supplied at $2.175m^3$/min, dissolved oxygen was controlled at 25% by varying agitation between 60-150IUM. At two thnepoints (EFT=4 and EFT8), bolus additions of additional nutrients were supplied. Each addition added. 5 .5kg of Sensient Amberferm 6400, 5.5kg of Sensient Tastone 154 and 4.4kg of

Diammonium Phosphate, in autoclaved solutions (1.00g1L solution for Amberfenn and Tastetorte,, 200g7/ 1, for Diammonium Phosphate). A sterile glucose solution of 55%w/w Glucose Monohydrate was fed into the bioreactor to maintain a level of residudual glucose of 2-5g/L. Once an OD of 25 was reached, the temperature was reduced to 25°C and the culture was induced with 0.648L of I M Isopropyl P-D-1-thiogalactopyranoside. The culture was allowed to grow for a total time of 25 hours, at which point the culture was diluted 1:1 with deionized water, then centrifuged, concentrating the centrate to 50%v/v solids content. Cell centrate was frozen at -20°C. Centrate was thawed to 4°C and diluted in 20 triM potassium phosphate pH 7.8, 100 mM NaCI, 10 ruM imidazole, and homogenized at 15,000 PSI. Homogenized cells were 0.2 urn filtered by tangential flow filtration (TEE) and filtered lysate was loaded directly onto a zinc-charged IMAC column (GE). Bound proteins were washed with 10 column volumes (CV) 20 mM potassium phosphate pH 7.4, 100 mM NaCI, 5 mM histidine and eluted with 10 CV 500 mM potassium phosphate monobasic, 100 mM NaCl. Eluted leghemoglobin was concentrated and diafiltered using a 3kDa molecular weight cutoff PES membrane and TEE. The concentrated sample was reduced with 20 m141 sodium dithionite and desalted using G-20 resin (GE). Desalted leghemoglobin samples were frozen in liquid nitrogen and stored at -20 C. Leghemoglobin concentration and purity were determined by SDS-PAGE and UV-vis analysis.

(xi) Oleosin. Sunflower oil bodies were purified from sunflower seeds. Sunflower seeds were blended in 100 mM sodium phosphate buffer pH 7.4, 50mM sodium chloride, 1 mM EDTA at 1:3 wt/v. Oil-bodies were collected by centrifugation (5000g, 20min), and resuspended at 1:5 (wt/v) in 50 mM sodium chloride, 2M urea and stir for 30min, 4°C. 2M urea wash and centrifugation steps were repeated. Oil-bodies collected by centrifugation were resuspended in 100 mM sodium phosphate buffer pH 7.4, 50mM sodium chloride. Centrifugation and washing steps were repeated once more, and the final washed oil-bodies fraction was obtained from a last centrifugation step. Oil-bodies were resuspended at 10% wt/w in 100 mM sodium phosphate buffer pH 7.4, 50mM sodium chloride, 2% wt/v vegetable oil fatty acid salts, homogenized at 5000 psi and incubated at 4°C for 12 hr. Solution was centrifuged (8000g, 30min), top layer removed

and soluble fraction collected. SDS-PAGE analysis suggested that oleosins are a major protein present in the soluble fraction. Oleosin concentration was 2.8 mg/ml.

(xii) Pea total proteins: Dry green or yellow pea flour was used to extract total pea proteins. The flour was suspended in 10 volumes of 20mM potassium phosphate buffer pH 8 and 100 mM sodium chloride and stirred for lhr. Soluble protein was separated from pea seed debris by centrifugation. The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 Kda cutoff PES membrane.

(xiii) Pea vicilin and Pea legumin: Dry green or yellow pea flour was used to extract total pea proteins as described above. The crude pea mixture obtained thereof was fractionated into pea vicilin and pea legumin using ion-exchange chromatography. Material was loaded on Q Sepharose FastFlow resin and fractions were collected as salt concentration was varied from 100 mM to 500 mM NaCl. Pea vicilin was collected at 350 mM sodium chloride while pea legumin was collected at 460 mM sodium chloride. The collected fractions were concentrated using a 10 KDa cutoff PES membrane.

(xv) Lentil total proteins: Air classified lentil flour was used to extract crude mixture of lentil proteins. Flour was suspended in 5 volumes of 20 mM potassium phosphate buffer pH 7.4 and 0.5 M sodium chloride and stirred for 1 hr. Soluble protein was separated from un-extracted protein and lentil seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(xvi) Lentil albumins: Air classified lentil flour was suspended in 5 volumes of 50 mM sodium chloride, pH 3.8 and stirred for 1 hr. Soluble protein was separated from un-extracted protein and lentil seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(xvii) Chickpea / Garbanzo bean total proteins: Garbanzo bean flour was suspended in 5 volumes of 20 mM potassium phosphate buffer pH 7.4 and 0.5 M sodium chloride and stirred for 1 hr. Soluble protein was separated from un-extracted protein and chickpea seed debris by centrifugation (8000 g, 20 minutes). The supernatant was

collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(xviii) Chickpea/ Garbanzo bean albumins: Garbanzo bean flour was suspended in 5 volumes of 50 mM sodium chloride, pH 3.8 and stirred for 1 hr. Soluble protein was separated from un-extracted protein and lentil seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(xix) Amaranth flour dehydrins: Amaranth flour was suspended in 5 volumes of 0.5 M sodium chloride, pH 4.0 and stirred for 1 hr. Soluble protein was separated from un-extracted protein and lentil seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 3 KDa cutoff PES membrane. Further enrichment of dehydrins from this fraction was obtained by boiling the concentrated protein material, spinning at 8000 g for 10 minutes and collecting the supernatant.

EXAMPLE 2: CONSTRUCTING A MUSCLE TISSUE ANALOG

To prepare a muscle tissue replica, 8 ml of moong bean protein solution (114 mg/ml in 20 mM phosphate buffer (pH 7.4) and 400 mM sodium chloride) were mixed with 16 ml of leghemoglobin solution (6 mg/ml leghemoglobin in 20 mM potassium phosphate, 400 mM NaC1, pH 7.3). The resulting mixture was concentrated using Amicon spin concentrators (10 kDa cut-off) to a final concentration of moong bean 8S globulin 61 mg/ml, and of leghemoglobin 6.5 mg/ml. Approximately 400 mg of transglutaminase powder were added to the solution, which were thoroughly mixed, and divided into two 50 ml Falcon tubes and incubated overnight at room temperature. Final total protein concentrations was 67.5 mg/ml total protein. The muscle tissue replica formed an opaque gel of reddish-brown color, with small amounts (< 1 ml) of inclusions of dark red, venous blood colored liquid.

EXAMPLE 3: INCREASED TENSILE STRENGTH ADIPOSE TISSUE REPLICA

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A 40 ml aliquot of rice bran oil and a 40 ml aliquot of moong bean protein (114 mg/ml) were combined in a 250m1 Pyrex beaker. The beaker was placed in a water bath and emulsified using a Branson Sonifer 450 sonicator with a 12mm tip for a 6 minute, 60% duty cycle at power level 5.

In an 18cm x 18cm x 2.5cm synthetic rubber Ikea plastic ice cube tray, 48 mg of electrospun fibers (from connective tissue Example 14) were laid longitudinally and as homogenously as possible across the bottom of one triangular 13.97cm x 1.27cm x 1.5875cm mold. Approximately 20 ml of the rice bran oil/moong bean protein emulsions then were poured on top of the fibers. An additional 20 ml of the emulsions were then poured into a similarly sized blank mold on the same tray to be used as a control.

The ice cube tray was floated in boiling water for 15 minutes, removed, and cooled to room temperature.

Using a razor blade, each of the resulting gels was cut into 3 segments, each 4.66cm long with a cross-sectional area of $1cm^2$. A Stable Micro Systems TA XTExpress Enhanced texture analyser with attached TA-96B probe was used to assess the tensile strength. The fiber containing the fat replica had a tensile strength of 23 kPa, whereas the fat replica with no fibers had a tensile strength of 20 kPa.

EXAMPLE 4 HIGH PERCENTAGE FAT ADIPOSE-REPLICA

An adipose tissue replica comprising a protein-oil emulsification formed with 3.3% wt/v pea globulin, 70% v/v oil that consisted of an equal mixture of coconut, cocoa, olive, and palm oils, and 0.5% wt/v lecitin, was cross-linked with a 2% transglutaminase (Ajinomoto Activa® TI). After draining and dehydrating, the resulting gel was medium soft and the fat content was confirmed to be 75% (wt/wt).

An adiose tissue matrix comprising a protein-oil emulsification was formed with 1.6 % wt/v Rubisco and 80% v/v cocoa butter. The resulting gel was soft.

EXAMPLE 5: METHOD OF PREPARING ADIPOSE TISSUE REPLICAS Oils are melted if necessary by warming to room temperature or gently heated. If the oils are solid at room temperature, they are kept near the melting point during the rest

of the procedure. Proteins are obtained per specified protocols (see Example 1). Lecithin is weighed and resuspended in water, then sonicated to create a homogenous solution. Components are combined at specified ratios and brought to volume with buffer if necessary (20 mM sodium phosphate pH 7.4 with 50 mM sodium chloride) then are subjected to homogenization or sonication to control particle size within an emulsion. Afterwards, emulsions are gelled by either: (a) heating/cooling, (b) cross-linking with a transglutaminase enzyme or (c) heating/cooling followed by addition of a transglutaminase enzyme. Control samples (no heating/cooling nor transglutaminase cross-linking treatments) were prepared for comparisons. Emulsions stabilized by heating/cooling treatment are prepared by placing emulsion in 90-100°C water bath for five minutes, then letting samples slowly cool to room temperature. Emulsions stabilized by transglutaminase cross-linking are prepared by adding transglutaminase to 2% wt/v and incubating at 37°C for 12-18hr. Emulsions stabilized by heating/cooling followed by addition of transglutaminase enzyme were prepared by first undergoing the heat/cool protocol, then adding the enzyme once the samples cooled to room temperature. All emulsifications are incubated for 8-12 hours at 37°C.

EXAMPLE 6: METHOD TO ANALYZE ADIPOSE TISSUE REPLICAS

After different gelling treatments, gelled emulsions are moved to room temperature for evaluation. The total volume of gelled emulsions, and volumes of phase separated water and/or oil volumes (if gelled emulsions are not in a single phase) are recorded. Firmness of the adipose tissue replica is evaluated by gentle poking of gelled emulsions. Cooking experiments are performed by transferring the mass to a heated surface and measuring the temperature of the liquid immediately after cooking.

EXAMPLE 7: ADIPOSE REPLICA OF BEEF FAT

An adipose tissue replica was made by gelling a solution of purified moong bean 8S protein emulsified with equal amounts of cocoa butter, coconut butter, olive oil and palm oil. Moong bean 8S protein was purified as described in Example 1, and had a concentration of 140 mg/ml in 20mM K-phosphate pH 7.4, 400mM NaCl. A fat mixture

was prepared by melting individual fats from solid to liquid state at 45°C for 30 min. Individual fats (cocoa butter, coconut butter, olive oil and palm oil) in liquid states were then mixed at a 1:1:1:1 (v/v) ratio. A protein-fat emulsion was formed by mixing a 70% v/v liquid fat mixture with 4.2% wt/v moong bean 8S protein, 0.4% wt/v soybean lecithin and emulsified by vortexing for 30 sec followed by sonication for 1 min. After homogenization, the fat-protein emulsion was in a single liquid phase as judged by visual observation.

One adipose tissue replica emulsion was stabilized by cross-linking with 0.2% wt/v transglutaminase enzyme at 37°C for l2hr. Another fat tissue replica was stabilized by gelling of proteins by heating to 100°C in a water bath followed by cooling to ambient room temperature. The resulting adipose tissue replicas were in a single phase. The adipose tissue replica matrix formed by the transglutaminase was a softer solid than the adipose tissue replica matrix formed by heat/cool induced gelling.

EXAMPLE 8: AN ADIPOSE REPLICA OF WAGYU BEEF FAT

An adipose tissue replica was made by gelling an emulsion of purified pea globulin proteins and equal amounts of cocoa butter, coconut butter, olive oil and palm oil. Pea globulin proteins were purified as described in Example 1 and had a concentration of 100 mg/ml, in 20mM K-phosphate pH 8, 400mM NaCl. A fat mixture was prepared by melting individual fats from solid to a liquid state at 45°C for 30 min. Individual fats (cocoa butter, mango butter, olive oil) in liquid state where then mixed at 2:1:1 (olive oil: cocoa butter: mango butter) v/v ratio. A protein-fat emulsion was formed by mixing a liquid fat mixture with a 5% wt/v solution of pea globulins in a 1:1 ration, and emulsifying using a hand-held homogenizer at the maximum setting for 30sec. After homogenization, the fat-protein emulsion was in a single liquid phase as judged by visual observation. Emulsion was stabilized by cross-linking with 0.2% wt/v transglutaminase enzyme at 37°C for l2hr. The resulting adipose tissue replica was in a single phase, was a soft solid and was salty in flavor.

EXAMPLE 9: ADIPOSE TISSUE REPLICA WITH FATTY ACID DISTRIBUTION OF BEEF:

An adipose tissue replica was made by gelling an emulsion of purified pea globulin proteins and equal amounts of cocoa butter, mango butter, olive oil and rice bran oil. Pea globulin proteins were purified as described in Example 1, and had a concentration of 100mg/ml, in 20mM K-phosphate pH 8, 400mM NaCl. A fat mixture was prepared by melting individual fats from solid to liquid state at 45°C for 30min. Individual fats (cocoa butter, mango butter, olive oil and rice bran oil) in a liquid state where then mixed at 1:1:1:1 v/v ratio. A protein-fat emulsion was formed by mixing 50% v/v liquid fat mixture with 5% wt/v Pea globulins, and emulsifying using a hand-held homogenizer at the maximum setting for 30sec. After homogenization, the fat-protein emulsion was in a single liquid phase as judged by visual observation. The emulsion was stabilized by cross-linking with 0.2% wt/v transglutaminase enzyme at 37°C for l2hr. The resulting adipose tissue replica was in a single phase, was a soft solid and was salty in flavor.

EXAMPLE 10: ADIPOSE TISSUE REPLICA WHERE FIRMNESS OF FAT TISSUE AT REFRIGERATION AND AMBIENT TEMPERATURES IS CONTROLLED BY MELTING TEMPERATURE OF FAT IN ADIPOSE TISSUE REPLICA.

A adipose tissue replica made as a stable emulsion of RuBisCo with sunflower oil is softer than a adipose tissue replica made as a stable emulsion of RuBisCo and cocoa butter. Adipose tissue replicas were formed with 0.18%, 1.6%, and 2.4% wt/v Rubisco with 70%, 80%, and 90% v/v sunflower or cocoa butters. Each adipose tissue replica that contained cocoa butter was firmer than the corresponding replicas that were formed with sunflower oil. Adipose tissue replicas comprising 0.18%, 1.6%, and 2.4% wt/v Rubisco with 70%, 80%, and 90% v/v cocoa butter were solid at room temperature but melted at close to mouth temperature. In the adipose tissue replicas formed with varying concentrations of Rubisco (0.18, 1.6 1.9 % wt/v) and 70-80% v/v sunflower oil, the replicas were firmer as the amount of protein in the adipose tissue replica matrix

increased. Adipose tissue replicas with 0.18% wt/v Rubisco were very soft; adipose tissue replicas with 1.6% wt/v Rubisco were soft; and adipose tissue replicas with 1.9% wt/v RuBisco were of medium firmness.

Adipose tissue replicas made as a stable emulsion of Moong Bean 8S protein with sunflower oil were softer than adipose tissue replicas made as a stable emulsion of Moong Bean 8S protein and cocoa butter. Adipose tissue replicas were formed with 2%, 1%, and 0.5% wt/v Moong Bean 8S protein with 70%, 80%, and 90% v/v sunflower or cocoa butters. Each adipose tissue replica that contained cocoa butter was firmer than the corresponding replica that was formed with sunflower oil.

Adipose tissue replicas made as stable emulsions of Moong Bean 8S protein with canola oil were softer than the corresponding adipose tissue replicas made as stable emulsions of Moong Bean 8S protein with an equal mixture of coconut, cocoa, olive, and palm oils. Adipose tissue replicas were formed with 1.4% wt/v Moong Bean 8S protein with 50%, 70%, and 90% v/v sunflower or mixture of oils. Each adipose tissue replica that contained a mixture of oils was firmer than the corresponding replica that was formed with sunflower oil. A adipose tissue replica comprising1.4% wt/v Moong Bean 8S protein with 50%, 70%, and 90% v/v of an equal mixture of coconut, cocoa, olive, and palm oil was solid at room temperature but melted at close to mouth temperature.

Adipose tissue replica made as a stable emulsion of soy proteins with sunflower oil was softer than the adipose tissue replica made as stable emulsion of soy proteins and cocoa butter. Adipose tissue replicas were formed with 0.6%, 1.6%, and 2.6% wt/v Soy with 50%, 70%, 80%, and 90% v/v sunflower or mixture of oils. Each adipose tissue replica that contained mixture of oils was firmer than the corresponding replica that was formed with sunflower oil. Adipose tissue replicas comprising 0.6%, 1.6%, and 2.6% wt/v soy proteins with 50%, 70%, 80%, and 90% v/v cocoa butter were solid at room temperature but melted at close to mouth temperature.

EXAMPLE 11: ADIPOSE TISSUE REPLICA COOKING: STRUCTURE OF FAT TISSUE MATRIX CONTROLS MELTING POINT DURING COOKING.

An adipose tissue comprising a stabilized protein-oil emulsion constructed as described

in Example 5 and Example 6 above, formed with 2% w/v Rubisco and 50%, 70%, and 90% v/v cocoa butter, melted at a higher temperature when formed upon heat/cool denaturation and at a lower temperature than when formed by cross-linking with a transglutaminase.

EXAMPLE 12: COOKING ADIPOSE TISSUE: ARRANGEMENT AND STRUCTURAL ORGANIZATION OF PROTEINS AND FAT WITHIN A FAT TISSUE MATRIX CONTROLS AMOUNT OF FAT RELEASED AND FAT RETAINED BY THE ADIPOSE TISSUE REPLICA DURING COOKING.

During cooking of a adipose tissue replica matrix comprising a protein-oil emulsion formed with 2% w/v Rubisco and 50%, 70%, or 90% v/v cocoa butter, more adipose tissue replica mass was retained after cooking when the adipose tissue replica was formed upon heat/cool denaturation than when formed by cross-linking with a transglutaminase. Released mass was liquid and appeared oily.

During cooking of adipose tissue replica matrix comprising a protein-oil emulsion formed with 2.6 and 0.6 % w/v Soybean protein and 50%, 70%, or 90% v/v cocoa butter, more adipose tissue replica mass was retained when formed upon heat/cool denaturation than when formed by cross-linking with transglutaminase. Released mass was liquid and appeared oily.

EXAMPLE 13: COOKING AN ADIPOSE TISSUE REPLICA: CONCENTRATION OF PARTICULAR PROTEINS WITHIN FAT TISSUE MATRIX CONTROLS MASS OF ADIPOSE TISSUE REPLICA THAT REMAINS AFTER COOKING

A series of adipose tissue replicas constructed from 1.4% wt/v moong bean 8S protein with 90% v/v canola oil and 0.45% wt/v soybean lecithin, were homogenized and increasing concentration of sunflower oleosins were added at varying concentrations to the emulsion. The concentration of oleosin varied from 1:10 to 1:10^6 oleosin to triglyceride molar ratio. An increase in mass retention after cooking was observed when the ratio of oleosins to oil in adipose tissue replica was greater.

A series of adipose tissue replicas formed with varying concentrations of Rubisco with 70% v/v sunflower oil, retained more mass upon cooking as the concentration of Rubisco increased. Adipose tissue replicas containing RuBisCo at 0% wt/v completely melted, while 1.9% wt/v Rubisco retained 10% mass, and adipose tissue replica containing 2.4% wt/v Rubisco retained 20% mass upon cooking

EXAMPLE 14: CONNECTIVE TISSUE ANALOG

Connective tissue fiber replicas were manufactured by electrospinning a solution of moong bean globulin (22.5 mg/ml) containing 400 mM sodium chloride, 6.75% w/v of poly(vinyl alcohol) and trace amounts of sodium azide (0.007% w/v). The resulting solution was pumped at 3 gl/min using a syringe pump, from a 5 ml syringe through a Teflon tube and a blunted 2l gauge needle. The needle was connected to a positive terminal of a Spellman CZE 30 kV high voltage supply set at 17kV and fixed 12 cm from a an aluminum drum (ca. 12 cm long, 5 cm in diameter) that was wrapped in aluminum foil. The drum was attached to a spindle that is rotated by an IKA RW20 motor at about 220 rpm. The spindle was connected to a ground terminal of the high voltage supply. The protein/polymer fibers that accumulated on the foil were scraped off and used as the connective tissue replicas.

EXAMPLE 15: EXTENDING THE LIFETIME OF REDUCED (HEME-FE2+) LEGHEMOGLOBIN

Equine myoglobin was purchased from Sigma. Myoglobin was resuspended at 10 mg/ml in 20 mM potassium phosphate, pH 8.0, 100 mM NaCl. SDS-PAGE analysis suggested that protein purity was — 90%.

Soy leghemoglobin was purified from *Glycine max* root nodules via ammonium sulfate precipitation (60%/90% fractionation) as detailed in Example 1. Resuspended 90% ammonium sulfate leghemoglobin was further purified by anion-exchange chromatography (HiTrap Q FF 5mL FPLC column) in 20 mM potassium phosphate, pH 8.0, 100 mM NaCl. The leghemoglobin eluted in flow through fractions. SDS-PAGE analysis suggested that protein purity was — 70%. Leghemoglobin was buffer exchanged

into 20 mM potassium phosphate, pH 7.4, 100 mM NaC1 and concentrated to 10 mg/ml on 3.5kDa membrane concentrators.

