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FISH & RICHARDSON

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July 10, 2015

Attorney Docket No.: 38767-0010001/M2CON-Track 1

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

Presented for filing is a new Continuation patent application for prioritized examination of:

Inventor(s): MARIJA VRLJIC, SERGEY SOLOMATIN, RACHEL FRASER, PATRICK O'REILLY BROWN M.D., PH.D., JESSICA KARR, CELESTE HOLZ-SCHIETINGER, MICHAEL EISEN AND RANJANI VARADAN

Title: METHODS AND COMPOSITIONS FOR CONSUMABLES

Enclosed are the following papers, including those required to receive a filing date under 37 C.F.R. § 1.53(b):

	Pages
Specification	139
Claims	4
Abstract	1
Drawing(s)	9

Enclosures:

— Application Data Sheet, 9 pages.
— Nucleotide and/or amino acid Sequence Listing (.txt format)
— Certification and Request for Prioritized Examination (Track I), 2 pages

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Basic Filing Fee			$280
Search Fee			$600
Examination Fee			$720
Publication fee			$0
Track I processing fee			$140
Track I prioritized examination fee			$4000
Total Claims (30)	over 20	10 x $80	$800
Independent Claims (4)	over 3	1 x $420	$420
Fee for Multiple Dependent claims			$0
Application size fee for each 50 pages over 100 Total Sheets: 153x .75 - 100/50 = 1x			$400
Surcharge – fee for Declaration			$140
Total Filing fee			$7500

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The filing fee is being paid concurrently herewith on the Electronic Filing System (EFS) by way of Deposit Account authorization. Please apply all charges or credits to Deposit Account No. 06-1050, referencing Attorney Docket No. 38767-0010001.

If this application is found to be incomplete, or if a telephone conference would otherwise be helpful, please call the undersigned at (612) 335-5070.

Please direct all correspondence to the following:

26191
PTO Customer Number

Respectfully submitted,

/M. Angela Parsons/

M. Angela Parsons, Ph.D.
Reg. No. 44,282

Enclosures
MAP/jaw

61186863.doc

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	METHODS AND COMPOSITIONS FOR CONSUMABLES
First Named Inventor/Applicant Name:	Marija Vrljic
Filer:	M. Angela Parsons/Judith Wasilkus
Attorney Docket Number:	38767-0010001

Filed as Large Entity

Filing Fees for Track I Prioritized Examination - Nonprovisional Application under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility application filing	1011	1	280	280
Utility Search Fee	1111	1	600	600
Utility Examination Fee	1311	1	720	720
Request for Prioritized Examination	1817	1	4000	4000
Pages:				
Utility Appl Size fee per 50 sheets >100	1081	1	400	400
Claims:				
Claims in Excess of 20	1202	10	80	800

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Independent claims in excess of 3	1201	1	420	420
Miscellaneous-Filing:				
Publ. Fee- Early, Voluntary, or Normal	1504	1	0	0
PROCESSING FEE, EXCEPT PROV. APPLS.	1830	1	140	140
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**7360**

Electronic Acknowledgement Receipt

EFS ID:	22889448
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:	M. Angela Parsons/Brenda Jurgens
Filer Authorized By:	M. Angela Parsons
Attorney Docket Number:	38767-0010001
Receipt Date:	10-JUL-2015
Filing Date:	
Time Stamp:	19:53:59
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$7360
RAM confirmation Number	5528
Deposit Account	061050
Authorized User	FISH & RICHARDSON P C
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	Application Data Sheet	ADS.pdf	1601932 999673bfd45357c02c9ffc401747b638f1cfcf9d	no	9
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
2	TrackOne Request	Request.pdf	154225 260ebdc056d2cc6f8aeaa58426fb6f10a0acb36b	no	2
Warnings:					
Information:					
3		Application.pdf	580899 9361daaa4ea85e40c477000d877745ccea3faf79	yes	143

Multipart Description/PDF files in .zip description

Document Description	Start	End
Specification	1	139
Claims	140	142
Abstract	143	143

Warnings:					
Information:					
4	Drawings-only black and white line drawings	Figures.PDF	11210115 fc86a8071290250713ce50c9c8b67f520e843ff8	no	9
Warnings:					
Information:					
5	Transmittal of New Application	Transmittal.pdf	82845 43f487e08b3323294d4ee214cf23d55a2bda02ed	no	3
Warnings:					
Information:					