Carbon monoxide treatment: Myoglobin at 10 mg/ml in 20 mM potassium phosphate, pH 8.0, 100 mM NaC1 and leghemoglobin at 10 mg/ml in 20 mM potassium phosphate, pH 7.4, 100 mM NaC1 were first degassed under vacuum for 1 hour at 4°C then perfused with carbon monoxide gas for 2 minutes. Globins were then reduced from heme-$Fe^{3'}$ to heme-Fe^{2} state by adding 10 mM sodium dithionite, 0.1mM sodium hydroxide for 2 minutes. Sodium dithionite and sodium hydroxide were removed from the protein solution by using size-exclusion chromatography (PD-10 desalting column) in 20 mM potassium phosphate, pH 8.0, 100 mM NaC1 and 20 mM potassium phosphate, pH 7.4, 100 mM NaC1 respectively. Globins fractions were collected as a peak red colored fractions as evaluated by visual estimation. UV-VIS spectra confirmed the presence of heme-Fe^{2} state for both proteins. After desalting, the solution was again perfused with gas for another 2 minutes. The color of the solutions was evaluated by taking UV-Vis spectra (250nm- 700nm) every 20 minutes using the nanodrop spectrophotometer. Control samples were not treated with carbon monoxide.

Sodium nitrite treatment: Myoglobin at 10 mg/ml in 20 mM potassium phosphate, pH 8.0, 100 mM NaC1 and leghemoglobin at 10 mg/ml in 20 mM potassium phosphate, pH 7.4, 100mM NaC1 were reduced from heme-$Fe^{3'}$ to heme —Fe^{2} by adding 10 mM sodium dithionite, 0.1mM sodium hydroxide for 2 minutes. Sodium dithionite and sodium hydroxide were removed from the protein solution by using size-exclusion chromatography (PD-10 desalting column) in 20 mM potassium phosphate, pH 8.0, 100 mM NaC1 and 20 mM potassium phosphate, pH 7.4, 100 mM NaC1, respectively. Globins fractions were collected as peak red colored fractions as evaluated by visual estimation. UV-VIS spectra confirmed the presence of heme-Fe^{2} state for both proteins. Sodium nitrite was then added to a final concentration of 1 mM from 100 mM nitrite in phosphate buffer pH 7.4. The lifetime of heme-Fe^{2} state was followed by recording UV-VIS spectra (250-700nm) using spectrophotometer as a function of time. Control samples were not treated with sodium nitrite.

Data analysis of heme-Fe^2 life-times for myoglobin and leghemoglobin samples treated with carbon monoxide and sodium nitrite was performed in Microsoft Excel by plotting the amplitude of the absorbance peak at the 540nm wavelength. The "baseline" of the 540 nm absorbance was determined by the state of the UV-vis spectrum of the globin solutions prior to the addition of any additives, dithionite reduction, or desalting. The built-in curve fit function was used to produce an exponential line-of-best-fit, the exponent of which directly relates to the half life of the peak amplitude.

The life-time of the heme-Fe^2 state and accompanying red color of myoglobin and leghemoglobin solutions in the absence of carbon monoxide and sodium nitrite were 6hr and — 4hr respectively. Addition of sodium nitrite extended the life-time of the heme-Fe^2 state and accompanying red color to more than seven days. Addition of carbon monoxide extended the life-time of heme-Fe2 ' state and accompanying red color to more than two weeks.

EXAMPLE 16: PREPARATION OF MEAT REPLICAS IN WHICH THE PARTICLE SIZE OF INDIVIDUAL TISSUE REPLICA UNITS IS VARIED TO CONTROL AROMA GENERATION DURING COOKING.

Muscle tissue replica and adipose tissue replica were prepared separately and then combined into a meat tissue replica such that the size of individual tissue replica units was varied to control aroma generation during cooking. Individual fat, muscle and connective tissue replicas were constructed in the following manner.

A muscle tissue replica was prepared as in Example 2. The muscle tissue replica formed an opaque gel of reddish-brown color, with small amounts (< 1 ml) of inclusions of dark red, venous blood colored liquid. A connective tissue replica was prepared as in Example 14. An adipose tissue replica was prepared as in Example 7.

Meat replicas with a lean-to-fat ratio 85/15 were prepared by combining individual muscle, connective and adipose tissues such that particle size of individual tissues replicas varied. (a) 2.1 g of muscle replica with 0.9 g of chunks of fat replica 5-10 mm in size ("coarse mix"); (b) 2.1 g of muscle replica with 0.9 g of fat replica chopped to 2-3 mm in size ("fine mix"); and (c) 2.1 g of muscle replica with 0.9 g of fat replica

thoroughly blended to <1 mm in size ("blend"). "Muscle only" control sample contained 3 g of muscle replica alone. "Fat only" control sample contained 3 g of fat replica alone as 5-10 mm sized particle. Meat, muscle and fat tissue samples were cooked in sealed glass vials at 150°C for 10 min. Aroma profiles of the samples were analyzed by a panel of testers, and by GC-MS.

Sensory olfactory analysis of meat replica samples performed by a panel of testers, suggested that the size of individual tissue units and the extent of their mixing within meat tissue replicas correlated with generation of different aromas. Muscle tissue replica cooked by itself generated aromas associated with store-bought gravy, faint citrus and star anise. Adipose tissue replica cooked by itself generated aromas associated with musty, rancid and sweet aromas. Cooked meat tissue replica (coarse particle size) generated aromas of store-bought gravy, sweet, slightly musty and star-anise. Cooked meat tissue replica (fine particle size) generated aromas associated with soy sauce, musty, slightly rancid and beef bouillon. Cooked meat tissue replica (very fine particle size) generated aromas associated with sweet, musty and soy-sauce. All samples with the exception of adipose tissue replica generated aromas associated with burnt meat smell, however to varying intensities.

Analysis of GCMS data indicated that the size of individual tissue units and the extent of their mixing within meat tissue replicas had profound effects on the generation of aromatic compounds upon cooking. In particular, multiple aromatic compounds associated with fruity/grean bean/metallic (2-pentyl-furan); nutty/green (4-methylthiazole); peanut butter/musty (pyrazine, ethyl); raw potato/roasted/earthy (Pyrazine, 2,3-dimethyl); vinegary (acetic acid); spicy/caramel/almond (5-methy1-2-furancarboxaldehyde); creamy (butyrolactone); sweet (2,5-dimethy1-3-(3-methyl butyl) pyrazine); fruity/stale beer (2-cyclopentene-l-one, 2-hydroxy-3-methyl); musty/nutty/coumarin/licorice/walnut/bread (3-acetyl-1H-pyrolline); coconut/woody/sweet (pantolactone); penetrating (1-H-pyrrole-2-2carboxaldehyde, 1-methyl); minty (caprolactam); toasty caramel (4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl) aromas appeared only in mixed meat replicas, but not in individual tissue replicas. Some other aromatic compounds, for example associated with gasoline-

like (nonane, 2,6-dimethyl), petroleum-like (3-hexene, 3-methyl); sour/putrid/fish-like (pyridine); bland/woody/yogurt (acetoin); fatty/honey/citrus (octanal); pungent/sweet/caramelic (2-propanone, 1-hydroxy) and nutty/burnt green (ethenyl pyrazine) aromas appeared only in individual tissue replicas, but did not accumulate in mixed meat replicas. Furthermore, the levels to which all of the above compounds accumulated during cooking depended on the sizes of tissue units and how they are mixed (coarse particle size, fine particle size, or very fine particle size (blended)).

Similar to meat tissue replica, it was found that structural organization and particle size of beef tissues modify response of beef tissues to cooking. For example, the flavor of meat is modified by the size of the particles. Beef samples were prepared as following: samples of beef muscle and beef fat were cut separately with a knife and: (a) "ground", where knife-cut tissue cubes were passed through standard meat grinder. 80/20 (wt/wt) lean/fat ground beef sample was prepared by mixing muscle and fat tissue cubes at appropriate ratio prior to grinding. This sample preparation is referred to as a "fine size particle mix". (b) Ground tissue particle size was further reduced by freezing ground tissue in liquid nitrogen and crushing it using mortar and pestle to a very fine powder (particle size <1mm). This sample preparation is referred to as a "very fine size particle mix". All samples were cooked in sealed glass vials at 150 °C for 10 min. Aroma profiles of the samples were analyzed by a panel of testers, and by GC-MS, as described in Example 1. "Muscle only" control sample contained 3 g of muscle tissue alone. "Fat only" control sample contained 3 g of fat tissue alone. Ground beef sample contained 3 g of a 80/20 (wt/wt) muscle/fat mixture.

Sensory olfactory analysis of beef samples performed by a panel of testers, suggested that the size of individual tissue units and the extent of their mixing within the samples correlated with generation of different aromas. Beef muscle cooked by itself generated typical aromas associated with cooked ground beef. Fat tissue replica cooked by itself generated slightly sweet aromas, and aromas associated with burnt mushrooms. Cooked ground beef with "fine size particle mix" generated typical aromas associated with cooked ground beef, with presence of slightly sweet aromas characteristic of cooked fat. Cooked ground beef with "very fine size particle mix" generated aromas associated

with cooked ground beef, but no slightly sweet aroma characteristic of cooked fat was detected.

Analysis of GCMS data indicated that the particle size of the individual tissue units has effect on generation of aromatic compounds upon cooking. In particular generation and/or amount of multiple aromatic compounds by individual tissue samples or ground beef sample varied in correlation with particle size of the tissue. Some of the aromatic compounds that differed between fine and very fine particle size of muscle tissue: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 3-Acetyl-1H-pyrolline, 1-(6-methy1-2-pyraziny1)-1-ethanone, 2,5-dimethy1-3-(3-methyl butyl) pyrazine, 2-furancarboxyaldehyde, 5-methyl, Acetic acid, Ethenyl pyrazine, Pyrazine, 2,3-dimethyl , 2-Propanone, 1-hydroxy, Octanal, Acetoin, 4-Methylthiazole, Pseudo-2-pentyl-furan, 2-pentyl-furan. Some of the aromatic compounds that differed between fine and very fine particle size of fat tissue: triethylene glycol: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, Caprolactam, 1-(6-methyl-2-pyraziny1)-1-ethanone, 2-Cyclopentene-l-one, 2-hydroxy-3-methyl, Butyrolactone, 2-furancarboxyaldehyde, 5-methyl, Ethanone, 1(2 furanyl), Acetic acid, 2-ethyl-5-methyl pyrazine, Pyrazine, 2,3-dimethyl, Pyrazine, ethyl, Octanal, Acetoin, 4-Methylthiazole, Pseudo-2-pentyl-furan, Pyridine, Nonane, 2,6-dimethyl. Some of the aromatic compounds that differed between fine and very fine particle size of 80/20 muscle/fat sample: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, Caprolactam, 1H-1Pyridine, 3-carbonitrile, 4-ethy1-2-oxo-2,5, 1-H-Pyrrole-2-2carboxaldehyde, 1-methyl, 2-Cyclopentene-l-one, 2-hydroxy-3-methyl , 2,5-dimethy1-3-(3-methyl butyl) pyrazine, Butyrolactone, 2-furancarboxyaldehyde, 5-methyl, Ethanone, 1(2 furanyl), Acetic acid, Ethenyl pyrazine, 2-ethyl-5-methyl pyrazine, Pyrazine, 2,3-dimethyl; 2-Propanone, 1-hydroxy, Octanal, Acetoin, 2-pentyl-furan.

EXAMPLE 17: LEGHEMOGLOBIN CONTRIBUTION TO FLAVOR

Beef flavors and aromas can be created in non beef consumables by addition of heme proteins. Ground chicken (90% lean, 10% fat) was strained with cheesecloth and mixed with recombinant soy leghemoglobin or recombinant bovine myoglobin to a final

concentration of 0.5-1.0% wt/wt. The recombinant heme proteins were expressed in *E. coli* and purified by nickel affinity purification as described in Example 1. Prior to being mixed with chicken, the heme proteins were reduced with 20 mM Na Dithionite. Na dithionite was removed from the sample with a Zeba desalting column (Thermo Scientific). Leghemoglobin was desalted into 20 mM potassium phosphate pH 7.4, 100 mM NaCl. Myoglobin was desalted into either 20 mM potassium phosphate pH 7.4, 100 mM NaC1 or 20 mM Na citrate pH 6.0, 100 mM NaCl. The reduced heme protein samples were divided in two, and half the sample was bubbled with carbon monoxide (CO) for 2 minutes. After mixing the heme protein samples with ground chicken, the mixture was poured into nugget-shaped molds and incubated overnight at 4°C. The nuggets were oven baked or pan fried at 165°C until each nugget reached an internal temperature of 165°C. A panel of judges tasted nuggets containing chicken alone, chicken mixed with buffer, chicken mixed with either leghemoglobin or myoglobin +/CO, or beef (90% lean, 10% fat). Judges filled out a survey to evaluate the aroma and flavor of each nugget. Judges rated the aroma and flavor of each nugget as follows: 1 = chicken, 2 = chicken + faint beef, 3 = 50/50 chicken + beef, 4 = beef + faint chicken, 5 = beef Shown in Table 2 are the average scores received for each nugget. Percentages indicate the final concentration of heme protein wt/wt (abbreviations: KP = 20 mM potassium phosphate pH 7.4, 100 mM NaC1 buffer. NC = 20 mM Na citrate pH 6.0, 100 mM NaC1 buffer. n/d = not determined). Adding recombinant leghemoglobin or myoglobin to chicken resulted in an increased beef aroma and flavor. The perceived levels of beef flavor and aroma increased with the myoglobin and leghemoglobin content. Leghemoglobin and myoglobin provide the same benefit to the flavor and aroma.

TABLE 2

	Oven Baked		Pan Fried	
	Aroma	Flavor	Aroma	Flavor
Chicken	1	1	1	1
Chicken KP	1	1	2.5	1.2
Chicken NC	1.5	1.5	1.5	1
Chicken 0.5% legH KP	1.5	2.5	3.67	3.2
Chicken 0.5% legH+CO KP	2.5	2.5	2.67	2.2

Chicken 0.5% Myo NC	2	2	1.5	2.4
Chicken 0.5% Myo+CO NC	2	2	2.5	3
Chicken 0.5% Myo+CO KP	2.5	2.5	2.33	2
Chicken 0.8% Myo+CO NC	2	3	4	2.6
Chicken 1% Myo NC	4.5	4	n/d	n/d
Chicken 1% legH NC	4	4	n/d	n/d
Beef	5	5	5	5

EXAMPLE 18: PREPARATION OF A NON-DAIRY CREAM LIQUOR

A cream liquor was made from a sunflower cream fraction, RuBsiCo and whiskey (Jameson). A sunflower cream fraction was made by blending sunflower seeds in 40 mM sodium phosphate pH 8.0, 400 mM sodium chloride buffer. Seed debris was pelleted by centrifugation at 5000g for 20 min, and the cream fraction collected. The cream fraction was resuspended in 10 mM potassium phosphate pH 7.4 buffer and collected by centrifugation at 5000g, 20 min. Rubsico was purified as described in Example 1 and used as 25mg/m1 stock solution in 20mM K-phosphate pH 7.0, 150mM NaCl.

In one example, cream liquor was made as follows: 11.4% wt/v of sunflower cream fraction, 40% v/v Jameson whiskey, 0.4-1.6% wt/v Rubsico, 0.5% wt/v vanilla extract, 0.5% v/v espresso coffee, and 1.5% wt/v chocolate powder. The resulting mixture was homogenized at 5000 psi.

In another example, cream liquor was made from sunflower cream fraction and whiskey (Jameson) and sugar: 11.4% wt/v of sunflower cream fraction, 40% v/v Jameson whiskey, 0.5% v/v vanilla extract, 0.5% v/v espresso coffee, 1.5% wt/v chocolate powder and 8% wt/v sugar.

Beverages were served either at ambient room temperature or chilled. Resulting beverages were beige to light chocolate in color. The tasting results suggested that the beverage had a very creamy alcoholic flavor similar to dairy cream liquor. Chilled product was preferred. The emulsion was stable at room temperature for at least 1 week (maximum time tested).

EXAMPLE 19 —CHOCOLATE SPREAD

A chocolate spread was made from 34% (wt/wt) cane sugar, 22 % (wt/wt) cocoa powder, 19 % (wt/v) pistachio cream fraction, and 12 % (v/v) almond milk. Cane sugar and cocoa powder (Ghirardelli) were commercially purchased. Almond skim milk was made in a following manner: Almonds were blanched by immersion into 100°C water for 30 sec. Blanched nuts were recovered and cooled by immersion into ice-cold water. Almonds were air dried. Almonds were then rehydrated by immersion in 2°C water for 16hr. Rehydrated almonds were drained, mixed with water at 1:2 wt/v ratio and blended in a Vitamix blender for 5 min. The blended slurry was collected in chilled container and stirred with a frozen cooling stick to cool. Once the slurry cooled to 10°C, the slurry was placed at 2°C for up to 12 hours. Almond skim and cream were separated by centrifugation at 7480g for 30min at 4°C. Almond milk separated into 3 layers, a dense pellet of insoluble solids, a clear to translucent aqueous layer (which is referred to as the "almond skim milk"), and a lighter, creamy, opaque layer (which is referred to as the "almond cream"). Almond milk was then was pasteurized at 75°C for 16 seconds, chilled and stored at 2°C.

A pistachio cream fraction was prepared by blending pistachios in 100 mM sodium carbonate pH 9.5 buffer with 400 mM sodium chloride and 1 mM EDTA, then centrifuging 5000xg for 20 min. The cream fraction was collected and washed into the same buffer once more. After centrifugation at 5000g for 20 min, the cream fraction was collected and washed into 20 mM sodium phosphate pH 7.4 buffer with 50 mM sodium chloride and 1 mM EDTA. After centrifugation at 5000g for 20 min, the cream fraction was collected an washed once more in the neutral (pH 7.4) buffer, centrifuged at 5000g for 20 min. Pistachio cream fraction was collected and stored at 4°C.

Chocolate spread was made in the following way. Cane sugar was melted in almond milk, cocoa powder was added to sugar-milk mixture with stirring and melted. Sugar, milk and cocoa were then added to the pistachio cream fraction and whisked together. Resulting mixture was then poured into molds and let sit for 24 hours at refrigeration and freezer temperatures.

In another example, chocolate spread was made from 42% (wt/wt) cane sugar, 27 % (wt/wt) cocoa powder, 31 % (wt/v) sunflower cream fraction, and 23% (v/v) almond skim milk. All ingredients and procedures apart from sunflower cream fraction were as described above.

The sunflower cream fraction was created from blending sunflower seeds in 5 times the weight to volume of a solution of 40 mM potassium phosphate pH 8, with 400 mM NaC1, 1 mM EDTA, then cooled to 20°C, and the slurry was then centrifuged. The top cream layer was removed and is blended in the same buffer, followed by heating for 1 hour at 40°C. The slurry is cooled down to 20°C then centrifuged; the cream layer is removed and mixed with 5 times the weight to volume of 100 mM sodium carbonate pH 10 with 400 mM NaC1, then centrifuge. The top layer is then mixed with 5 times the weight to volume of water and centrifuged again, the resulting cream fraction is very creamy, white, and neutral tasting.

In another example, a chocolate spread was made from 37% (wt/wt) cane sugar, 23 % (wt/wt) cocoa powder, 13 % (wt/v) sunflower cream fraction, and 7 % (wt/wt) cocoa butter, and 20 % v/v almond skim milk.

In another example, a chocolate spread was made from 37% (wt/wt) cane sugar, 23 % (wt/wt) cocoa powder, 13 % (wt/v) sunflower cream fraction, and 7 % (wt/wt) coconut oil 20 % v/v almond skim milk.

In another example a chocolate spread was made from 37% (wt/wt) cane sugar, 23 % (wt/wt) cocoa powder, 13 % (wt/v) sunflower cream fraction, and 7 % (wt/wt) palm oil, and 20 % v/v almond skim milk.

In another example a chocolate spread was made from 1.8% (wt/wt) cane sugar, 1.13% (wt/wt) cocoa powder, 88 % (wt/v) pistachio cream fraction, and 9 % almond skim milk, by whisking equal amounts of spread described above and pistachio oil bodies.

In another example, a chocolate spread was made from 8.5 % (wt/wt) cane sugar, 5.4 % (wt/wt) cocoa powder, 81 % (wt/v) sunflower cream fraction, and 4.6 % (v/v) almond skim milk, by mixing chocolate spread described above with sunflower cream at ratio 2:1.

Visual and textural inspection of all products suggested that they formed stable, solid, creamy spreads at room temperatures. All products were firm solids at refrigeration and freezer temperatures. Tasting results of all products suggested very pleasant, rich creamy texture with product melting in the mouth reviewed positively by tasters. Individual tasters preferences varied with respect to like or dislike of pistachio flavor, coconut flavor, preference for more or less sweet product and more or less cocoa flavor. One particular sample was described as being similar to a milk chocolate spread, as the sunflower cream fraction contributed a neutral flavor.

EXAMPLE 20 - Generation of Adipose Tissue Replica

Adipose tissue replicas were generated using the ingredients recited in Table 3.

TABLE 3

Adipose Tissue Replica

Ingredient	%
Coconut oil	65
Pea vicillin protein in buffer	21.3
Cocoa butter	10
Buffer	2.7
Lecithin slurry, 50 mg/ml	1
Total	100

Lecithin (SOLEC™ F Deoiled Soy Lecithin, The Solae Company, St. Louis, MO) was prepared at a concentration of 50 mg/ml in 20 mM potassium phosphate, 100 mM NaC1, pH 8.0 buffer and sonicated (Sonifier Analog Cell Disruptor model 102C, BRANSON Ultrasonics Corporation, Danbury, Connecticut) for 30 seconds.

The pea vicilin protein was supplied as a liquid containing approximately 140 mg/g pea vicilin in 20 mM potassium phosphate, 100 mM NaC1, pH 8.0 buffer.

Coconut oil (Shay and Company, Milwaukie, OR) and cocoa butter (Cocoa Family, Duarte, CA) were melted by heating to 50 - 70°C and then combined and held warm until needed.

The buffered protein solution, additional buffer, and lecithin slurry were mixed in a 32 ounce sized metal beaker and equilibrated to room temperature. An emulsion was formed using a hand held homogenizer (OMNI model GLH fitted with G20-195ST

20mm generator probe, OMNI International, Kennesaw, GA). The homogenizer probe was put into the protein lecithin mixture and turned on to speed 4. The warmed oil was then added slowly over the course of about 2 minutes while continuously moving the probe around in the mixture.

The emulsion was then heat set by placing the metal beaker into a 95°C waterbath. Using a clean spatula, the emulsion was stirred every 20 seconds for 3 minutes total. The beaker was then removed from the waterbath and stored at 4°C for several hours until thoroughly cooled.

EXAMPLE 21 - Generation of Raw Tissue Replica

A raw tissue replicawas generated using the ingredients recited in Table 4.

TABLE 4:

Raw Tissue Replica

Ingredient	**%**
Buffer	41.6
Heme protein in buffer	26.7
Pea legumin in buffer, dried	12.1
Pea vicillin in buffer, dried	9.4
Flavor precursor mix, 17x	6.2
Transglutaminase preparation	4
Total	100

The buffer was 20 mM potassium phosphate, 100 mM NaC1, pH 7.4. The heme protein was prepared at a concentration of 55 mg/g in 20 mM potassium phosphate, 100 mM NaC1, pH 7.4 buffer. The 17x flavor precursor mix precursor is described in Example 27. The pea legumin was prepared in 20 mM potassium phosphate, 500 mM NaC1, pH 8 buffer and then freeze dried prior to use. The final protein concentration of the dried material was 746 mg/g. The pea vicilin was prepared in 20 mM potassium phosphate, 200mM NaC1, pH 8 buffer and then freeze dried prior to use. The final protein concentration of the dried material was 497 mg/g.

The liquid ingredients (buffer, heme, and flavor precursor mix) were mixed in a plastic beaker. The dry pea legumin and pea vicilin were then added and allowed to fully rehydrate while gently stirring for 1 hour at room temperature. The dry transglutaminase preparation (ACTIVA® TI, Ajinomoto, Fort Lee, NJ) was then added and stirred for about 5 minutes until dissolved. The stirring was then turned off and the mixture was allowed to gel at room temperature until firm. After the gel had formed the raw tissue replicawas stored refrigerated until used.

EXAMPLE 22 - Hard Connective tissue replica

A hard connective tissue replica was made as following using soy protein isolate (Supro Ex38, Solae), wheat gluten (Cargill), and bamboo fiber (Alpha-Fiber B-200, The Ingredient House). The purified proteins were freeze-dried and milled using a standard coffee grinder. Commercially available powders of soy protein isolate and wheat gluten were used as received.

The connective tissue replica contained 49% soy protein isolate, 49% wheat gluten and 2% bamboo fiber. The ingredients were thoroughly mixed and loaded into the loading tube of the extruder's batch feeder. A twin-screw extruder (Nano 16, Leistritz Extrusion Corp.), with a high-pressure water injection pump (Eldex) and custom-made die nozzles (stainless steel tube, 3 mm ID, 15 cm length, pressure rating 3000+ PSI) attached using a Hy-Lok two-ferule tube fitting and a custom-made die with a threaded nozzle and 10 mm ID, 20 mm long flow channel was used.

The dry mixture was fed into the extruder at the rate 1 g/min. Water was fed by the pump into the second zone of the extruder's barrel. The rate of water feeding is adjusted to the rate of dry mixture feeding such as to provide 55% moisture level in the final extrudiate. A temperature gradient was maintained along the extruder barrel as follows: feed zone - 25°C, zone 1 — 30°C, zone 2 — 60°C, zone 3 — 130°C, zone 4 —130°C. The die plate was neither actively heated, nor cooled. The die nozzle was actively cooled (by applying moist tissue replica) to maintain extrudate temperature below 100°C.

Hard connective tissue replica obtained by this process was dark off-white ("cappuccino") colored material shaped into 3 mm thick filaments that had tensile strength similar to animal connective tissue (3 MPa).

Example 23. Soft Connective tissue replica

A soft connective tissue replica was made as in Example 22, except that the rate of water feeding was adjusted to the rate of dry mixture feeding to provide 60% moisture level in the final extrudate. A temperature gradient was maintained along the extruder barrel as follows: feed zone - 25°C, zone 1 — 30°C, zone 2 — 60°C, zone 3 — 115°C, zone 4 — 115°C. The die plate was neither actively heated, nor cooled. The die nozzle was actively cooled (by applying moist tissue) to maintain extrudate temperature below 100°C.

Soft connective tissue replica obtained by this process was light off-white colored material shaped into 3 mm thick filaments that had low tensile strength (< 0.1 MPa) and had a significant propensity to split longitudinally into bands and thin fibers

EXAMPLE 24. Thin connective tissue replica (zein fibers) process:

A thin connective tissue replica was prepared using zein protein powder, glycerol (FCC grade), polyethylene glycol (PEG 400 or PEG3350), ethanol, sodium hydroxide (FCC grade), and water. The zein powder and PEG3350 at a 35% w/w ratio to zein were dissolved in 85% aqueous ethanol to reach a final zein concentration of 57% w/w. The pH of the solution was adjusted to 7.0 with 1M solution of sodium hydroxide in ethanol. A syringe pump with A 1-12 ml syringe, spinning nozzle (hypodermic needle, 18-27 gauge, or a plastic nozzle, 18-24 gauge), heating silicon tape, and a heating fan was used, with a collector assembled using a computer-controlled motor rotating a Delrin rod, which serves as a collector.

This solution was loaded into the syringe, which was mounted onto the syringe pump, with an 18 gauge plastic tip attached. A silicon heating tape was looped around the tip to maintain it at elevated temperature. After solution was extruded out of the tip and forms a drop, it was picked up with a spatula and carefully transferred towards the

collector rod to form a filament between the tip and the collector. The extrusion rate was optimized to produce even flow of material from the tip (5 ml/h for a 12 ml syringe and 18 gauge tip). The collector rotation speed was 3 RPM. The heating fan was positioned to blow hot air onto the spooling fiber. After spooling, the fibers were cured in a 120°C oven for 24 hours.

Thin connective tissue replica obtained by this process was semi-clear yellow colored material shaped into 300-micrometer thick fibers that were semi-flexible in air, and became very flexible and elastic in presence of water, maintaining high tensile strength similar to animal connective tissue (6 MPa)

EXAMPLE 25. Noodles

Noodles were prepared using purified pea vicilin (freeze-dried), and soy protein isolate (Supro EX38 by Solae, Solbar Q842 (CHS)) or soy protein concentrate (Hisolate, Harvest Innovations). To prepare the noodles, 67% soy protein concentrate or isolate, and 33% ground pea vicilin powder were thoroughly mixed and loaded into the loading tube of the extruder's batch feeder. The dry mixture was fed into the extruder at the rate in the range of 1-2 g/min. Water was fed by the pump into the second zone of the extruder's barrel at the rate 3.6-5.3 ml/min such as to maintain the final moisture content in the extrudate at 72.5%. A temperature gradient was maintained along the extruder barrel as follows: feed zone - 25 °C, zone 1 — 30 °C, zone 2 — 60 °C, zone 3 — 100 °C, zone 4 — 100 °C. Zone 1 temperature can be varied in the range of 25 — 45 °C. Zone 2 temperature can be varied in the range of 45 — 65°C. Zone 3 and 4 temperatures can be varied in the range of 95 — 100°C. The die plate was neither actively heated, nor cooled. The die nozzle was passively cooled by ambient air ensuring extrudate temperature below 100°C.

Noodles obtained by this process were light yellow colored material shaped into 1.5 mm thick filaments that had low tensile strength (< 0.1 MPa) and a moderately sticky texture.

Example 26. Sticky Tissue Replica Preparation

Sticky tissue replica was prepared using purified pea vicilin (freeze-dried) and purified pea legumin (freeze-dried). To prepare the sticky tissue replica, 50% ground pea vicilin powder and 50% ground pea legumin powder were thoroughly mixed and loaded into the loading tube of the extruder's batch feeder. The dry mixture was fed into the extruder at the rate in the range 0.4 — 0.8 g/min. Water was fed by the pump into the second zone of the extruder's barrel at the rate in the range 1.6 — 3.2 ml/min, such that the final moisture content of the extrudate was maintained at 80%. As the total throughput increased from 2 g/min to 4 g/min, the screw speed was increased from 100 to 200 RPM. Larger die diameters (4 mm and above) are also helpful for preventing backflow at higher throughputs. A temperature gradient was maintained along the extruder barrel as follows: feed zone - 25 °C, zone 1 — 30 °C, zone 2 — 60 °C, zone 3 — 90 °C, zone 4 — 90 °C. The die plate was neither actively heated, nor cooled. The die nozzle was passively cooled by ambient air. The die nozzle is kept free of obstructions by solidifying gel material.

Sticky tissue replica obtained by this process was semi-transparent water-white colored material shaped into irregular 1-5 cm sized bulbs that had sticky paste-like texture.

Example 27. Preparation of the flavor precursor mix

A flavor precursor mix was prepared by mixing concentrated stock solutions of each additive to make a 17X solution. Table 5 contains the chemical composition of the mix and the mM concentration of each component in the final burger. The concentrated flavor precursor mix was sterile filtered and adjusted to pH 5.5 — 6.0 using NaOH, and used at a 1X concentration in burgers.

TABLE 5.

Chemical composition of the flavor precursor mix.

Chemical entity	mM
Alanine	5.6
Arginine	0.6
Asparagine	0.8

Aspartate	0.8
Cysteine	0.8
Glutamic acid	3.4
Glutamine	0.7
Glycine	1.3
Histidine	0.6
Isoleucine	0.8
Leucine	0.8
Lysine	0.7
Methionine	0.7
Phenylalanine	0.6
Proline	0.9
Threonine	0.8
Tryptophan	0.5
Tyrosine	0.6
Valine	0.9
glucose	5.6
Ribose	6.7
Maltodextrin	5.0
Thiamine	0.5
GMP	0.2
IMP	0.6
Lactic acid	1.0
creatine	1.0
NaC1	10
KC1	10
Kphos pH 6.0	10

Example 28 - Freeze-alignment to produce texturized protein for use as muscle replicas

This example describes a non-extrusion based method to produce texturized protein materials that can be used in meat replicas.

A muscle tissue-replica was prepared by first preparing a gel of lentil proteins by mixing a 4.5% (w/v) solution of lentil proteins in 20mM potassium phosphate buffer pH 7.4 + 100mM sodium chloride with 20% (v/v) canola oil (from Jedwards International). The mixture was gelled by heating at 95°C for 15 minutes and slow cooled to room

temperature (at the rate of 1°C/minute). The gel was then poured into a vessel and frozen at -40°C by placing above a liquid nitrogen bath until completely frozen. The frozen material was then dried in a freeze-dryer. When the material was completely dried, the material was stabilized by autoclaving (121°C, 15 minutes). The resulting material is a texturized muscle tissue replica formed with plant proteins.

The aligned muscle replica then was presoaked in water for 5 minutes, cut into pieces of length 3-4mm and then combined with lOg adipose-replica, lOg connective tissue replica and 5g cold-set gels to form 50g beef patty replicas. An in-house sensory panel attributed inclusion of freeze-aligned tissue to impart improved fibrous texture to the patty.

A muscle-tissue replica also was prepared by first forming freeze-aligned material as described above. After the tissue replica was steam cooked at 121°C for 10 minutes, the material was allowed to soak in a solution of heat-denatured pea vicilins (at 6% w/v in 20mM potassium phosphate buffer pH 7.4 + 100mM sodium chloride, heat denatured by heating at 95°C for 30 minutes), 1% equine myoglobin (w/v) (Sigma), and 40% (v/v) canola oil (from Jedwards International). Gelation of the medium was induced by addition of calcium chloride at 20 mM. The sample was allowed to sit for 5 minutes at room temperature to allow for gel formation. The resulting muscle-replica contained aligned material in a cold-set gel reminiscent of beef muscle in steak.

EXAMPLE 29 - Cold gelation of proteins for meat applications

In one example, a cold-set gel comprising myoglobin was prepared by first heat denaturing a 6% (w/v) solution of pea-vicilins in 20mM potassium phosphate buffer, pH 7.4 with 100mM sodium chloride at 100°C for 30 minutes. The solution was allowed to cool back to room temperature. Canola oil (from Jedwards International) and equine myoglobin (Sigma) were added to a final concentration of 20% (v/v) and 1% (w/v), respectively. Gel formation was induced by adding calcium chloride at 20mM. A 50g beef patty replica was formed by combining 5g of the cold gel with lOg of adipose tissue-replica, lOg of connective tissue-replica, and 25g of muscle tissue-replica. Five mls of a

7% (w/w) solution of crude lentil protein was added to the mixture and patties were formed.

Example 30 - Binding materials in meat replicas

In one example, a beef replica was made by first preparing a coacervate from a 3% (w/v) solution of pea vicilins and legumins (vicilin:legumin ratio of 3:1) in 20 mM potassium phosphate pH 7.4 + 100 mM sodium chloride. Melted palm oil (from Jedwards International) was added to the solution to a final concentration of 5% and mixed by vortexing. The emulsion then was acidified by adding hydrochloric acid while stirring to a pH of 5. The slurry was then centrifuged at 5000 x g for 10 minutes and the liquid top layer was decanted from the coacervate.

A 50g beef patty replica was formed by combining the coacervate at 10% with adipose tissue-replica (20%), connective tissue-replica (20%) and muscle tissue-replica (50%). Five mls of a 7% solution of crude lentil protein was added to the mixture and patties were formed. Patties that included coacervate as binding material were observed to be more cohesive than patties that did not.

EXAMPLE 31 - Assembly of a sticky and noodle type ground tissue replica and burger replica

Ground tissue replica and burger replicas were prepared using the ingredients in Table 6. During all pre-processing steps, the temperature of the materials was maintained cold (4 — 15°C).

TABLE 6

Composition of sticky and noodle type formulation

Ingredient	%
Adipose	26
Soft connective	20.6
Sticky	12
Raw	10
Noodles	10

Hard connective	10
Flavor and heme solution	10
k-carrageenan	1.4
Total	100

Adipose tissue replica from Example 6 was chilled following the final heat-cool step into a solid block. Optionally 0.2% by weight of heme protein can be added as a 20 mg/ml liquid solution and manually worked into the adipose. The adipose was then crumbled manually into small bits 3 — 7 mm in diameter while cold.

Soft connective tissue replica from Example 23 was produced by an extrusion process as long string-like pieces. The soft connective was chopped with a mini chopper (Mini-Prep® Plus Processor model DLC-2L Cuisinart, Stamford, CT) in a single step process. Approximate 200g of soft connective was placed in the mini chopper and processed on the chop setting for 60 seconds to yield pieces of 1 — 3 mm in length with ragged edges.

Sticky tissue replica and noodles tissue replica (see Examples 25 and 26) were produced by an extrusion process as long noodle-like pieces or amorphous pieces, respectively. Raw tissue replica of Example 21 was provided from the enzymatic crosslinking process as a solid block. All three of these tissue replicas were manually broken down into pieces 1 - 3 cm in diameter.

Hard connective tissue replica from Example 22 was produced from an extrusion process as long string-like pieces. The hard connective tissue replica was chopped to three levels, named coarse, intermediate, and fine, in a mini chopper (Mini-Prep® Plus Processor model DLC-2L Cuisinart, Stamford, CT). 160 — 200 g of hard connective tissue replica was placed in the mini chopper and processed on the chop setting for 90 seconds. One third of the material was removed as the coarse chopped fraction. The material remaining in the chopper was then processed for an additional 60 seconds and one third of the original weight was removed as the intermediate chopped fraction. The material remaining in the chopper was then processed for an additional 30 seconds to produce the fine fraction.

Leghemoglobin was freeze-dried and then reconstituted in a 17x flavor precursor mix (see Example 27) adjusted to pH 6.0 with 10 N NaOH to make the flavor and heme protein solution.

Following pre-processing described above, the soft connective, sticky, raw, noodles, hard connective, and 2/3 of the adipose were mixed by hand in a bowl. A typical batch size was 100 g to 2000 g. The flavor and heme solution was then dribbled onto the mixed tissue replicas and mixed gently by hand, then k-carrageenan powder was tsprinkled over the mixture and mixed in by hand. During the assembly, grinding, and forming all materials were kept cold (4 — 15°C). The mixture was ground using a stand mixer fitted with a food grinder attachment (KitchenAid® Professional 600 Series 6 Quart Bowl-Lift Stand Mixer model KP26M1XER and KitchenAid® Food Grinder model FGA, St. Joseph, MI) on speed setting 1. The food grinder material was fed by a screw conveyor past a rotating knife installed in front of a fixed hole plate.

The ground tissue replica mix was collected in a bowl and the remaining 1/3 of the crumbled adipose was then added to the ground tissue replica mix and mixed in by hand. Approximately 30 g or 90 g portions of ground tissue replica were then formed by hand into round patty shapes. Typical dimensions for 30 g patties were 50 mm x 12 mm. Typical dimensions for 90 g patties were 70 mm x 18 mm. Patties were refrigerated until cooked. Cooked patties had appearance, texture, and flavor similar to ground beef as judged by a trained sensory panel. In addition to cooking in patty format, the ground tissue replica can also be used in a variety of dishes such as taco filling, casseroles, sauces, toppings, soups, stews, or loaves.

EXAMPLE 32 - Assembly of a Wheat Gluten containing ground tissue replica and burger replicas

Ground tissue replica and burgers were prepared using the ingredients in Table 7.

TABLE 7:

Composition of Wheat Gluten Type formulation

Ingredient	%
Adipose	25
Soft connective	30
Raw tissue replica with heme and flavor	35
Wheat gluten	5
Hard connective	5
Total	100

Adipose, soft connective, and hard connective were preprocessed as described in Example 31. During all pre-processing steps, the temperature of the materials was maintained cold (4 — 15°C).

Raw muscle with heme protein and flavor was then prepared as follows. Freeze-dried pea vicilin and pea legumin were dissolved in water and 16x flavor stock. The freeze-dried heme protein was dissolved in this mixture and the pH was adjusted with citric acid to 5.8. A dry transglutaminase preparation (ACTIVA® TI Ajinomoto Fort Lee, NJ) was then added and mixed for about 5 min to fully dissolve. The mixture was then allowed to stir for an additional 10 minutes until some increase in viscosity was observed. The soft connective and hard connective were then added and the mixture was allowed to sit for 1 hour at room temperature to cure and form a solid mass.

Wheat gluten powder (vital wheat gluten, Great Northern, item 131100, Giusto's Vita-Grain, South San Francisco, CA) then was added to the gelled raw muscle and mixed to distribute. This mixture was then immediately ground using a stand mixer fitted with a food grinder attachment as described in the previous example. Ground tissue replica was then chilled for 5 min at -20°C. Finally, the chopped adipose, pre-chilled to 4°C, was added to the chilled ground tissue replica.

Ground tissue replica mix with adipose tissue replica added was then formed by hand into two 90 g round patties. 90 g patties are typically 70 mm x 18 mm. A typical batch size was 180 - 200 g and produced two patties. The patties were then allowed to rest at room temperature for 30 minutes. After resting the patties can be cooked or refrigerated until ready to cook.

EXAMPLE 33: Generation of beef flavor in replica burgers by the addition of Heme and flavor precursors.

Characteristic flavor and fragrance components in meat are mostly produced during the cooking process by chemical reactions molecules (precursors) including amino acids, fats and sugars that are found in plants as well as meat. Flavor precursors along with 1% Leghemoglobin were added to the muscle component of the burger replicas as indicated in Table 8. Three replicas, one with no precursors, and two different mixtures of precursors, along with 80:20 beef were cooked then served to a trained sensory panel to describe the flavor attributes shown in Table 9. The addition of precursors increased the beefy flavor, the bloody notes, overall flavor quanity, and decreased the off notes in the replica. The replicas and beef sample also were analyzed by GCMS by adding 3 grams of uncooked replica or beef into a GCMS vial. All samples were cooked at 150°C for 3 mins, cooled to 50°C to extract for 12 minutes using GCMS (SPME fiber sampling of headspace). A search algorithm analyzed the retention time and mass fingerprint information to assign chemical names to peaks. In the replica burger with 1% Leg hemeoglobin, and precursor mixture 2, 136 beef compounds were created. In Table 10, all the compounds created in the replica burger that were also identified by GCMS in the beef samples are indicated.

TABLE 8

Flavor precursors added to the beef replicas before cooking.

Samples	767	804	929
Additive	Precursor No (mm) Mix 1	Precursor Precursors Mix 2	
Alanine	5.61	5.61	
Cysteine	0.83	0.83	
Glutamic acid	3.40	3.40	
Leucine	0.76	0.76	
Lysine	0.68	0.68	
Methionine	0.67	0.67	
Tryptophan	0.49	0.49	
Tyrosine	0.55	0.55	
Valine	0.85	0.85	

Glucose	5.55	5.55
Ribose	6.66	6.66
Lactic acid	1.00	1.00
creatine	1.00	1.00
Thiamine	0.50	0.50
IMP + GMP	0.40	0.40
Sucrose		2.00
Fructose		2.00
Xylose		2.00
Maltodextrin	0.50%	0.50%

TABLE 9

The sensory score determined by the sensory panel for the replica burgers and 80:20 beef sample.

Sample #		Beef	767	804	929
Flavor Quality	STDEV 0.0	1.3	1.0	1.2	
Flavor Intensity	STDEV 0.8	1.2	1.4	1.2	
Flavor: Beefy	STDEV 1.0	1.2	0.8	1.2	
Flavor: bloody/Metallic	STDEV 1.2	1.0	0.9	1.4	
Flavor: Savory	STDEV 1.2	1.2	1.2	1.8	
Off Flavors:					
chemical/oxidized/beany	STDEV 0.8	1.2	1.8	1.0	

TABLE 10

ref flavor compounds created in replica burger with 1% LegH and precursor mix 2 as detected by GCMS.

octen-2-one	octanoic acid	(Z)-2-decenal,
penten-3-ol	octane	carbon disulfide

:,aproic acid vinyl ester	octanal	butyrolactone
icetylthiazole	nonanal	butanoic acid
ophene	4,7-dimethyl-undecane,	3-methyl-butanal,
,Ahyl-thiirane,	methyl ethanoate	2-methyl-butanal,
azole	methional	butanal
Trene	methacrolein	3,6,6-trimethyl-bicyclo[3.1.1]hept-2-ene,
	isovaleric acid	benzyl alcohol
rrole	isopropyl alcohol	1,3-dimethyl-benzene
ridine	hexanoic acid	benzene
methyl-pyrazine	2,2,4,6,6-pentamethyl-heptane,	benzaldehyde
ramethyl-pyrazine,	2-methyl-heptane,	acetophenone
Ahyl-pyrazine,	heptane	acetonitrile
iyl-pyrazine	heptanal	acetone
„thy1-2,5-dimethyl-pyrazine	furfural	acetoin
5-dimethyl-pyrazine	furaneol	acetic acid ethenyl ester
3-dimethyl-pyrazine	3-methyl-furan	acetic acid
„thy1-5-methyl-pyrazine	2-propyl-furan	acetamide
„theny1-6-methyl-pyrazine,	2-pentyl-furan	acetaldehyde
razine	2-methyl-furan	4-methyl-5-thiazoleethanol
methyl-propanal,	2-ethyl-furan	6-methyl-5-hepten-2-one
Dpanal	furan	trans-2-(2-pentenyl)furan
enylacetaldehyde	formamide	(E)-4-octene,
enol	ethyl acetate	4-cyclopentene-1,3-dione
ntanoic acid	1-(2-furany1)-ethanone	4-cyanocyclohexene
„thy1-2,2-dimethyl-pentane	1-(1H-pyrrol-2-y1)-ethanone	dihydro-2-methyl-3(2H)-furanone,
ntanal	dimethyl trisulfide	(E,E)-3,5-octadien-2-one
:xesol	dimethyl sulfide	3,5-octadien-2-one
alic acid, butyl propyl ester	d-limonene	2,2-dimethyl-undecane
heptene	1-octen-3-ol	toluene
„thy1-5-methylcyclopentene	1-octanol	1-pentanol
butanol	1-hexanol	1-octen-3-one
I-pyrrole-2-carboxaldehyde	2-methyl- 1H-pyrrole	2-butanone
honanone	3-methyl-2-butenal	2-thiophenecarboxaldehyde
a-butylacrolein	3-ethylcyclopentanone	2-pyrrolidinone
methyl-2-heptene	2(5H)-furanone	2-propenal
)-2 -hex enal,	dihydro-5-penty1-2(3H)-furanone	1-hydroxy-2-propanone

E)-2-heptenal,	5-ethyldihydro-2(3H)-furanone	1-(acetyloxy)-2-propanone
-methyl-2-heptanone	5-acetyldihydro-2(3H)-furanone	2-pentanone
-heptanone	2,6-dimethylpyrazine	(E)-2-octenal
-furanmethanol	(E,E)-2,4-nonadienal	2-octanone
-ethyl-2-1,4-dioxin	(E,E)-2,4-heptadienal	(E)-2-nonenal
-ethy1-2-methy1-1,3-:xadiene	(E,E)-2,4-decadienal	8-methyl-1-undecene
-butenal	2,3-dimethy1-5-ethylpyrazine	1-propanol
-penten-3-one		

EXAMPLE 34 - Removal of off-flavors from plant protein solutions

i) Synthesis of ligand modified LOX removal resin

A 1 mL settled volume of CM Sepharose resin (Sigma Aldrich Catalog # 7,F100) was loaded into a BioRad minicolumn. 3 mL of 50 mM MES (2-)rpholinoethanesulfonic acid) buffer preset to a pH range of 5.5 to 6 was allowed to ss through the resin bed. Separately, to 1 mL of the same buffer was added in cession, 0.044 mL of 4,7,10- trioxa-1,13-tridecanediamine, 0.030 mL of 12 N HC1, 23 of NHS (N-hydroxy-succinimide), and 38 mg of EDC (1-ethy1-3-(3-nethylaminopropyl)carbodiimide hydrochloride) with dissolution after each addition. Le resulting solution was added to the top of the column bed and allowed to gravity lw through and the effluent was collected. The resulting effluent was returned to the) of the column bed. The cycle of addition of solution, elution and return was done ar times. When the last elution was finished, 3 mL of 50 mM MES buffer preset to a I of 5.5 to 6 was allowed to gravity flow through the column. Linoleic acid (0.03 mL) is dissolved in 0.5 mL DMF (N,N-dimethylformamide), followed in sequence by 12 NHS, 19 mg EDC, and 0.5 mL of 50 mM MES buffer preset to a pH of 5.5 to 6. The -IS/EDC mixture was shaken to mix, resulting in a two-phase liquid which was applied the top of the column and eluted through the resin bed, allowed to gravity flow through column and effluent collected. The effluent was returned to the top of the column d. The cycle of addition of solution, elution and return was done four times. Once the ;t collection was finished a solution of 70% ethanol in water (5 mL) was added to the

top of the column, followed by 3 mL of 0.1 M sodium hydroxide. This was followed by 0.1 M buffer of potassium phosphate preadjusted to pH 7 to 8.

ii) Removal of off-flavors from pea proteins using the ligand modified LOX removal resin.

A solution of pea proteins (30 mls at 20 mg/ml protein concentration in 20 mM potassium phosphate buffer pH 7.4, 100mM sodium chloride) was passed through a 100 ml resin bed described above. All unbound material was collected and the resin further washed with 200 mls of buffer. Both fractions were combined and resin was stripped of bound protein by washing with 2 bed volumes of 20 mM potassium phosphate, 1M sodium chloride. Resin bed was regenerated by washing with 2 bed volumes of 0.1M NaOH followed by 3 bed volumes of water and re-equilibration with buffer.

Depletion of LOX activity in the pooled unbound fraction was confirmed by assaying for enzyme activity. Sodium linoleate was used as substrate for LOX and formation of hydroperoxide intermediates was monitored by absorbance at 234nm. Assays were performed at pH9 in 50mM sodium borate buffer. Assays confirmed depletion of LOX activity in the pooled unbound fraction. LOX was removed from resin in washes with 0.5M and 1M sodium chloride.

Improvement in flavor in the LOX depleted protein solutions (both as-is and incubated with canola oil at 10%) was confirmed by a panel of 4 tasters. Samples of pea proteins at same concentration, but not depleted of LOX was used at control. Tasters described LOX -depleted samples as mild tasting and control samples as having beany, planty taste. In addition, GC-MS analysis of samples showed 5X reduction in overall volatiles in the LOX-depleted samples.

Reduction of off-flavors using dialysis and activated carbon

A solution of pea albumins (30 mls at 40 mg/ml in 20mM potassium phosphate buffer pH 7.4, 100 mM sodium chloride) was dialyzed against 100 volumes of buffer overnight at 4°C. The solution was then poured over a bed of activated carbon (100-mesh, Sigma-Aldrich) prewetted with buffer. The slurry was centrifuged at 5000 x g for

10 minutes and the supernatant containing protein was decanted off. This solution was tested for improvement in flavor by both taste and GC-MS. Improved flavor (less bitter, less soapy flavor) was noted by tasters and GC-MS confirmed a >2X reduction in volatile compounds; in particular, samples treated with activated carbon showed a decrease in C6- and C7 compounds (e.g., 1-heptanal, 2-heptenal, or 2-heptanone) that are associated with green/grassy/planty flavors.

Reduction of off-flavors using antioxidants and/or LOX inhibitors

A 7% solution of pea-vicilins was heated with coconut oil (20%) to 95°C for 15 min in the presence of antioxidants or LOX inhibitors and compared for off-flavors sample against a control without any antioxidants or LOX inhibitors. In a similar experiment, soymilk was heated in the presence of antioxidants or LOX inhibitors and tasted in-house for off-flavors. Table 11 summarizes off-flavors noted by tasters. Both epigallocetechin gallate and propyl gallate were effective at minimizing off-flavors in pea samples. However, in case of soymilk, epigallocatechin gallate did not appear to reduce beaniness; propyl gallate and a-tocopherol were found to slightly improve flavor in soymilk.

Table 11

Compound	soymilk	**Pea**
a-tocopherol	Slightly improved flavor	Oxidized oil
caffeic acid (0.02%)	Beany	Oxidized oil
epigallocatechin gallate	Beany	Improved flavor
propyl gallate (0.02%)	Slightly improved flavor	Improved flavor
13-carotene	Beany	Oxidized oil

EXAMPLE 35 - ADIPOSE REPLICA WITH LECITHIN GRADIENT

Lecithin (SOLEC^{Tm} F Deoiled Soy Lecithin, The Solae Company, St. Louis, MO) was prepared at a concentration of 50 mg/mL in 20 rnM potassium phosphate, 100 rriM NaCI, pH 8M buffer and sonicated (Sonifier Analog Cell Disruptor model 102C,

BRANSON Ultrasonics Corporation, Danbury, Connecticut) for 30 seconds. Moong protein was supplied as a liquid in 20 mM potassium phosphate, 100 niM NaC I, p1:1 8.0 buffer, Coconut oil was melted by heating to 50 - 70°C and held warm until needed. The coconut oil, buffered protein solution, additional buffer, and lecithin slurry were mixed at 70°C, and an emulsion was formed using a hand held homogenizer, The emulsion was then heat set by placing the tube into a 95°C waterbath for a total of 5 mins. The tube was then removed from the waterbath and stored at 4°C for twelve hours or longer prior to analysis.

To observe the effect of lecithin on the adipose tissue replica replica, a formulation was prepared that contained 1% NOT rnoong protein and 75% v/v coconut oil, with the lecithin amount increasing from 0%, 0.05%, 0.25%, 0.5%, and 1.0% w/v. Properties of the adipose replica were measured by weighing small portions of the material and forming uniform round balls, which were then cooked on a non-stick pan with temperature slowly ramping up to 150°C. The temperature of the pan at which fat visibly released from the balls was measured as the fat release temperature. After cooking to completion, the point at which fat no longer released, the total fat released was measured.

An increase in the amount of lecithin in the adipose replica correlated with an increase in the percent fat released, and with a decrease in the fat release temperature (see FIGS. 2A and 2B). With 0% lecithin, there was an average of 40% fat released, and when lecithin was increased to 0.05%, there was an average of 82% fat released, which further increased to 88% with 0.25% lecithin, then leveled off to an average of 60% with further increases in lecithin. With 0% lecithin, a high temperature of 217°C was required to begin the fat release. The fat release temperature decreased to 122°C with 0.25% lecithin, then leveled off to an average of 62°C with further increases of lecithin.

Firmness of the adipose replica was measured by texture analyzer (TA XT plus). A probe penetrated a flat surface of the adipose replica, and the force at 2 mm penetration was recorded. A small amount of lecithin (0.05%) increased the firmness, and at 0.25% and above, the firmness of the adipose replica decreased. See FIG. 2C.

EXAMPLE 36 - ADIPOSE REPLICA WITH VARYING VEGETABLE OIL TYPE

To observe the effect of vegetable oil type on the adipose replica, a formulation was prepared that contained a **1.5%** w/v moong protein and 0.05% w/v lecithin, and 75% v/v of different vegetable oils (canola oil, cocoa butter, coconut oil, and olive oil) using the same methodology as Example 35. Properties of the adipose replica were measured by weighing small portions of the material and forming uniform round balls, which were then cooked on a non-stick pan with temperature slowly ramping up to 250°C. The temperature of the pan at which fat visibly released from the balls was measured as the fat release temperature. After cooking to completion, the point at which fat no longer released, the total fat released was measured.

Varying the vegetable oil type had a large effect on the adipose replica. Oils with a higher amount of unsaturated fats, including canola and rice bran oils, released very little fat (1 and 2% fat released, see FIG. 3), while oils with a higher amount of saturated fats, including cocoa butter and coconut oil released significantly more fat (30% and 50% fat released, see FIG. 4). The adipose replicas with canola and rice bran oils required a temperature greater than 250°C to melt (not measured), while the adipose replicas with cocoa butter and coconut oil released fat at a lower temperature (82°C and 137°C).

EXAMPLE 37 - ADIPOSE REPLICA MADE WITH COACERVATE

Lecithin (SOLECTM F Deoiled Soy Lecithin, The Solae Company, St. Louis, MO) was prepared at a concentration of 50 mg/ml in 20 mM potassium phosphate, 100 mM NaC1, pH 8.0 buffer and sonicated (Sonifier Analog Cell Disruptor model 102C, BRANSON Ultrasonics Corporation, Danbury, Connecticut) for 30 seconds. Pea legumin and pea vicilin proteins in 20 mM potassium phosphate, 100 mM NaC1, pH 8.0 buffer, were mixed to a 1:1 ratio. Cocoa butter was melted by heating to 70°C and held warm until needed. The cocoa butter was added to 2% and 10% w/v to the protein mixtures, and was added at 60°C to maintain cocoa butter in the liquid state. While the solutions were still warm, the mixtures were sonicated 1-3 minutes, until cocoa butter particles were visibly emulsified. The pH of the samples was adjusted to 5.5 with HC1,

and the mixtures turned into a milky white color, then centrifuged at 5,000xg for ten minutes. After centrifugation, a pellet was collected, comprising a coacervate of protein and cocoa butter. The 2% fat coacervates were sticky and stretchy, while the 10% fat coacervates were fatty and pliable. The coacervates were sealed in plastic and subjected to high pressure processing (HPP). The sample was sealed in a heat-sealable food-saver plastic bag and then subject to high pressure processing (85k psi for 5 minutes in an Avure 2L Isostatic Food Press). After HPP, the 2% coacervate samples formed a semi-firm, cohesive material. The 10% coacervate samples were crumbly, soft, and oily.

Properties of the processed coacervate samples were measured by breaking off small portions of the material and cooking on a non-stick pan with temperature slowly ramping up to 250°C. The coacervate samples did not release any fat upon cooking to this temperature.

OTHER EMBODIMENTS

While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

EXHIBIT 1E

WHAT IS CLAIMED IS:

1. A consumable product comprising an isolated and purified plant protein, wherein the isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2°C and about 32°C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90°C and 110°C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM.

2. The consumable product of claim 1, wherein said product is a beverage, a protein supplement, a baked good, a condiment, a meat product, or a meat substitute product.

3. The consumable product of claim 2, wherein said beverage is an alcoholic beverage or a protein drink.

4. ~~166. The meat substitute~~ The consumable product of claim ~~163, comprising:~~ 3, wherein said alcoholic beverage is a cream liquor.

5. The consumable product of claim 4, said cream liquor further comprising a non-dairy lipid emulsion, wherein said cream liquor is free of animal products.

6. The consumable product of claim 3, wherein said protein drink is a meal replacement beverage, a beer supplemented with said protein, or distilled alcoholic beverage supplemented with said protein.

7. The consumable product of claim 2, wherein said condiment is a mayonnaise replica.

8. The consumable product of claim 2, wherein said meat product is a pate, a sausage replica, or a meat substitute.

9. The consumable product of any one of claims 1-8, wherein said isolated and purified plant protein is at least 10 kDa in size.

10. The consumable product of any one of claims 1-9, wherein said isolated and purified plant protein is not fully denatured.

11. The consumable product of any one of claims 1-10, wherein said isolated and purified plant protein is not derived from soy.

12. The consumable product of any one of claims 1-11, wherein said isolated and purified plant protein comprises one or more of RuBisCo, Moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin.

13. The consumable product of any one of claims 1-12, wherein said isolated and purified plant protein comprises a dehydrin, a hydrophilin, an intrinsically disordered protein, or a protein identified based on its ability to stay soluble after boiling at a pH and a salt concentration comparable to a food.

14. The consumable product of any one of claims 1-13, said consumable product further comprising a plant derived lipid or a microbial-derived lipid.

15. The consumable product of any one of claims 1-14, further comprising a second isolated and purified protein.

16. The consumable product of any one of claims 1-15, further comprising a seasoning agent, a flavoring agent, an emulsifier, a gelling agent, a sugar, or a fiber.

17. A consumable product comprising a coacervate comprising one or more isolated and purified proteins.

18. The consumable product of claim 17, wherein said consumable product is a meat replica.

19. The consumable product of claim 17 or claim 18, said product further comprising a plant derived lipid or a microbial-derived lipid.

20. The consumable product of claim 19, wherein said plant derived lipid or microbial-derived lipid comprises lecithin and/or an oil.

21. The consumable product of claim 20, said product comprising up to about 1% lecithin by weight.

22. The consumable product of claims 20 or claim 21, wherein said product comprises lecithin and said oil.

23. The consumable product of any one of claims 20-22, wherein said oil is canola oil, palm oil, or cocoa butter.

24. The consumable product of any one of claims 20-23, said product comprising about 1% to about 9% of said oil.

25. The consumable product of any one of claims 17-24, wherein said one or more isolated and purified proteins comprise plant proteins.

26. The consumable product of claim 25, wherein said one or more plant proteins comprise one or more pea proteins, chickpea proteins, lentil proteins, lupine proteins, other legume proteins, or mixtures thereof

27. The consumable product of claim 26, wherein said one or more pea proteins are legumins, vicilin, a convicilin, or a mixture thereof.

~~lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phos phoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde 3 phosphate dehydrogena ses, pyruvate decarboxylases, actin, translation elongation factors, ribulose 1,5 bisphosphate carboxylase oxygenase (rubisco), ribulose 1,5 bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglyci nins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteino plast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.~~

~~a) The meat substitute product of claim **163,** further comprising one or more isolated and purified iron containing proteins.~~

~~b) The meat substitute product of claim **173,** wherein said one or more isolated and purified iron containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, pro toglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, G1b3, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, and flavohemoglobins.~~

~~a) 60 90% water;~~

~~b) 5 30% protein content;~~

~~c) 1 20% of a fat or fat replica;~~

~~wherein said protein content comprises one or more iso lated, purified plant proteins.~~

~~**167.** The meat substitute product of claim **166,** wherein said protein content is derived from a non animal source.~~

~~**168.** The meat substitute product of claim **167,** wherein said non animal source is a plant source, or a genetically modified yeast or bacteria.~~

~~**169.** (canceled)~~

~~**170.** The meat substitute product of claim **163,** wherein 50% or more of said protein content by weight are one or more isolated purified proteins.~~

~~**171.** The meat substitute product of claim **170,** wherein each of said one or more isolated proteins is isolated and purified separately from different plant species.~~

~~25.~~28. ~~**172.** The meat substitute product of claim **170,** wherein one or more of said isolated proteins is selected from the group consisting of: hemoglobin, myoglobin, chlorocruorin, eryth rocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglo bins, flavohemoglobins, ribosomal proteins, actin, hexoki nase,~~ A meat replica comprising a muscle replica, a connective tissue replica; an adipose tissue replica, and a coacervate comprising one or more isolated and purified proteins.

29. The meat replica of claim 28, wherein said coacervate further comprises a plant-derived lipid or microbial-derived lipid.

30. The meat replica of claim 29, wherein said plant derived lipid or microbial-derived lipid is lecithin and/or an oil.

31. The meat replica of any one of claims 28-30, wherein said meat replica is a ground beef replica.

32. A consumable product comprising a comprising a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt.

33. The consumable product of claim 32, wherein said isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin.

34. The consumable product claim 32, wherein said isolated and purified plant protein comprises a dehydrin, a hydrophilin, or an intrinsically disordered protein.

35. The consumable product of any one of claims 32-35, wherein said cold set gel further comprises a plant derived lipid or microbial derived lipid.

36. The consumable product of claim 35, wherein said plant derived lipid or microbial derived lipid is lecithin and/or oil.

37. An adipose tissue replica comprising one or more isolated plant proteins, one or more plant or algal derived oils, and optionally a phospholipid.

38. The adipose tissue replica of claim 37, wherein said phospholipid is lecithin.

39. The adipose tissue replica of claim 37 or claim 38, wherein said plant based oils are selected from the group consisting of corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, palm oil, palm kernel oil, coconut oil, babassu oil, Shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, and combinations thereof

40. The adipose tissue replica of any one of claims 37-39, wherein the fat release temperature of said adipose tissue replica is between 23°C to 33°C, 34°C to 44°C, 45°C to 55°C, 56°C to 66°C, 67°C to 77°C, 78°C to 88°C, 89°C to 99°C, 100°C to 110°C, 111°C to 121°C, 122°C to 132°C, 133°C to 143°C, 144°C to 154°C, 155°C to 165°C, 166°C to 167°C, 168°C to 169°C, 170°C to 180°C, 181°C to 191°C, 192°C to 202°C, 203°C to 213°C, 214°C to 224°C, 225°C to 235°C, 236°C to 246°C, 247°C to 257°C, 258°C to 268°C, 269°C to 279°C, 280°C to 290°C, or 291°C to 301°C.

41. The adipose tissue replica of any one of claim 37-40, wherein the percent fat release of said adipose tissue replica is 0 to 10%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, 50% to 60%, 60% to 70%, 70% to 80%, 80% to 90%, or 90% to 100% upon cooking.

42. The adipose tissue replica of any one of claims 37-41, wherein said isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin.

43. The adipose tissue replica of any one of claims 37-42, wherein said adipose tissue replica comprises from about 40% to about 90% of said oil.

44. The adipose tissue replica of any one of claims 37-43, wherein said adipose tissue replica comprises from about 1% to about 6% of said isolated and purified plant protein.

45. The adipose tissue replica of any one of claims 37-44, wherein said adipose tissue replica comprises from about 0.05 to about 2% of said phospholipid.

46. The adipose tissue replica of any one of claims 37-45, wherein the firmness of said adipose tissue replica is similar to that of beef adipose tissue.

47. A consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, wherein said consumable product does not comprise meat.

48. The consumable product of claim 47, wherein said heme-containing protein accounts for at least 0.01% of the composition.

49. The consumable product of claim 47 or claim 48, further comprising one or more ammonium, sodium, potassium, or calcium salts.

50. The consumable product or meat replica of any one of claims 17-49, wherein said isolated and purified proteins are crosslinked.

51. A consumable product comprising a gelled emulsion, said gelled emulsion comprising:

a) an isolated and purified protein;

b) a first lipid that when not in said consumable product is solid at a selected temperature range; and

c) a second lipid that when not in said consumable product is liquid at said selected temperature range; wherein the melting temperature of the mixture of said first and second lipids is similar to the melting temperature of lipids found in meat, and wherein said first and second lipids are plant derived lipids or microbial derived lipids.

52. A method for making a consumable product comprising:

a) preparing a solution comprising an isolated and purified plant protein, wherein said isolated and purified plant protein has (i) a solubility in said solution of at least 25 at a temperature between about 2°C and 32°C, wherein said solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in said solution of at least 1 mg/ml at a temperature of between 90°C and 110°C, wherein said solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM; and

b) adding said solution to a beverage.

53. The method of claim 52 wherein said solution comprises two or more isolated and purified plant proteins.

54. The method of claim 52 or claim 53, wherein said beverage is clear.

55. The method of any one of claim 52-54, wherein said isolated and purified plant protein is at a concentration of at least 1% by weight in said solution.

56. The method of any one of claims 52-55, wherein said isolated and purified plant protein is selected from the group consisting of RuBisCo, a moong globulin, a soy globulin, a pea globulin, a pea albumin, a prolamin, a lentil protein, a dehydrin, a hydrophilin, and an intrinsically disordered protein.

57. The method of any one of claims 52-56, wherein said isolated and purified plant protein is lyophilized prior to making said solution.

58. The method of any one of claims 52-57, wherein said beverage has an improved mouthfeel compared to a corresponding beverage without said isolated and purified protein.

59. A method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions.

~~53.~~60. ~~The meat substitute composition of claim 174 wherein said iron-containing~~ The method of claim 59, wherein said heme-containing protein comprises an amino acid sequence with at least 70% homology to ~~SEQ ID NO 1.~~an amino acid sequence set forth in any one of SEQ ID NOs: 1-27.

61. A method for making a meat replica comprising a cold set gel comprising:

a) denaturing a solution comprising at least one isolated and purified protein from a non-animal source under conditions wherein said isolated and purified protein does not precipitate out of said solution;

b) optionally adding any heat-labile components to said solution of denatured protein;

c) gelling said solution of denatured protein at about 4°C to about 25°C by increasing the ionic strength of said solution to form a cold set gel, and

d) incorporating said cold set gel into a meat replica.

62. The method of claim 61, wherein gelling is induced using 5 to 100 mM sodium or calcium chloride.

63. The method of claim 61 or claim 62, wherein said heat-labile components are proteins or lipids, or mixtures thereof.

64. The method of claim 63, wherein said protein is a heme-containing protein.

65. The method of claim 63, wherein said cold set gel is formed in a matrix comprising a freeze-aligned plant protein.

66. The method of any one of claims 61-65, wherein said isolated and purified protein from a non-animal source is a plant protein.

67. The method of claim 66, wherein said plant protein is selected from the group consisting of RuBisCo, a moong globulin, a soy globulin, a pea globulin, a pea albumin, a prolamin, a lentil protein, a dehydrin, a hydrophilin, and an intrinsically disordered protein.

68. An adipose tissue replica, comprising

a) an isolated and purified non-animal protein;

b) a non-animal lipid; and

c. a three-dimensional matrix comprising fibers derived from non-animal sources, wherein said lipid and said protein are dispersed in said three-dimensional matrix, and wherein said three-dimensional matrix stabilizes the structure of the adipose tissue replica.

69. A connective tissue replica comprising one or more isolated and purified proteins assembled into fibrous structures by a solution spinning process.

70. The connective tissue replica of claim 69 wherein the fibrous structures are stabilized by a cross-linking agent.

71. A method for imparting a beef like flavor to a consumable product, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

72. A method for making a poultry or a fish composition taste like beef, said method comprising adding a heme protein to said poultry or fish composition, respectively.

73. The method of claim 71 or claim 72, wherein said heme-containing protein has an amino acid sequence with at least 70% homology to any one of the amino acid sequences set forth in SEQ ID NOs: 1-27.

74. A method of making a coacervate, said method comprising

a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of sodium chloride; and

b) isolating said coacervate from said solution.

75. The method of claim 74, wherein said pH is between 4 and 5.

76. The method of claim 74 or claim 75, wherein said plant proteins comprise one or more pea proteins, chickpea proteins, lentil proteins, lupine proteins, other legume proteins, or mixtures thereof

77. The method of claim 76, wherein said pea proteins comprise isolated and purified legumins, isolated and purified vicilins, isolated and purified convicilins, or combinations thereof

78. The method of claim 77, wherein said isolated and purified pea proteins comprise isolated and purified vicilins and isolated and purified convicilins.

79. The method of any one of claims 74-78, wherein said acidifying step is done in the presence of a plant derived lipid or microbial derived lipid.

80. The method of claim 79, wherein said plant derived lipid or microbial derived lipid comprises oils and/or phospholipids.

81. A method of making an adipose tissue replica, said method comprising forming an emulsion comprising one or more isolated plant proteins, one or more plant or algal derived oils, and optionally a phospholipid.

82. The method of claim 81, wherein said phosoholipid is included, and is lecithin.

83. The method of claim 81 or claim 82, wherein said plant based oils are selected from the group consisting of corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil,

sunflower oil, flax seed oil, palm oil, palm kernel oil, coconut oil, babassu oil, Shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, and combinations thereof

84. The method of any one of claims 81-83, wherein the fat release temperature of said adipose tissue replica is between 23°C to 33°C, 34°C to 44°C, 45°C to 55°C, 56°C to 66°C, 67°C to 77°C, 78°C to 88°C, 89°C to 99°C, 100°C to 110°C, 111°C to 121°C, 122°C to 132°C, 133°C to 143°C, 144°C to 154°C, 155°C to 165°C, 166°C to 167°C, 168°C to 169°C, 170°C to 180°C, 181°C to 191°C, 192°C to 202°C, 203°C to 213°C, 214°C to 224°C, 225°C to 235°C, 236°C to 246°C, 247°C to 257°C, 258°C to 268°C, 269°C to 279°C, 280°C to 290°C, or 291°C to 301°C.

85. The method of any one of claim 81-84, wherein the percent fat release of said adipose tissue replica is 0 to 10%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, 50% to 60%, 60% to 70%, 70% to 80%, 80% to 90%, or 90% to 100% upon cooking.

86. The method of any one of claims 81-85, wherein said isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin.

87. The method of any one of claims 81-86, wherein said emulsion comprises from about 40% to about 90% of said oil.

88. The method of any one of claims 81-87, wherein said emulsion comprises from about 1% to about 4% of said isolated and purified plant protein.

89. The method of any one of claims 81-88, wherein said adipose tissue replica comprises from about 0.05 to about 1% of said phospholipid.

90. The method of any one of claims 81-89, wherein the emulsion is formed by high-pressure homogenization, sonication, or hand homogenization.

91. A method of minimizing undesirable odors in a composition comprising plant proteins, said method comprising contacting said composition with a ligand having affinity for one or more lipoxygenases.

92. A method of minimizing undesirable odors in a composition comprising plant proteins, said method comprising contacting said composition with activated carbon then removing the activated carbon from said composition.

93. A method of minimizing undesirable odors in a composition comprising plant proteins, said method comprising adding a lipoxygenase inhibitor and/or an antioxidant to said composition.

94. A chocolate flavored spread comprising:

a) sugar

b) a chocolate flavoring, and

c) a cream fraction from a plant based milk.

95. A method for altering the texture of a consumable during or after cooking comprising incorporating within the consumable one or more plant proteins with a low denaturation temperature.

96. The method of claim 95, wherein at least one of the one or more plant proteins is isolated and purified.

97. The method of claim 95, wherein the one or more plant proteins are selected from the group consisting of rubisco, pea proteins, lentil proteins, or other legume proteins.

98. The method of claim 97, wherein the pea proteins comprise pea albumin proteins.

99. The method of claim 95, wherein the consumable becomes firmer during or after cooking.

100. A tissue replica comprising a freeze-aligned non-animal protein.

101. The tissue replica of claim 100, wherein the non-animal protein is a plant protein.

102. The tissue replica of claim 101, wherein the non-animal protein is isolated and purified.

103. The tissue replica of claim 100, which is a muscle tissue replica.

104. A meat replica comprising a tissue replica comprising a freeze-aligned non-animal protein.

~~c) The meat substitute product of claim **163,** wherein said meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum ara bic, and no acacia gum.~~

EXHIBIT 1E

~~d) The meat substitute product of claim **174,** wherein said meat substitute product contains less than 1% wheat gluten.~~

~~e) The meat substitute product of claim **175,** wherein said meat substitute product contains no wheat gluten.~~

~~f) The meat substitute product of claim **176,** wherein said meat substitute product is characterized by one or more of the following contains no soy protein isolate, no soy protein concentrate, or no tofu.~~

~~g) (canceled)~~

~~h) (canceled)~~

~~i) The meat substitute product of claim **179,** wherein said meat substitute product contains less than 5% carbohydrates.~~

~~j) (canceled)~~

~~k) The meat substitute product of claim **163,** wherein said meat substitute product is characterized by one or more of the following: contains no tofu, no soy protein, contains less than 1% cellulose, contains less than 5% insoluble carbohydrates, or no wheat gluten.~~

~~l) (canceled)~~

~~m) The meat substitute product of claim **163,** wherein said meat substitute product contains no animal products and less than 5% carbohydrates.~~

~~n) (canceled)~~

~~o) (canceled)~~

~~p) (canceled)~~

~~Q) (canceled)~~

~~R) (canceled)~~

~~S) The meat substitute product of claim **163,** wherein said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.~~

~~**193-222.** (canceled)~~

* * * * *

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Rachel Fraser	Art Unit	:	1791
Patent No.	:	9,943,096	Examiner	:	Elizabeth A. Gwartney
Issue Date	:	April 17, 2018	Conf. No.	:	2358
Serial No.	:	15/398,479			
Filed	:	January 4, 2017			

Title : METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES

Attention Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION

Please issue a Certificate of Correction in accordance with the attached form.

One or more of the errors to be corrected were made by Applicant. The fee of $150 is being paid with this request. Please apply any other necessary charges or credits to Deposit Account No. 06-1050, referencing the above-indicated Attorney Docket Number.

Respectfully submitted,

Date: /May 2, 2018/

/M. Angela Parsons/
M. Angela Parsons, Ph.D.
Reg. No. 44,282

Customer Number 26191
Fish & Richardson P.C.
Telephone: (612) 335-5070
Facsimile: (877) 769-7945

61675261.doc

PTO/SB/44 (09-07)
Approved for use through 08/31/2013. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.	:	9,943,096
APPLICATION NO.:		15/398,479
ISSUE DATE	:	April 17, 2018
INVENTOR(S)	:	Rachel Fraser, Patrick O'Reilly Brown M.D., Ph.D., Jessica Karr, Celeste Holz-Schietinger and Elysia P. Cohn

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In Column 1, Line 8, delete “10, 2105” and insert --10, 2015--, therefor.

In Column 73, Line 65, In Claim 2, delete “histoglobin,” and insert --histaglobin-- therefor.

In Column 75, Line 11, In Claim 10, after “glucose,” insert --ribose,--.

In Column 75, Line 12, In Claim 10, delete “glactose,” and insert --galactose,--, therefor.

In Column 75, Line 13, In Claim 10, delete “thereof” and insert --thereof;--, therefor.

In Column 75, Line 14, In Claim 10, delete “cysteine,” and insert --cystine,--, therefor.

In Column 75, Line 18, In Claim 10, delete “a” and insert --the--, therefor.

In Column 75, Line 28, In Claim 11, after “flavoring” delete “wherein the food flavoring”.

In Column 76, Line 7, In Claim 15, delete “histoglobin,” and insert --histaglobin,--, therefor.

In Column 76, Line 40, In Claim 22, delete “and or” and insert --and/or--, therefor.

MAILING ADDRESS OF SENDER (Please do not use customer number below):
Fish & Richardson P.C.
P.O. Box 1022
Minneapolis, MN 55440-1022

This collection of information is required by 37 CFR 1.322, 1.323, and 1.324. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1.0 hour to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Attention Certificate of Corrections Branch, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and selection option2.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,943,096 B2
APPLICATION NO. : 15/398479
DATED : April 17, 2018
INVENTOR(S) : Rachel Fraser et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

In Column 1, Line 8, delete "10, 2105" and insert --10, 2015--, therefor.

In the Claims

In Column 73, Line 65, In Claim 2, delete "histoglobin," and insert --histaglobin--, therefor.

In Column 75, Line 11, In Claim 10, after "glucose," insert --ribose,--.

In Column 75, Line 12, In Claim 10, delete "glactose," and insert --galactose,--, therefor.

In Column 75, Line 13, In Claim 10, delete "thereof" and insert --thereof;--, therefor.

In Column 75, Line 14, In Claim 10, delete "cysteine," and insert --cystine,--, therefor.

In Column 75, Line 18, In Claim 10, delete "a" and insert --the--, therefor.

In Column 75, Line 28, In Claim 11, after "flavoring" delete "wherein the food flavoring".

In Column 76, Line 7, In Claim 15, delete "histoglobin," and insert --histaglobin,--, therefor.

In Column 76, Line 40, In Claim 22, delete "and or" and insert --and/or--, therefor.

Signed and Sealed this
Twenty-ninth Day of May, 2018

Andrei Iancu
Director of the United States Patent and Trademark Office

only when notice and comment are required by the APA or other law, are not applicable. These amendments do not contain any collection of information requirements as defined by the Paperwork Reduction Act of 1995. *See* 5 CFR 1320.3. Further, because the amendments impose no new burdens on private parties, the Commission does not believe that the amendments will have any impact on competition for purposes of Section 23(a)(2) of the Securities Exchange Act of 1934. 15 U.S.C. 78w(a)(2).

III. Statutory Authority

This rule is adopted pursuant to statutory authority granted to the Commission, including Section 19 of the Securities Act of 1933, 15 U.S.C. 77s; Sections 4A, 4B, and 23 of the Exchange Act, 15 U.S.C. 78d–1, 78d–2, and 78w; Section 38 of the Investment Company Act of 1940, 15 U.S.C. 80a–37; Section 211 of the Investment Advisers Act of 1940, 15 U.S.C. 80b–11; and Section 3 of the Sarbanes-Oxley Act of 2002, 15 U.S.C. 7202.

List of Subjects in 17 CFR Part 200

Administrative practice and procedure, Authority delegations (Government agencies).

For the reasons set out in the preamble, the Commission is amending Title 17, Chapter II of the Code of Federal Regulations as follows:

PART 200—ORGANIZATION; CONDUCT AND ETHICS; AND INFORMATION AND REQUESTS

Subpart A—Organization and Program Management

■ 1. The general authority citation for part 200, subpart A continues to read in part as follows:

Authority: 15 U.S.C. 77c, 77o, 77s, 77z–3, 77sss, 78d, 78d–1, 78d–2, 78o–4, 78w, 78*ll*(d), 78mm, 80a–37, 80b–11, 7202, and 7211 *et seq.*, unless otherwise noted.

* * * * *

■ 2. Amend § 200.30–14 by:
■ a. Redesignating paragraphs (f) through (o) as paragraphs (g) through (p); and
■ b. Adding new paragraph (f).

The addition reads as follows.

§ 200.30–14 Delegation of authority to the General Counsel.

* * * * *

(f) In bankruptcy cases, to take the following actions with respect to plan or settlement provisions that have the effect of releasing, exculpating, discharging, or permanently enjoining actions against non-debtor third parties in contravention of Section 524(e) of the Bankruptcy Code or applicable law:

(1) Object to approval of disclosure statements, including on the basis that the disclosure statement lacks adequate information under Section 1125(b) to support such release provisions;

(2) Object to confirmation of bankruptcy plans; or

(3) Object to approval of settlements.

* * * * *

By the Commission.

Dated: February 19, 2020.

Vanessa A. Countryman,

Secretary.

[FR Doc. 2020–03705 Filed 2–28–20; 8:45 am]

BILLING CODE 8011–01–P

DEPARTMENT OF COMMERCE

Patent and Trademark Office

37 CFR Part 1

[Docket No. PTO–P–2019–0035]

Clarification of the Practice for Requiring Additional Information in Petitions Filed in Patent Applications and Patents Based on Unintentional Delay

AGENCY: United States Patent and Trademark Office, Department of Commerce.

ACTION: Clarification.

SUMMARY: The United States Patent and Trademark Office (USPTO) is clarifying its practice as to situations that will require additional information about whether a delay in seeking the revival of an abandoned application, acceptance of a delayed maintenance fee payment, or acceptance of a delayed priority or benefit claim was unintentional.

DATES: The clarification of practice set forth is applicable to any petition decided on or after March 2, 2020.

FOR FURTHER INFORMATION CONTACT: Christina Tartera Donnell, Attorney Advisor, Office of Petitions, by telephone at 571–272–3211; or Douglas I. Wood, Attorney Advisor, Office of Petitions, by telephone at 571–272–3231; or by mail addressed to: Mail Stop Comments-Patents, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313–1450.

SUPPLEMENTARY INFORMATION: Title II of the PLTIA amended the provisions of title 35, United States Code (U.S.C.), to implement the Patent Law Treaty (PLT). *See* Public Law 112–211, § 201–203, 126 Stat. 1527, 1533–37 (2012). Section 201(b) of the PLTIA added a new 35 U.S.C. 27, which expressly provides that the director of the USPTO may establish procedures to revive an unintentionally abandoned application for patent or accept an unintentionally delayed issue fee payment, upon petition by the applicant for patent or patent owner. *See* Public Law 112–211, 201(b)(1), 126 Stat. at 1534. Section 202(b)(1)(B) of the PLTIA amended 35 U.S.C. 41(c)(1) to provide that the director may accept the payment of any maintenance fee required by 35 U.S.C. 41(b) after the six-month grace period if the delay is shown to the satisfaction of the director to have been unintentional. *See* Sec. 202(b)(1)(B), Public Law 112–211, 126 Stat. at 1535–36. The 18-month publication provisions of the American Inventors Protection Act of 1999 (AIPA) amended 35 U.S.C. 119 and 120 to provide that a priority claim for a foreign or international application and a benefit claim to an earlier domestic provisional or nonprovisional application must be filed within the period required by the USPTO, but that the USPTO may establish procedures to accept an unintentionally delayed priority or benefit claim. *See* Public Law 106–113, 113 Stat. 1501, 1501A–563 through 1501A–564 (1999).

The USPTO revised the rules of practice to implement the 18-month publication provisions of section 4503 of the AIPA in September 2000. This included revising the rules of practice pertaining to foreign priority and domestic benefit claims (37 CFR 1.55 and 1.78) to set a time period within which such priority and benefit claims must be filed, and to provide for the acceptance of unintentionally delayed priority or benefit claims. *See* Changes to Implement Eighteen-Month Publication of Patent Applications, 65 FR 57023, 57024–25, 57030–31, 57053–55 (September 20, 2000). The USPTO revised the rules of practice for consistency with the PLT and title II of the PLTIA in October 2013. This included revising the rules of practice pertaining to the revival of abandoned applications (37 CFR 1.137) and acceptance of delayed maintenance fee payments (37 CFR 1.378) to provide for the revival of abandoned applications and acceptance of delayed maintenance fee payments solely on the basis of "unintentional" delay, as well as revisions to the rules of practice pertaining to foreign priority and domestic benefit claims (37 CFR 1.55 and 1.78). *See* Changes to Implement the Patent Law Treaty, 78 FR 62368, 62377–78, 62380–83, 62399–400, 62402–07 (October 21, 2013).

The provisions for the revival of an abandoned application (37 CFR 1.137)

EXHIBIT 1G

require a petition including, inter alia, a statement that the entire delay in filing the required reply, from the due date of the reply until the filing of a grantable petition, was unintentional, but also provide that "[t]he Director may require additional information where there is a question whether the delay was unintentional" (37 CFR 1.137(b)(4)). The provisions for the acceptance of a delayed maintenance fee payment (37 CFR 1.378) similarly require a petition including, inter alia, a statement that the delay in payment of the maintenance fee was unintentional, but also provide that "[t]he Director may require additional information where there is a question whether the delay was unintentional" (37 CFR 1.378(b)(3)). The provisions for the acceptance of a delayed priority or benefit claim (37 CFR 1.55 and 1.78) likewise require a statement that the delay between the date the claim was due and the date the claim was filed was unintentional, but also provide that "[t]he Director may require additional information where there is a question whether the delay was unintentional" (37 CFR 1.55(e)(4), 1.78(c)(3) and (e)(3)).

The USPTO is clarifying its practice as to situations that will require additional information about whether a delay in seeking the revival of an abandoned application, acceptance of a delayed maintenance fee payment, or acceptance of a delayed priority or benefit claim was unintentional. Specifically, the USPTO will require additional information in these cases, first, when a petition to revive an abandoned application is filed more than two years after the date the application became abandoned; second, when a petition to accept a delayed maintenance fee payment is filed more than two years after the date the patent expired for nonpayment; and third, when a petition to accept a delayed priority or benefit claim is filed more than two years after the date the priority or benefit claim was due. *See, e.g.,* Changes to Patent Practice and Procedure, 62 FR 53131, 53158–59, 53161 (October 10, 1997) (the length of the delay in filing a petition to revive may itself raise a question as to whether the delay was unintentional, and thus the USPTO may require additional information as to the cause of the delay when a petition to revive is not filed promptly). The reason for requiring additional information in cases where there has been an extended delay—a delay of more than two years from the date the application became abandoned, the patent expired, or a priority or benefit claim was due—until the filing of a petition, is to ensure that, in situations where there has been such an extended delay in filing the petition, the USPTO is provided with sufficient information of the facts and circumstances surrounding the entire delay to support a conclusion that the entire delay was "unintentional."

Section 711.03(c) of the Manual of Patent Examining Procedure (MPEP) discusses the "unintentional" delay standard with respect to petitions to revive an abandoned application, but its discussion of the "unintentional" delay is generally applicable to any petition under the "unintentional" delay standard. The USPTO usually relies upon the applicant's duty of candor and good faith and accepts the statement that the entire delay was unintentional without requiring further information because the applicant or patentee is obligated under 37 CFR 11.18 to inquire into the underlying facts and circumstances when providing this statement to the USPTO. *See* MPEP § 711.03(c), subsection II.C. An extended period of delay (*i.e.,* more than two years from the date the application became abandoned, the patent expired, or a priority or benefit claim was due) in filing a petition to revive an application, accept a delayed maintenance fee payment, or accept a delayed priority or benefit claim, however, raises a question as to whether the entire delay was unintentional. This may create uncertainty and unpredictability relating to patent rights in that there is a greater likelihood that the entire delay may not be "unintentional" within the meaning of 37 CFR 1.55, 1.78, 1.137, and 1.378, as compared to a petition that was filed within a shorter time period after the abandonment of the application, expiration of the patent, or due date for a priority or benefit claim. An applicant or patentee cannot meet the "unintentional delay" standard in 37 CFR 1.55(e), 1.78(c) and (e), 1.137(a), or 1.378(b) if the entire delay is not unintentional. *See* MPEP 711.03(c), subsections II.C. through F.

Furthermore, providing an inappropriate statement that the delay was "unintentional" may have an adverse effect when attempting to enforce the patent. *See In re Rembrandt Technologies LP Patent Litigation,* 899 F.3d 1254, 1272–73 (Fed. Cir. 2018) (patents held unenforceable due to a finding of inequitable conduct in submitting an inappropriate statement that the delay was unintentional). Revival of an application, reinstatement of a patent, or acceptance of a priority or benefit claim after an extended delay (*i.e.,* more than two years since the date of abandonment, expiration of the patent, or due date of the priority claim) can also create uncertainty and unpredictability relating to patent rights because the abandoned status of an application, or the expired status of a patent, or an absence of the priority or benefit claim, may be relied upon by other parties. Requiring additional information in these situations will improve the reliability and predictability of patent rights by ensuring that only applications and patents in which the entire delay was unintentional are revived or reinstated, and only priority or benefit claims for which the entire delay was unintentional are accepted.

Accordingly, any applicant filing a petition to revive an abandoned application under 37 CFR 1.137 more than two years after the date of abandonment, any patentee filing a petition to accept a delayed maintenance fee under 37 CFR 1.378 more than two years after the date of expiration for nonpayment of a maintenance fee, and any applicant or patent owner filing a petition to accept a delayed priority or benefit claim under 37 CFR 1.55(e) or 1.78(c) and (e) more than two years after the due date of the priority or benefit claim should expect to be required to provide an additional explanation of the circumstances surrounding the delay that establishes that the entire delay was unintentional. This requirement is in addition to the requirement to provide a statement that the entire delay was unintentional in 37 CFR 1.137(b)(4) and 1.378(b)(3), or 1.55(e)(4), or 1.78(c)(3) and (e)(3).

The USPTO may revisit the two-year time period established in this notice for requiring an additional explanation as to whether a delay is unintentional at a future point and may adjust the time period based on an evaluation of whether a two-year time period is appropriate for requesting additional information when determining whether a period of delay is unintentional. Nothing in this notice should be taken as an indication that the USPTO will only require additional information in consideration of a petition to revive an abandoned application under 37 CFR 1.137 filed more than two years after the date the application became abandoned, a petition to accept a delayed maintenance fee payment in an expired patent under 37 CFR 1.378 filed more than two years after the date the patent expired, or a petition under 37 CFR 1.55(e) or 1.78(c) or (e) to accept a delayed priority or benefit claim filed more than two years after the due date of the priority or benefit claim. The USPTO may require additional information whenever there is a

EXHIBIT 1G

question as to whether the delay was unintentional.

Dated: February 18, 2020.

Andrei Iancu,

Under Secretary of Commerce for Intellectual Property and Director of the United States Patent and Trademark Office.

[FR Doc. 2020–03715 Filed 2–28–20; 8:45 am]

BILLING CODE 3510–16–P

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 31

[FRL–10005–45–OECA]

RIN 2020–AA53

On-Site Civil Inspection Procedures

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: EPA is promulgating this rule of Agency procedure to fulfill the objectives outlined in the October 9, 2019 Executive Order (E.O.) 13892, *Promoting the Rule of Law Through Transparency and Fairness in Civil Administrative Enforcement and Adjudication.* This rule describes certain Agency procedures for conducting on-site civil inspections, as contemplated by section 7 of E.O. 13892, *Ensuring Reasonable Administrative Inspections.* This rule applies to on-site civil inspections conducted by federally credentialed EPA civil inspectors, federally credentialed contractors and Senior Environmental Employment (SEE) employees conducting inspections on behalf of EPA.

DATES: This rule is effective March 2, 2020.

FOR FURTHER INFORMATION CONTACT: Chad Carbone (202–564–2523), Office Enforcement and Compliance Assurance (2221A), U.S. Environmental Protection Agency, Washington, DC 20460; telephone number: (202) 564–2523; fax number: (202) 564–0050; email: *carbone.chad@epa.gov.*

SUPPLEMENTARY INFORMATION: The following outline is provided to assist the reader in locating topics of interest in the rule.

Table of Contents

I. General Information
A. Does this action apply to me?
B. What action is the Agency taking?
C. What is the Agency's authority for taking the action?
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1. Timing of Inspections and Facility Notification
2. Inspector Qualifications
3. Obtaining Consent to Enter
4. Opening Conference
5. Physical Inspection
6. Managing Confidential Business Information (CBI)
7. Interview Facility Personnel
8. Records Review
9. Sampling
10. Closing Conference
B. Inspection Report
III. Statutory and Executive Order Reviews

I. General Information

A. Does this action apply to me?

This rule applies to all federally credentialed EPA civil inspectors, federally credentialed contractors and Senior Environmental Employment (SEE) employees conducting inspections on behalf of EPA. As an internal rule of Agency procedure, the rule does not apply to federally credentialed state and tribal inspectors conducting inspections on EPA's behalf. This rule describes certain important aspects of the Agency's process of conducting on-site civil inspections and does not alter the rights or interests of parties or any person or entity outside the EPA.

B. What action is the Agency taking?

The purpose of this rulemaking is to provide direction to agency personnel on how to conduct EPA on-site civil administrative inspections, as required by section 7 of E.O. 13892, entitled *Ensuring Reasonable Administrative Inspections,* and which states: "Within 120 days of the date of this order, each agency that conducts civil administrative inspections shall publish a rule of agency procedure governing such inspections, if such a rule does not already exist. Once published, an agency must conduct inspections of regulated parties in compliance with the rule." This rulemaking addresses the common elements applicable to on-site civil inspections for compliance with all of the environmental laws that EPA implements. The specific activities that may occur during an inspection may vary depending on the facility and the statutory authority upon which the inspection is based. It is also important to note that EPA inspections are only one type of compliance monitoring activity that EPA conducts in order to help evaluate compliance. The primary focus of inspections is on recording observations and gathering information. As such, compliance determinations are an independent process that rely upon a myriad of information and are reviewed by Agency attorneys and management.

C. What is the Agency's authority for taking the action?

EPA's authority to issue this procedural rule is contained in the: Clean Air Act (CAA): 42 U.S.C. 7414, 7525, 7542, 7603, 7621; Clean Water Act (CWA): 33 U.S.C. 1318, 1321, 1364, 1367; Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) (Superfund): 42 U.S.C. 9604, 9606, 9622, Executive Order 12580, section 2(j)(2); Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA): 7 U.S.C. 136; Resource Conservation and Recovery Act (RCRA): 42 U.S.C. 6908, 6912, 6927, 6928, 6934, 6971, 6973, 6991, 6992; Safe Drinking Water Act (SDWA): 42 U.S.C. 300; and the Toxic Substances Control Act (TSCA): 15 U.S.C. 2610. EPA is also issuing this rule under its housekeeping authority. Section 301 of Title 5 U.S.C. authorizes an agency head to prescribe regulations governing his or her department and the performance of its business, among other purposes. EPA gained housekeeping authority through the Reorganization Plan No. 3 of 1970, 84 Stat. 2086 (July 9, 1970), as recognized by the U.S. Department of Justice Office of Legal Counsel. *See* "Authority of EPA to Hold Employees Liable for Negligent Loss, Damage, or Destruction of Government Personal Property," 32 O.L.C. 79, 2008 WL 4422366 at *4 (May 28, 2008). As a rule of Agency procedure this rule is exempt from the notice and comment requirements set forth in the Administrative Procedure Act. *See* 5 U.S.C. 553(b)(A).

II. Background

A. General Overview of On-Site Civil Inspections

Below is a general overview of the process for conducting on-site civil inspections. To ensure greater transparency and clearer direction to its inspectors, the Agency is codifying the major elements of inspections it carries out in its civil enforcement of environmental laws. (This rule does not apply to investigations of environmental crimes.) This overview also provides information regarding additional activities that may occur during the civil inspection process.

1. Timing of Inspections and Facility Notification

EPA inspectors should generally conduct inspections during the facility's normal work hours. However, there may be circumstances which require EPA inspectors to access, monitor, or observe specific operations or activities at other times. Where possible, for announced

EXHIBIT 1G

02570 U.S. PTO
071211

PTO/SB/16 (12-08)
Approved for use through 06/30/2010. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

U.S. PTO
61/572205
07/12/2011

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EM 615 168 247 US; Mailed July 12, 2011

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Patrick	**Brown**	**Stanford, CA**

TITLE OF THE INVENTION (500 characters max):

METHODS AND COMPOSITIONS FOR CONSUMABLES

Direct all correspondence to: CORRESPONDENCE ADDRESS

☒ The address corresponding to Customer Number: 021971

OR

☐ Firm or Individual Name

Address

City	State	Zip
Country	Telephone	Email

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Application Data Sheet. See 37 CFR 1.76

☐ CD(s), Number of CDs _____

☒ Drawing(s) *Number of Sheets* 1 Gray Scale; 3 Color

☒ Other (specify) PETITION UNDER 37 CFR 1.183 FOR WAIVER OF REQUIREMENTS UNDER 37 CFR 1.84 AND PROPOSED AMENDMENT

☒ Specification (e.g. description of the invention) *Number of Pages* 10

Fees Due: Filing Fee of $220 ($110 for small entity). If the specification and drawings exceed 100 sheets of paper, an application size fee is also due, which is $270 ($135 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

METHOD OF PAYMENT OF THE FILING FEE AND APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fee and application size fee (if applicable).

☐ Payment by credit card. Form PTO-2038 is attached

220.00

TOTAL FEE AMOUNT ($)

☒ The Director is hereby authorized to charge the filing fee and application size fee (if applicable) or credit any overpayment to Deposit Account Number: 23-2415

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No

☐ Yes. the name of the U.S. Government agency and the Government contract number are:

SIGNATURE Michael A. Willis Date July 12, 2011

TYPED or PRINTED NAME Michael A. Willis REGISTRATION NO. 53,913 *(if appropriate)*

TELEPHONE 650.493.9300 Docket Number: 41751-701.101

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	41751-701.101
	Application Number	
Title of Invention	METHODS AND COMPOSITIONS FOR CONSUMABLES	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
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Applicant Information:

Applicant 1

Applicant Authority ◉ Inventor ○ Legal Representative under 35 U.S.C. 117 ○ Party of Interest under 35 U.S.C. 118

Prefix	Given Name	Middle Name	Family Name	Suffix
	Patrick		Brown	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Stanford	State/Province	CA	Country of Residence	US

Citizenship under 37 CFR 1.41(b) US

Mailing Address of Applicant:

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Address 2	

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Postal Code	94305	Country	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

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Application Information:

Title of the Invention	METHODS AND COMPOSITIONS FOR CONSUMABLES		
Attorney Docket Number	41751-701.101	Small Entity Status Claimed	☐
Application Type	Provisional		
Subject Matter	Utility		
Suggested Class (if any)		Sub Class (if any)	
Suggested Technology Center (if any)			
Total Number of Drawing Sheets (if any)	4	Suggested Figure for Publication (if any)	

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Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

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Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)

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Application Number	Country[i]	Parent Filing Date (YYYY-MM-DD)	Priority Claimed
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Assignee 1

If the Assignee is an Organization check here. ☐

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	41751-701.101
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Title of Invention	METHODS AND COMPOSITIONS FOR CONSUMABLES	

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Signature:

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Signature				Date (YYYY-MM-DD)	2011-07-12
First Name	Michael	Last Name	Willis	Registration Number	53913

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PATENT APPLICATION

METHODS AND COMPOSITIONS FOR CONSUMABLES

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Entity: Large business concern

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METHODS AND COMPOSITIONS FOR CONSUMABLES

BACKGROUND OF THE INVENTION

[0001] Animal farming had a profound negative environmental impact. Currently it is estimated that 30% of Earth's land surface is dedicated to animal farming and that livestock account for 20% of total terrestrial animal biomass. Due to this massive scale animal farming accounts for more than 18% of net greenhouse gas emissions. Animal farming may be the largest human source of water pollution, and animal farming is by far the world's largest threat to biodiversity. If the worlds human population could shift from a meat containing diet to a vegetarian diet 26% of Earth's land surface would be freed for other uses. Furthermore the shift to a vegetarian diet would massively reduce water and energy consumption.

[0002] The consumption of meat has a profound negative impact on human health. The heath benefits of a vegetarian diet are well established. If the human population would shift to a vegetarian diet the cost savings in health care would be staggering.

[0003] Hunger is a worldwide problem, yet the world's 4 major commodity crops (soybeans, maize, wheat, and rice) already supply more than 100% of the human population's requirements for calories and protein, including every essential amino acid.

[0004] Plant based meat substitutes have largely failed to cause a shift to a vegetarian diet. Usually meat substitutes involve extrusion of soy/grain mixture. Texture too homogenous. Sold pre-cooked - taste/aroma built in (not a product of cooking). These meat substitutes fail to replicate the experience of eating meat.

[0005] Thus, it is clear that there is a great need in the art for an improved method and system for reducing animal farming while avoiding the shortcomings and drawbacks of the previous meat substitutes.

SUMMARY OF THE INVENTION

INCORPORATION BY REFERENCE

[0006] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0008] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0009] **Figure 1** depicts a sliced open pea root nodule.

[0010] **Figure 2** depicts leghemoglobin isolated from 1 oz of pea roots. The red color commonly attributed to meat is evident in the color photo.

DETAILED DESCRIPTION OF THE INVENTION

[0010] Methods and compositions for the production of consumables are described herein. The consumables can be for human or animal consumption. For example the consumable can be food fit for human consumption. The consumable can be approved by suitable regulatory authorities. The consumables can be sold in grocery stores or prepared in restaurants, similar to already existing human foods. The consumables could also be food for domestic animals. For instance, dog food could be produced according to the present inventions. The consumables may also be food for wild animals. For instance, the consumables could be provided to non-domesticated predatory animals.

[0011] The consumables of the present invention compete with, supplement or replace animal based foods. For instance the consumables can be meat replicas made entirely from plant sources. The consumables can be made to mimic the cut or appearance of meat as it is currently sold. For instance a consumable may be visually indistinguishable from ground beef or a particular cut of beef. Alternatively, the consumables can be made with a unique look or appearance. For instance the consumable could contain patterns or lettering that is based upon the structure of the consumable. In some instances the consumables look like traditional meat products after they are prepared. For example a consumable may be produced which is larger than a traditional cut of beef but which, after the consumable is sliced and cooked the product appears the same as a traditional cooked meet.

[0012] The consumable may be made entirely from plant based sources. In some instances the consumable can be made from organic sources. The consumables may also be made from a combination of plant based sources and animal based sources. For instance, the consumable may be a ground beef product supplemented with plant based products of the invention.

[0013] The consumables can be made from local products. For instance the consumables can be made from plants grown within a certain radius of the eventual consumer. That radius could be 1, 10, 100, or 1000 miles for example. So, in some embodiments, the invention provides a method for producing a meat replica which does not contain products which have been shipped over 1, 10, 100, or 1000 miles prior to producing the meat replica.

[0014] The present invention provides methods for producing consistent properties from the consumables when they are produced from various sources. So, for example, a plant based meat replica produced from local plants in Iowa, USA, will have substantially similar taste, odor, and texture as a plant based meat replica produced from local plants in Lorraine, France. This consistency allows for methods for advertising locally grown foods with consistent properties.

[0015] The consumables require fewer resources to produce than animal farming. Accordingly, the present invention provides for meat replicates which require less water or energy to produce than meat. For example a consumable can require 10, 50, 100, 200, 300, 500, or 1000 gallons of water per pound of consumable. For comparison beef can require over 2000 gallons of water per pound of meat.

[0016] The consumable can also be shipped in components and produced or assembled at a different location. When available local components can be used for production of the consumable. These can be supplemented with components which are not locally available. This allows for methods of producing consumables, for instance meat replicates, using less energy in shipment than is required for meat. For example, local water can be used in combination with a kit which provides other components of the consumable. Using local water will reduce shipping weight thereby reducing cost and environmental impact.

[0017] The consumables can be produced or assembled wholly or in part in areas where animal farming is not practical or is not allowed. The consumable can be produced or assembled within an urban environment. For example a kit may be provided to a user to enable the user to produce the consumable. The user could use local water or use plants from a rooftop garden, for instance in Shanghai. In another example, the consumables could be produced aboard a space craft, space station, or lunar base. Accordingly, the present invention provides methods and systems for the production of meat replicas for use in space travel or for training for the same. For instance the present invention could be used in earth based training for space travel. The consumables could also be produced on an island or upon a manmade platform at sea where the keeping of livestock is difficult or prohibited.

[0018] The consumables are, in some embodiments, designed to replicate the experience of eating meat. The look, texture, and taste of the consumable can be such that it is similar or indistinguishable from meat. The invention therefore provides in certain embodiments methods for determining whether a animal or human can distinguish the consumable from meat.

[0019] One method to determine whether the consumable is comparable to meat is to a) define the properties of meat and b) determine whether the consumable has similar properties. Properties of meat that can be tested include mechanical properties such as hardness, cohesiveness, brittleness, chewiness, gumminess, viscosity, elasticity, and adhesiveness. Properties of meat that can be tested also include geometric properties such as particle size and shape, and particle shape and orientation. Additional properties can include moisture content and fat content. These properties can be described using terms such as "soft," "firm" or "hard" describe hardness; "crumbly," "crunchy," "brittle," "chewy," "tender," "tough," "short," "mealy," "pasty," or "gummy," to describe cohesiveness; "thin" or "viscous" to describe viscosity; "plastic" or "elastic" to describe elasticity; "sticky," "tacky" or "gooey" to describe adhesiveness; "gritty," "grainy" or "course" to describe particle shape and size; "fibrous," "cellular" or "crystalline" to describe particle shape and orientation, "dry," "moist," "wet," or "watery" to describe moisture content; or "oily" or "greasy" to describe fat content. So, in one embodiment a group of people can be asked to rate a certain meat, for instance ground beef, according to properties which describe the meat. These ratings can be used as an indication of the properties of the meat. The consumables of the present invention can then be compared to the meat to determine how similar the consumable is to the meat. In some instances the properties of the consumables are then altered to make the consumable more similar to the meat. So, in some embodiments, the consumable is rated similar to meat according to human evaluation. In some embodiments the consumable is indistinguishable from real meat to a human.

[0020] In some embodiments the consumable is compared to meat using fMRI. For example, the experience of eating meat is measured using fMRI. A map of regions of the brain associated with eating meat is created. The brain map can be refined by giving the person other types of foods and determining meat fMRI patterns. Additionally the person can be given pleasurable and distasteful foods to further refine the map. fMRI literature can be used to further refine the map. Once the experience of eating meat is defined statistically, meat replicas can be tested to determine how similar the meat replica is to meat. This process can be used to test various versions of the consumables to best approximate the experience of eating meat. In some embodiments the consumable is a meat replica that, when consumed, causes an fMRI pattern similar to the pattern found when the person consumes meat.

[0021] In some embodiments the consumable is compared to real meat based upon olfactometer readings. In various embodiments the olfactometer can be used to assess odor concentration and odor thresholds, odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of appreciation, or relative intensity of odors. In

some embodiments the olfactometer allows the training and automatic evaluation of expert panels. So in some embodiments the consumable is a product that causes similar olfactometer readings.

[0022] Gas chromatography–mass spectrometry (GCMS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to separate and identify different substances within a test sample. GCMS can, in some embodiments, be used to evaluate the properties of a consumable. For example volatile chemicals can be isolated from the head space around meat. These chemicals can be identified using GCMS. A profile of the volatile chemicals in the headspace around meat is thereby created. In some instances each peak of the GCMS can be further evaluated. For instance, a human could rate the experience of smelling the chemical responsible for a certain peak. This information could be used to further refine the profile. GCMS could then be used to evaluate the properties of the consumable. The GCMS could be used to refine the consumable. In some embodiments the consumable has a GCMS profile similar or identical to that of meat.

[0023] Characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions molecules including amino acids, fats and sugars which are found in plants as well as meat. Therefore in some embodiments the consumable is tested for similarity to meat during or after cooking. In some embodiments human ratings, fMRI, olfactometer readings, or GCMS measurements, or combinations thereof, are used to create an olfactory map of cooked meat. Similarly, an olfactory map of the consumable, for instance a meat replica, can be created. These maps can be compared to assess how similar the cooked consumable it so meat. In some embodiments the olfactory map of the consumable during or after cooking is similar to or indistinguishable from cooked or cooking meat.

[0024] The consumable is, in some embodiments, a meat replica principally or entirely composed of ingredients derived from non-animal sources, including a muscle tissue replica, an adipose tissue replica and and/or a connective tissue replica, where these elements are combined in a manner that approximates the physical organization of meat. The meat can be white meat or dark meat. The meat can be from an animal, for example from farmed animals such as cattle, sheep, pigs, chickens, or turkey. Meat is typically from skeletal muscle of an animal but it is understood that meat can also come from other muscles or organs of the animal. For instance, in some embodiments the consumable is a meat replica that simulates a kidney. Meat can also come from fish or other seafood. For example, in some embodiments, the consumable is a meat replica that simulates crab, lobster, oysters, fish, or scallops.

[0025] In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, including a muscle tissue replica, an adipose tissue replica and and/or a connective tissue replica, where these elements are combined in a manner that approximates the physical organization of natural ground meat. Alternatively, the consumable can be a meat product which is supplemented with non-animal sources. For example, in some embodiments ground beef can be supplemented with ingredients derived from non-animal sources, for instance a muscle tissue replica, an adipose tissue replica, and and/or a connective tissue replica. This supplementing can result in a product with properties similar to ground beef but which costs less and has a smaller environmental impact. In another example, in some embodiments meat from game animals such as venison or elk is supplemented with ingredients derived from non-animal sources to improve upon the properties of the meat. For example an adipose tissue replica can be added to improve the qualities of the cooked game.

[0026] The physical organization of the consumable may be manipulated by controlling the localization, organization, assembly, or orientation of different components of the consumable. In some embodiments the consumable is designed so the components are associated with one another as in meat. In some embodiments the consumable is designed so that after

cooking the components of the consumable are associated with one another as in cooked meat. In some embodiments a soft, elastic gel made from plant or microbial proteins and/or carbohydrates containing droplets of fat suspended in the proteinaceous gel is used to approximate the organization adipose tissue in meat. In some embodiments some or all of the components of the consumable are suspended in a gel. In various embodiments the gel can be a hydrogel, an organogel, or a a xerogel, The gel can be made thick using an agent based on polysaccharides or proteins. For example fecula, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, alginin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, or proteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, of fungi can be used alone or in combination to form an architecture or structure for the consumable. Enzymes that catalyze reactions leading to covalent crosslinks between proteins can also be used alone or in combination to form an architecture or structure for the consumable. For example transclutaminase, lysyl oxidases, or other amine oxidases (e.g. Pichia pastoris lysyl oxidase (PPLO)) can be used alone or in combination to form an architecture or structure for the consumable. In some embodiments multiple gels with different components are combined to form the consumable. For example a gel containing a plant-based protein can be associated with a gel containing a plant-based fat. In some embodiments fibers or stings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

[0027] The percentage of different components may also be controlled. For example plant-based substitutes for muscle, fat tissue, connective tissue, and blood components can be combined in different ratios and physical organizations to best approximate the look and feel of meat. The various can also components can be arranged to insure consistency between bites of the consumable. The components can be arranged to insure that no waste is generated from the consumable. For example, while a traditional cut of meat may have portions that are not typically eaten, a meat replicate can improve upon meat by not including these inedible portions. Such an improvement allows for all of the product made or shipped to be consumed, which cuts down on waste and shipping costs. Alternatively, a meat replica may include inedible portions to mimic the experience of meat consumption. Such portions can include bone, cartilage, connective tissue, or other materials commonly referred to as gristle, or materials included simulating these components. In some embodiments the consumable may contain simulated inedible portions of meat products which are designed to serve secondary functions. For example a simulated bone can be designed to disperse heat during cooking, making the cooking of the consumable faster or more uniform than meat. In other embodiments a simulated bone may also serve to keep the consumable at a constant temperature during shipping.

[0028] In some embodiments the consumable contains components to replicate the components of meat. The main component of meat is typically skeletal muscle. Skeletal muscle typically consists of roughly 75 percent water, 19 percent protein, 2.5 percent intramuscular fat, 1.2 percent carbohydrates and 2.3 percent other soluble non-protein substances. These include nitrogenous compounds, such as amino acids, and inorganic substances such as minerals. Accordingly, some embodiments of the present invention provide for replicating approximations of this composition for the consumable. F or example, in some embodiments the consumable is a plant-based meat replica can comprise roughly 75% water, 19% protein, 2.5% fat, 1.2% carbohydrates; and 2.3 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprises between 60-90% water, 10-30% protein, 1-20% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. Some meat also contains myoglobin which accounts for most of the red color and iron content of some meat. It is understood that these percentages can vary in meat and the meat replicas can be produced to approximate the natural variation in meat. Additionally, in some instances, the present invention provides for

improved meat replicas, which comprise these components in typically unnatural percentages. For example a meat replica can be produced with a higher than typical average fat content. The percentages of these components may also be altered to increase other desirable properties.

[0029] In some instances a meat replica is designed so that, when cooked, the percentages of components are similar to cooked meat. So, in some embodiments, the uncooked consumable has different percentages of components than uncooked meat, but when cooked the consumable is similar to cooked meat. For example, a meat replica may be made with a higher than typical water content for raw meat, but when cooked in a microwave the resulting product has percentages of components similar to meat cooked over a fire.

[0030] In some embodiments the consumable is a meat replica with a lower that typical water content for meat. In some embodiments the inventions provides for methods for hydrating a meat replica to cause the meat replica to have a content similar to meat. For example a meat replica with a water content that would be low for meat, for example 1%, 10%, 20%, 30%, 40% or 50% water, is hydrated to roughly 75% water. Once hydrated, in some embodiments, the meat replica is then cooked for human consumption.

[0031] The consumable can have a protein component. In some embodiments the protein content of the consumable is 10%, 20%, 30%, or 40%. In some embodiments the protein content of the consumable is similar to meat. In some embodiments the protein content in the consumable is greater than that of meat. In some embodiments the consumable has less protein than meat.

[0032] The protein in the consumable can come from a variety or combination of sources. Non-animal sources can provide some or all of the protein in the consumable. Non-animal sources can include vegetables, fruits, nuts, grains, algae, bacteria, or fungi. The protein can be isolated or concentrated from one or more of these sources. In some embodiments the consumable is a meat replica comprising protein only obtained from non-animal sources.

[0033] In some embodiments protein is formed into asymmetric fibers for incorporation into the consumable. In some embodiments these fibers replicate muscle fibers. In some embodiments the protein are spun fibers. Accordingly, the present invention provides for methods for producing asymmetric or spun protein fibers.

[0034] In some embodiments the consumable contains a protein or proteins that have all of the essential amino acids. In some embodiments the proteins added to the consumable are supplemented with amino acids.

[0035] Fat is important for the experience of eating cooked meat. Accordingly, in some embodiments the consumable has a fat component. In some embodiments the fat content of the consumable is 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, or 60% fat. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a replica of animal fat consisting of a soft, elastic gel made from plant or microbial proteins (and/or carbohydrates), containing droplets of fat suspended in the proteinaceous gel.

[0036] Fat used is some embodiments of the present invention can be from a variety of sources. For example fats used in the consumable may be derived from coconuts, palm oil, palm kernel oil, cocoa, sunflowers, corn, rapeseed, soy beans, peanuts, tree nuts, algae, olives, cotton, or flax. In some embodiment the fat is derived from pulp or seed oil. Yeast or molds may also be sources of fat for certain embodiments of the invention. For instance, in one embodiments the consumable is a meat replica comprising triglycerides derived from *Mortierella isabellina*.

[0037] In some embodiments plant oils are modified to resemble animal fats. The plant oils can be modified to taste and smell most similar to meat when cooking. Accordingly, some aspects of the invention involve methods for testing the qualitative similarity between the cooking properties of animal fat and the cooking properties of plant oils in the consumable.

[0038] In some embodiments.a variety of plant lipids are combined to approximate the properties of animal fat. In some embodiments the lipids that are not used in making the consumable are used for other applications, for example applications in cosmetics, lubricants, waxes, polymers, surfactants, water repellents, or biofuels. Accordingly the present invention provides for a methods for saving and recycling plant lipids which are byproducts of the production of the consumable.

[0039] The fat can be suspended in a gel. In some embodiments the present invention provides for methods for producing droplets of fat suspended in the proteinaceous gel. The fat can isolated and homogenized. For example an organic solvent mixture can be used to help mix a lipid. The solvent can then be removed. At this point the lipid can be frozen, lyophilized, or stored. So in some aspects the invention provides for a method for isolating and storing a lipid which has been selected to have characteristics similar to animal fat. The lipid film or cake can then be hydrated. The hydration can utilize agitation or temperature changes. The hydration can occur in a precursor solution to a gel. After hydration the lipid suspension can be sonicated or extruded to further alter the properties of the lipid in the solution. At this point, in some embodiments other components of the consumable are added to the solution followed by a gelling agent. In some embodiments crosslinking agents (e.g. transglutaminase or lysyl oxidase) are added to bind the components of the consumable. In other embodiments the gelling agent is added and the lipid/gel suspension is later combined with additional components of the consumable.

[0040] Odorants released during cooking are generated by reactions between fats and protein & carbohydrates. In some embodiments the odorants that combine during the cooking of meat are identified and located near one another in the consumable, such that upon cooking of the consumable the odorants combine. So, in some embodiments, the characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions of amino acids, fats and sugars found in plants as well as meat.

[0041] The color of meat is an important part the experience of eating meat. The main determinant of the nutritional definition of the color of meat is the concentration of myoglobin. It is estimated that the white meat of chicken has under 0.05%; pork and veal have 0.1-0.3%; young beef has 0.4-1.0%; and old beef has 1.5-2.0%. So, in some embodiments, the consumable is a meat replica which comprises myoglobin. In some embodiments the consumable is a plant based meat replica that has animal myoglobin added. So, for example a replica of young beef can have about 0.4-1% myoglobin.

[0042] Hemoglobin is the iron-containing oxygen-binding protein in the red blood cells of vertebrates. Hemoglobin is similar in color to myoglobin. In some embodiments the invention provides a method of saving and recycling blood from animal farming to supplement the color of a consumable. For example blood is saved from a slaughter house, hemoglobin from the blood is used to enhance the color of a consumable. In some aspects the consumable is a plant-based meat replica containing hemoglobin.

[0043] Additional iron containing proteins exist in nature. In some embodiments the consumable comprises an iron containing protein that is not myoglobin. In some embodiments the consumable does not contain myoglobin. In some embodiments the consumable does not contain hemoglobin. In some embodiments the consumable is a meat replica that comprises an iron containing protein other than myoglobin or hemoglobin.

[0044] Examples of iron containing proteins include hemerythrin and leghemoglobin. In various embodiments these iron containing proteins are added to the consumable to alter the visual characteristics or iron content of the consumable. In some

embodiments oxygen binding proteins (e.g. hemocyanin, hemerythrin, chlorocruorin, vanabins, erythrocruorin, pinnaglobin, and leghemoglobin) are added to the consumable. In some embodiments the consumable comprises a hemoprotein (e.g. hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin).

[0045] Leghemoglobin, similar in structure and physical properties to myoglobin, is readily available as an unused by-product of commodity legume crops (eg., soybean, pea). The leghemoglobin in the roots of these crops in the US exceeds the myoglobin content of all the red meat consumed in the US. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, including a muscle tissue replica, an adipose tissue replica, a connective tissue replica, and leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a heme protein. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a member of the globin protein family. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a high iron content from a heme protein. In some embodiments the iron content is similar to meat. In some embodiments the consumable has the distinctive red color of meat, such color provided by leghemoglobin.

[0046] Leghemoglobin is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.

[0047] In some embodiments leghemoglobin is added to meat to enhance the properties of meat. For example, a leghemglobin containing solution can be injected into raw or cooked meat. In another example a leghemoglobin solution is dripped over meat or a consumable of the invention to enhance appearance. In one embodiment advertising, photography, or videography of food products such as meat or a meat substitute is enhanced with leghemoglobin.

[0048] In some embodiments the present invention provides methods for obtaining leghemoglobin from plants. Leghemoglobin can be obtained from a variety of plants. For example leghemoglobin can be isolated from the roots nodules of legumes such as soy beans. The root nodule is obtained and homogenized in an aqueous solution , soluble protein includeing leghemoglobin are removed after insoluble matter is removed by precipitation or filtration. Leghemoglobin can be purified by selective precipitation and/or chromatography and/or the use of molecules with specific affinity for leghemoglobin .

[0049] Heme proteins, for example leghemoglobin, can be combined with other plant based meat replica components. In some embodiments the heme proteins are captured in a gel which contains other components, for example lipids and or proteins. In some aspects a multiple gels are combined with non-gel based heme proteins. In some embodiments the combination of the heme proteins and the other compounds of the consumable are done to insure that the heme proteins are able to diffuse through the consumable. In some embodiments the consumable is soaked in a heme containing solution, for instance a leghemoglobin solution, until the consumable is saturated. In some embodiments the consumable is soaked in a heme protein containing solution, for instance a leghemoglobin solution for 1, 5, 10, 15, 20 or 30 hours. In some embodiments the consumable is soaked in a heme containing solution, for instance a leghemoglobin solution for 1, 5, 10, 15,

30, or 45 minutes. In some embodiments the consumable is injected with a heme containing solution, for instance a leghemoglobin solution, until the consumable is the color of uncooked meat.

[0050] A meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a heme protein, a muscle tissue replica, an adipose tissue replica and and/or a connective tissue replica, wherein the heme protein is within the muscle tissue replica.

[0051] The release of odorants upon cooking is an important aspect of meat consumption. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with an odorant that is released upon cooking. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing mixtures of proteins, peptides, amino acids, nucleotides, sugars and polysaccharides and fats in combinations and spatial arrangements that enable these compounds to undergo chemical reactions during cooking to produce odorants and flavor-producing compounds. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a volatile or labile odorant that is released upon cooking. In some embodiments the consumable is a method for preparing a meat replica where meat replicas principally or entirely composed of ingredients derived from non-animal sources are heated to release a volatile or labile odorant.

[0052] Given the usefulness of heme proteins for coloring consumables it will be useful to detect whether a product contains a particular heme protein. Accordingly the present invention includes in some embodiments methods to determine whether a product contains a heme protein. Methods for detecting proteins are well known in the art. For example an ELISA or proximity-ligation assoacy or luninex assay or western blot analysis can be performed to determine whether leghemoglobin is present in a food product such as meat or a meat replica. In one embodiment the detection methods are performed to determine whether meat has been altered with leghemoglobin.

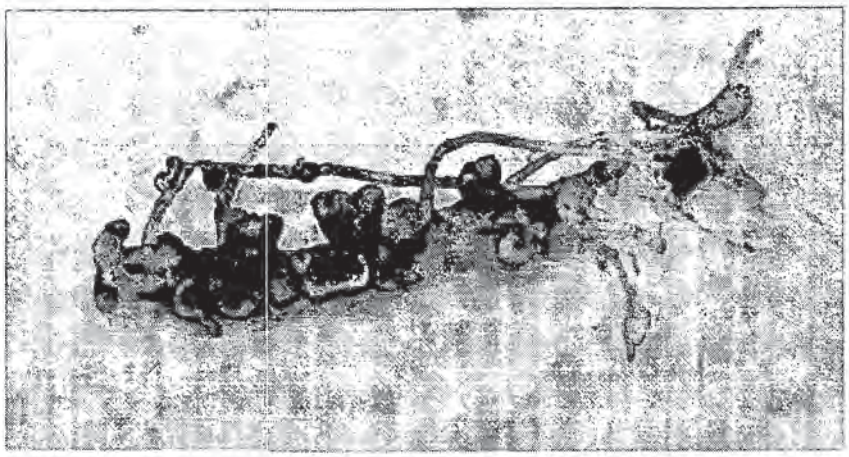


Figure 1.

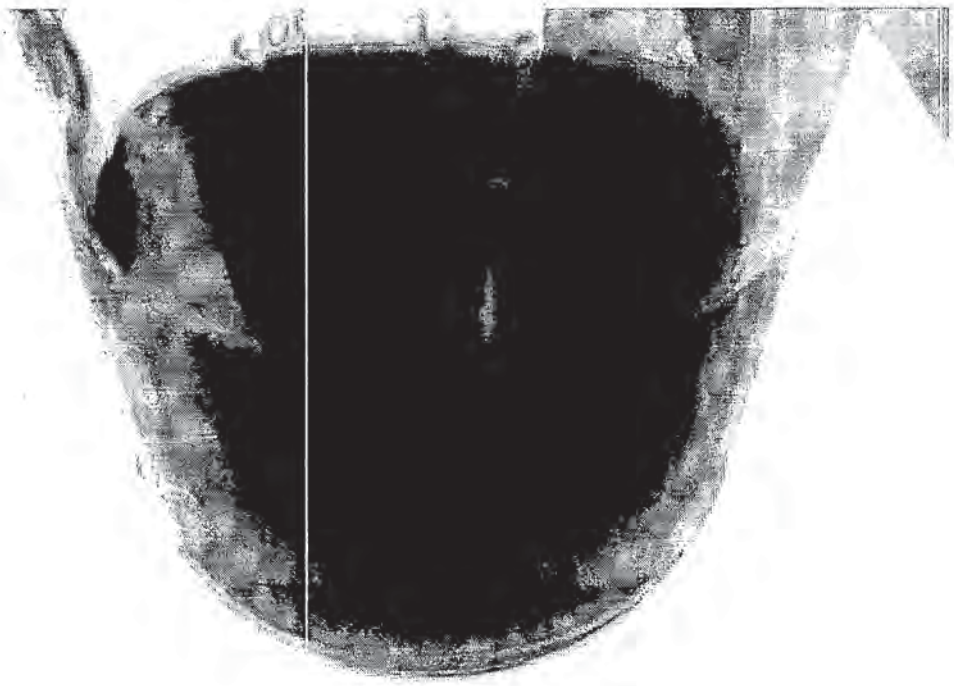


Figure 2.

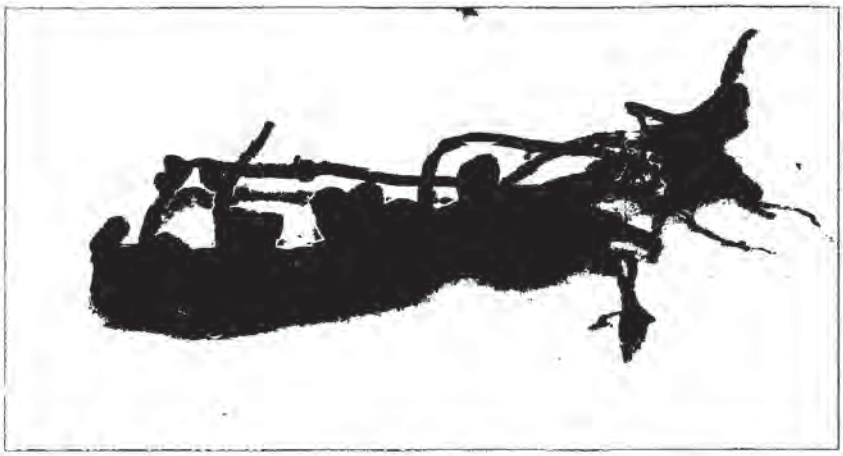


Figure 1.

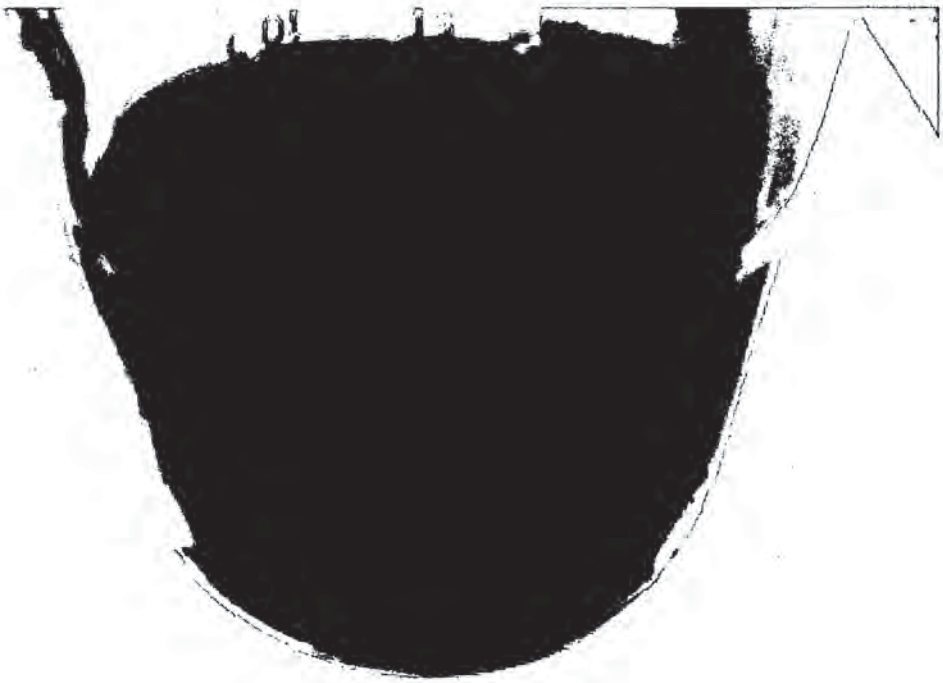


Figure 2.

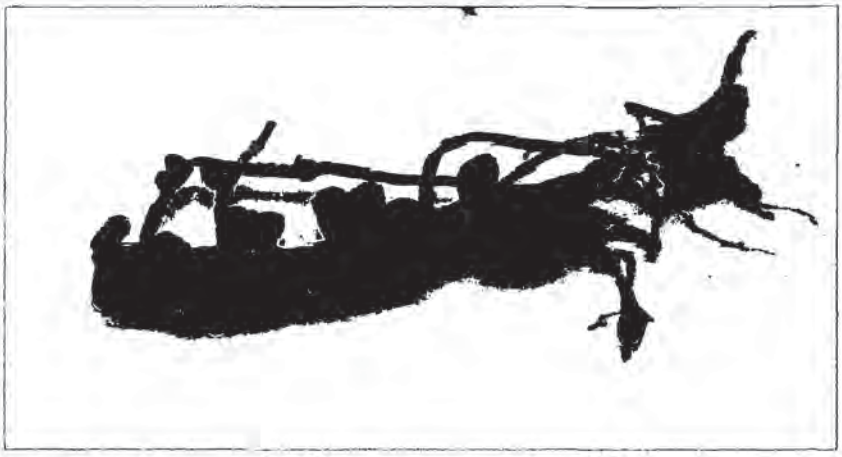


Figure 1.

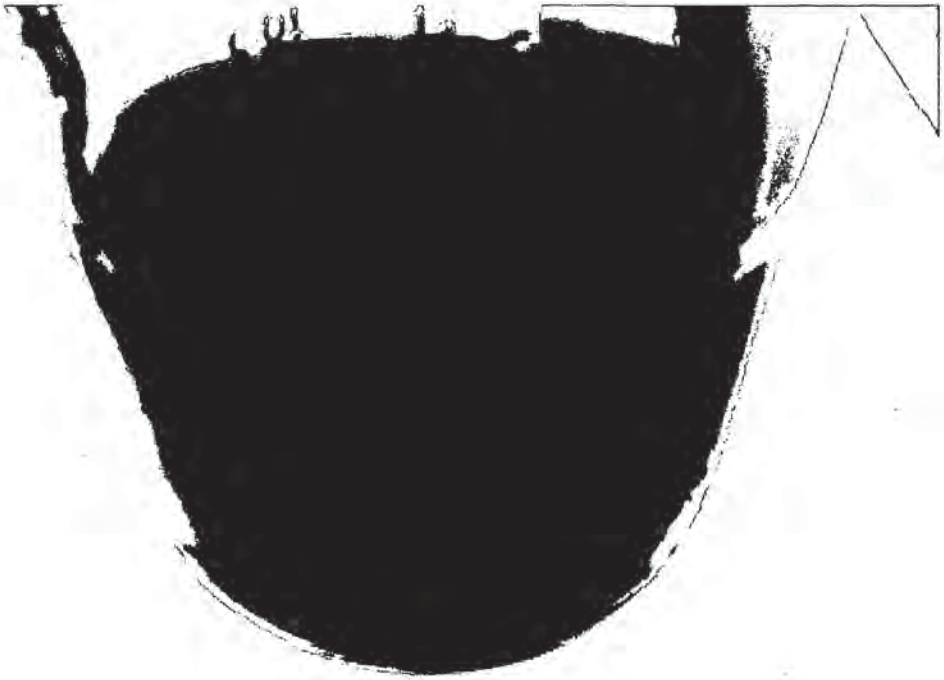


Figure 2.

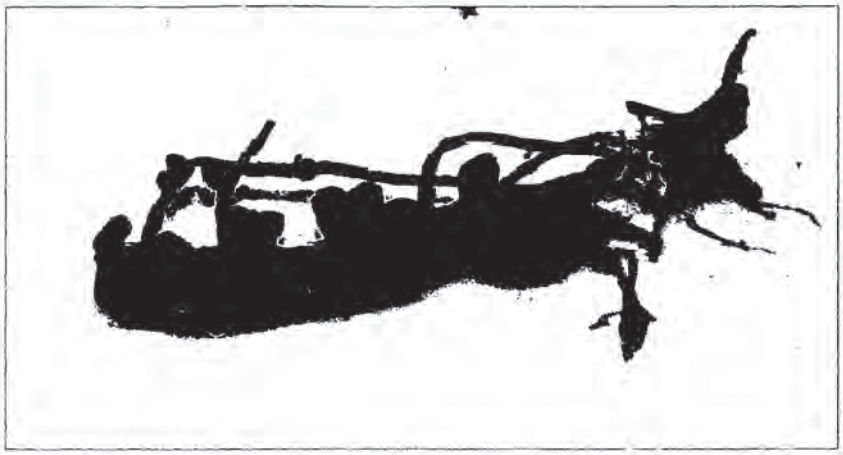


Figure 1.



Figure 2.

EXHIBIT 1H

PATENT APPLICATION SERIAL NO._____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

07/14/2011 MBELETE1 00000040 232415 61572205
01 FC:1005 220.00 DA

PTO-1556
(5/87)

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PETITION UNDER 37 CFR 1.183 FOR WAIVER OF REQUIREMENTS UNDER 37 CFR 1.84 AND PROPOSED AMENDMENT	Docket Number (Optional) 41751-701.101

First named inventor: Patrick BROWN

Application No.: Not yet assigned Group Art Unit: NA

Filed: Herewith Examiner: NA

Title: METHODS AND COMPOSITIONS FOR CONSUMABLES

Commissioner for Patents
P.O. Box 1450, Alexandria
VA 22313-1450

APPLICANT HEREBY PETITIONS UNDER 37 CFR 1.183 FOR WAIVER OF THE REQUIREMENTS OF 37 CFR 1.84 AND SUBMITS A PROPOSED AMENDMENT TO THE SPECIFICATION PURSUANT TO MPEP 608.02 (COLOR DRAWINGS)

1. Petition fee
   - ☐ is enclosed herewith – fee $
   - ☒ charge to Deposit Account 23-2415 (Docket No. 41751-701.101) – fee $ 130.00

2. Petition for Extension of time and fee
   - ☐ Applicant petitions the Commissioner for an extension of time of month
     - ☐ small entity – fee $ (37 CFR 1.17(m))
     - ☐ other than small entity – fee $ (37 CFR 1.17(m))

3. Statement.
   - ☒ Three (3) sets of color drawings are enclosed herewith.
   - ☐ Color drawings were submitted in informal form with the original application.

It is asserted that the color drawings are necessary because: Gray scale drawings do not enable differentiation of structures due to the reduced range of scale available to the eye.

July 12, 2011
Date

Michael A. Willis, Reg. No. 53,913

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07/14/2011 MBELETE1 00000040 232415 61572205
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571-272-7822

Paper 15
Entered: May 22, 2023

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

MOTIF FOODWORKS, INC.,
Petitioner,

v.

IMPOSSIBLE FOODS, INC.,
Patent Owner.

IPR2023-00206 (Patent 9,943,096 B2)
IPR2023-00207 (Patent 11,224,241 B2)
IPR2023-00208 (Patent 11,013,250 B2)
IPR2023-00209 (Patent 10,039,306 B2)[1]

Before DONNA M. PRAISS, CHRISTOPHER M. KAISER, and JEFFREY W. ABRAHAM, *Administrative Patent Judges*.

PRAISS, *Administrative Patent Judge*.

ORDER
Granting Patent Owner's Motion for Leave to
Seek a Certificate of Correction
37 C.F.R. § 1.323, 42.20

[1] This Order addresses issues that are the same in all identified cases. We exercise our discretion to issue one Order to be filed in each case. The parties are not authorized to use this style heading in subsequent papers.

Pursuant to our authorization (Ex. 2014), Patent Owner filed a Motion Pursuant to 37 C.F.R. § 1.323 for leave to request a certificate of correction for the challenged patent in each of the above-identified proceedings. Paper 9 ("Motion" or "Mot.," collectively "Motions").[2] Petitioner filed an Opposition. Paper 11 ("Opp.," collectively "Oppositions"). For the reasons that follow, Patent Owner's Motions are granted.

Background

U.S. Patent No. 9,943,096 ("the '096 patent," Ex. 1001) claims priority to U.S. Application No. 13/941,211 filed on July 12, 2013 ("the '211 application"). Ex. 1161, code (21). Specifically, the '096 patent states:

> This application is a continuation of U.S. application Ser. No. 14/797,006 filed on Jul. 10, 2015 which is a continuation of and claims priority to PCT/US2014/011347 which claims priority to U.S. application Ser. No. 13/941,211, filed Jul. 12, 2013, U.S. Application Ser. No. 61/908,634, filed Nov. 25, 2013, and to U.S. Application Ser. No. 61/751,816, filed Jan. 11, 2013, and is related to the following patent applications: Application Serial No. PCT/US12/46560; Application Serial No PCT/US12/46552; Application Ser. No. 61/876,676, filed Sep. 11, 2013; and Application Ser. No. 61/751,818, filed Jan. 11, 2013, all of which are incorporated herein by reference.

Ex. 1001, 1:7–18;[3] *see id.* at codes (60), (63).

The '211 application, in turn, claims priority to U.S. Provisional Application 61/671,069 filed on July 12, 2012 (Ex. 2012 "the '069

[2] For expediency, we refer to the '096 patent and Paper numbers filed in IPR2023-00206. Similar Papers were filed in IPR2023-00207, IPR2023-00208, and IPR2023-00209, and, unless otherwise specified, our reasoning applies to all of the patents challenged in those proceedings.

[3] As corrected by a Certificate of Correction dated May 29, 2018. Ex. 1165.

provisional"), International Application PCT/US2012/046560 (Ex. 1012 "Brown"), and U.S. Provisional Application 61/572,205 filed on July 12, 2011 ("the '205 provisional"). Ex. 1161, code (60), (63).

Patent Owner asserts "[p]riority to the '069 provisional, Brown, and the '205 provisional was unintentionally omitted from the priority chain of" the '096 patent and other patents in the family stemming from the '211 application. Mot. 2. Accordingly, Patent Owner seeks to file a certificate of correction adding language to the priority statement above to specify that the '211 application claims the benefit of the '069 application, Brown, and the '205 provisional. *See* Ex. 2001 (Patent Owner's Request for Certificate of Correction). Patent Owner contends that, even though 37 C.F.R. § 1.323 does not apply here because this *inter partes* review proceeding is in the pre-institution phase and, thus, not involved in a "trial" before the Board, the applicable standard has been met. *Id.* at 6 (quoting *Honeywell Int'l Inc. v. Arkema Inc.*, 939 F.3d 1345, 1349 (Fed. Cir. 2019); citing *Edwards Lifesciences Corp. v. Evalve, Inc.*, IPR2019-01285, Paper 19 at 7–8 (Jan. 9, 2020)).

Patent Owner asserts that "[g]ranting of a petition and request for a certificate of correction would disqualify Brown as prior art and thus provide a basis for rejecting these grounds [in IPR2023-00206 and IPR2023-00207]." Mot. 9 (citing *Edwards*, IPR2019-01285, Paper 19 at 5–8; *Parsons Xtreme Gold v. Taylor Made Golf Co.*, IPR2018-00657, Paper 14 at 11 (Aug. 14, 2018)). Accordingly, the correction sought could bear on whether art cited in the Petition (Ex. 1012 "Brown") is prior art to the '096 patent. Pet. 13–14; Mot. 9; Opp. 9.

Discussion

35 U.S.C. § 255 provides, in relevant part, that a certificate of correction may be issued by the Director "[w]henever a mistake of a clerical or typographical nature, or of minor character, which was not the fault of the Patent and Trademark Office, appears in a patent and a showing has been made that such mistake occurred in good faith." According to the Federal Circuit, "the Director—not the Board—will evaluate the merits of the patentee's petition, including whether the mistake is of 'minor character' or 'occurred in good faith.'" *Honeywell*, 939 F.3d at 1349 (citing 35 U.S.C. § 255). That statute gives this authority to the Director, and the Director has not delegated this authority to the Board. *Id.* As the parties acknowledge, the Board's role on Patent Owner's Motion under 37 C.F.R. § 1.323 is simply "to determine whether there is sufficient basis supporting Patent Owner's position that the mistake may be correctable." *Id.* (internal quotation marks and citation omitted); Mot. 6; Opp. 2.

Patent Owner relies on the '096 patent's previously claimed priority to the '211 application, and the '211 application's claimed priority to the '069 provisional, Brown, and the '205 provisional, to support its assertion that the omission of the '069 provisional, Brown, and the '205 provisional in the '096 patent's priority claim before now was "unintentional." Mot. 2. Patent Owner directs us to the petition and certificate of correction filed for child applications claiming priority through the same chain and asserts that it "seeks to submit identical requests for certificate of correction and petitions to accept the unintentionally delayed claims for priority in each of the challenged patents currently subject to an IPR." *Id.* at 2–3. Patent Owner's

Request for Certificate of Correction in one of the mentioned child applications states "it is clear from the record that the revised priority claims are appropriate." Ex. 2001, 3; Mot. 2. According to Patent Owner, the omission "initially occurred in the filing . . . and was inadvertently carried through later-filed applications." Mot. 6. Patent Owner asserts "applicant first became aware of the omissions upon review of these IPR petitions." *Id.*

Petitioner argues that Patent Owner's Motion does not provide a "sufficient basis . . . that the mistake may be correctable" because (1) the existing priority claim is defective, (2) Patent Owner's "mistake" was not made in "good faith," (3) the delay between now and the filing of the Petition was not adequately explained to support being "unintentional," and (4) the disclosure of the prior-filed application must provide adequate support for the claimed subject matter of the later-filed application in order to be entitled to the benefit of an earlier-filed application. Opp. 2–7. Petitioner also asserts that it will be prejudiced if we grant Patent Owner's Motion because "Petitioner could not have known PO would attempt to 'correct' priority for the Challenged Patents." *Id.* at 9.

Petitioner provides redline comparisons between the disclosure of the '211 application and the disclosure of PCT/US2014/011347 ("PCT '347) to show that PCT '347 is not a continuation of the '211 application because they have different specifications and figures. Opp. 2–3 (citing *Applied Material, Inc. v. Advanced Semiconductor Materials America, Inc.*, 98 F.3d 1563, 1579 (Fed. Cir. 1996)); Ex. 1160 (PCT '347); Ex. 1161 (publication of the '211 application); Ex. 1162 (redline comparison of Ex. 1160 and Ex. 1161). According to Petitioner, Patent Owner's Motion seeks to "exploit

the defect by further extending the priority claim back from the '211 application to earlier-filed applications." *Id.* at 4.

After reviewing the arguments in the Motion and the Opposition, we conclude that "there is a sufficient basis supporting Patent Owner's position that the mistake may be correctable." *Honeywell*, 939 F.3d at 1349.

Because the '211 application is already part of the '096 patent's priority claim, and because the '211 application itself claims the benefit of the '069 application, Brown, and the '205 provisional, Patent Owner's assertion that the '069 provisional, Brown, and the '205 provisional were unintentionally omitted from the priority chain has a substantial basis. Ex. 1161, codes (60), (63). Petitioner does not argue that the '211 application is not entitled to claim priority to the omitted applications.

Petitioner's arguments directed to whether the omission was "unintentional" and made "in good faith" go to the merits of Patent Owner's requested correction, which is in the purview of the Director and not the Board. Accordingly, we cede exclusive jurisdiction over the '096 patent and grant Patent Owner permission to file a petition for a certificate of correction to the Director.

Although Petitioner's comparison of the '211 application and PCT '347 demonstrates that the disclosures of the '211 application and PCT '347 are not the same and, therefore, PCT '347 is not a continuation of the '211 application, both PCT '347 and the '211 application already appear in the '096 patent's existing priority claim. Ex. 1001, 1:9–10, code (63). Furthermore, Petitioner's argument that the disclosure of the prior-filed applications must provide adequate support for the claimed subject matter of

the later-filed application in order to be entitled to the benefit of an earlier-filed application goes to the merits of the challenges in the Petition and is separate from the issue whether the '096 patent can claim priority to the omitted earlier-filed applications.

In that regard, Petitioner requests, in the event the Motion is granted, that it have the opportunity to brief the issue of whether the challenged claims are entitled to the benefit of the earlier-filed applications omitted from the '096 patent's priority claim. Opp. 10. We agree with Petitioner that it could not have known Patent Owner would seek to change the priority claims for each of the four challenged patents in these proceedings. Accordingly, we grant Petitioner's request for additional briefing on this issue. In the interest of time, we authorize Petitioner and Patent Owner to file, simultaneously, a brief addressing the question of whether the challenged claims in each proceeding are entitled to the benefit of the filing dates of the '069 provisional, Brown, and the '205 provisional. Each brief shall be not more than fifteen (15) pages in length as suggested by Petitioner. *Id.* The parties shall have the opportunity to file a responsive brief of not more than five (5) pages in length.

Second, Petitioner requests that we instruct Patent Owner to include Petitioner's Opposition and any Board order concerning the Motion with its petition to the Director. *Id.* In our view, Petitioner's Opposition, including its exhibits, may be useful to the Director in determining whether it is appropriate to issue a certificate of correction. Therefore, we order Patent Owner to include a copy of Petitioner's Opposition in addition to this Order with the petition for a certificate of correction submitted to the Director.

Order

In consideration of the foregoing, it is hereby

ORDERED that Patent Owner's Motions are *granted*;

FURTHER ORDERED that Patent Owner shall file with the Director a Petition for a Certificate of Correction within five (5) business days of the entry of this Order, shall serve a copy of the Petition on Petitioner, and shall file a copy of the Petition as an exhibit in this proceeding;

FURTHER ORDERED that Patent Owner shall include a copy of Petitioner's Opposition and accompanying exhibits along with this Order with the Petition submitted to the Director;

FURTHER ORDERED that when a decision is rendered on Patent Owner's Petition for a Certificate of Correction, Patent Owner shall file, within five (5) business days of such decision, a copy of the document as an exhibit to this proceeding;

FURTHER ORDERED that within seven (7) business days of the entry of this Order, Petitioner is authorized to file a brief of no more than fifteen (15) pages in length to address the effect, if any, on the present proceedings of Patent Owner's requested corrections to the challenged patents, particularly whether the challenged claims are entitled to claim the benefit of the filing date of the '069 application, Brown, and the '205 provisional, as well as what the earliest effective filing date properly should be;

FURTHER ORDERED that within seven (7) business days of the entry of this Order, Patent Owner is authorized to file a brief of no more than

fifteen (15) pages in length to address the effect, if any, on the present proceedings of Patent Owner's requested corrections to the challenged patents, particularly whether the challenged claims are entitled to claim the benefit of the filing date of the '069 application, Brown, and the '205 provisional, as well as what the earliest effective filing date properly should be; and

FURTHER ORDERED that the parties are authorized to file a responsive brief of no more than five (5) pages in length within seven (7) business days following the filing of the briefs concerning the effect of Patent Owner's requested corrections.

EXHIBIT 2
0712

IPR2023-00206 (Patent 9,943,096 B2)
IPR2023-00207 (Patent 11,224,241 B2)
IPR2023-00208 (Patent 11,013,250 B2)
IPR2023-00209 (Patent 10,039,306 B2)

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Electronic Acknowledgement Receipt

EFS ID:	48074801
Application Number:	14796970
International Application Number:	
Confirmation Number:	8501
Title of Invention:	METHODS AND COMPOSITIONS FOR CONSUMABLES
First Named Inventor/Applicant Name:	Marija Vrljic
Customer Number:	26191
Filer:	Heather L. Flanagan/Emily Palmer
Filer Authorized By:	Heather L. Flanagan
Attorney Docket Number:	38767-0010001
Receipt Date:	30-MAY-2023
Filing Date:	10-JUL-2015
Time Stamp:	17:02:33
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$ 2680
RAM confirmation Number	E20235TH03558968
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Petition for review by the Office of Petitions	Petition-ExpeditedReview.pdf	72502 c4195647ed29cc838e6d6c8dec76a89567238861	no	2
Warnings:					
Information:					
2	Request for Certificate of Correction	ReqCOC.pdf	145809 e9702976e511f588624f07678d0fbaa88fc5c761	no	5
Warnings:					
Information:					
3	Application Data Sheet	SuppADS.pdf	188825 a3b842ccd6290269f50dcae69b0840325b988719	no	2
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
4	Miscellaneous Incoming Letter	Exhibit1.pdf	188200 b3355bf729cb48b438e2d18e872429fe691e276c	no	16
Warnings:					
Information:					
5	Miscellaneous Incoming Letter	Exhibit1A.pdf	5368390 272fd6d6789e22fbd82ff72eccfe0d7e51678982	no	83
Warnings:					
Information:					
6	Miscellaneous Incoming Letter	Exhibit1B.pdf	1313311 49ccd2881a470d3cbda10146f1156ff2d57eb57a	no	38
Warnings:					

Information:					
7	Miscellaneous Incoming Letter	Exhibit1C.pdf	11992711 df1cf50bec953833003680a6c28119d82de5a6a7	no	131
Warnings:					
Information:					
8	Miscellaneous Incoming Letter	Exhibit1D.pdf	8808941 7b16facbba9e6e7825451e207f37325125c88c82	no	160
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9	Miscellaneous Incoming Letter	Exhibit1E.pdf	14615270 61f9d627b443b015ae0d0d45ae29b855d749847b	no	233
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10	Miscellaneous Incoming Letter	Exhibit1F.pdf	648732 07a59f46ad4b7efb055be305d047a6e08c5c079c	no	3
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Information:					
11	Miscellaneous Incoming Letter	Exhibit1G.pdf	4141765 b2fcd510cb509b575b803b462b982fe13edb2c19	no	3
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12	Miscellaneous Incoming Letter	Exhibit1H.pdf	18420022 39455806d62947b329551d9c46a07ba76ea2af4b	no	20
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Information:					
13	Miscellaneous Incoming Letter	Exhibit2.pdf	668938 5ab9185606ed4704b898b21faf481af01d4edbbb	no	10
Warnings:					
Information:					

14	Petition for review by the Office of Petitions	Petition-Delayed-Priority.pdf	107132 aaada534946488a012e09c652164e1a8c92e19ed	no	7
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Information:					
			Total Files Size (in bytes):	66723422	

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Electronic Patent Application Fee Transmittal

Application Number:	14796970
Filing Date:	10-Jul-2015
Title of Invention:	METHODS AND COMPOSITIONS FOR CONSUMABLES
First Named Inventor/Applicant Name:	Marija Vrljic
Filer:	Heather L. Flanagan/Emily Palmer
Attorney Docket Number:	38767-0010001

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
PETITION FEE- 37 CFR 1.17(F) (GROUP I)	1462	1	420	420
PET. DELAY SUB OR RESTORE PRIORITY-CLAIM	1454	1	2100	2100
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
CERTIFICATE OF CORRECTION	1811	1	160	160
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**2680**