6	Sequence Listing (Text File)	SEQ.TXT	47557 e80f58c31bf66765448787883a7215ef66437289	no	0
Warnings:					
Information:					
7	Fee Worksheet (SB06)	fee-info.pdf	45341 e80f58c31bf66765448787883a7215ef66437289	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	13722914	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

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.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	38767-0010001
	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.
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.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Marija		Vrljic	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	San Mateo	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	461 West 25th Avenue		
Address 2			
City	San Mateo	State/Province	CA
Postal Code	94403	Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	sergey		Solomatin	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Mountain View	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	422 Hillwood Court		
Address 2			
City	Mountain View	State/Province	CA
Postal Code	94040	Country	US

Inventor 3 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Rachel		Fraser	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

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

City	San Francisco	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	3653 24th Street Apt 2		
Address 2			
City	San Francisco	State/Province	CA
Postal Code	94110	Country	US

Inventor 4 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
Dr.	Patrick	O'Reilly	Brown	

Residence Information (Select One) (•) US Residency ( ) Non US Residency ( ) Active US Military Service

City	Stanford	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	76 Peter Coutts Circle		
Address 2			
City	Stanford	State/Province	CA
Postal Code	94305	Country	US

Inventor 5 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Jessica		Karr	

Residence Information (Select One) (•) US Residency ( ) Non US Residency ( ) Active US Military Service

City	San Francisco	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	456 Kansas Street		
Address 2			
City	San Francisco	State/Province	CA
Postal Code	94107	Country	US

Inventor 6 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Celeste		Holz-Schietinger	

Residence Information (Select One) (•) US Residency ( ) Non US Residency ( ) Active US Military Service

City	East Palo Alto	State/Province	CA	Country of Residence	US

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

Mailing Address of Inventor:

Address 1	1159 Sage Street		
Address 2			
City	East Palo Alto	**State/Province**	CA
Postal Code	94303	**Country**	US

Inventor 7 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Michael		Eisen	

Residence Information (Select One) (•) US Residency ( ) Non US Residency ( ) Active US Military Service

City	Berkeley	**State/Province**	CA	**Country of Residence**	US

Mailing Address of Inventor:

Address 1	2408 McGee Avenue		
Address 2			
City	Berkeley	**State/Province**	CA
Postal Code	94703	**Country**	US

Inventor 8 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Ranjani		Varadan	

Residence Information (Select One) (•) US Residency ( ) Non US Residency ( ) Active US Military Service

City	Fremont	**State/Province**	CA	**Country of Residence**	US

Mailing Address of Inventor:

Address 1	1580 Skelton Court		
Address 2			
City	Fremont	**State/Province**	CA
Postal Code	94536	**Country**	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	26191

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

Email Address	apsi@fr.com	Add Email	Remove Email

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 complete 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

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.

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).
Either enter 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	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	26191		

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

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 blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
	Continuation of	PCTUS2014011361	2014-01-13
Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCTUS2014011361	Claims the benefit of	13941211	2013-07-12
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCTUS2014011361	Claims benefit of provisional	61908634	2013-11-25
Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)
PCTUS2014011361	Claims benefit of provisional	61751816	2013-01-11

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

PTO/AIA/14 (12-13)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	38767-0010001
	Application Number	
Title of Invention	METHODS AND COMPOSITIONS FOR CONSUMABLES	

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☐ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date o f filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	38767-0010001
	Application Number	
Title of Invention	METHODS AND COMPOSITIONS FOR CONSUMABLES	

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

○ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
◉ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. ☒

Organization Name	Impossible Foods Inc.		
Mailing Address Information For Applicant:			
Address 1	525 Chesapeake Drive		
Address 2			
City	Redwood City	**State/Province**	CA
Country	US	Postal Code	94063
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not subsitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication . An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

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

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Signature	/M. Angela Parsons/			Date (YYYY-MM-DD)	2015-07-10
First Name	M. Angela	Last Name	Parsons Ph.D.	Registration Number	44282

Additional Signature may be generated within this form by selecting the Add button.

This collection of information is required by 37 CFR 1.76. 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 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet 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: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent C o o p eration Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (03-14)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Marija Vrljic	Non provisional Application Number (if known):	
Title of Invention:	METHODS AND COMPOSITIONS FOR CONSUMABLES		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1), the prioritized examination fee set forth in 37 CFR 1.17(c), and if not already paid, the publication fee set forth in 37 CFR 1.18(d) have been filed with the request. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.

2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims.

3. The applicable box is checked below:

I. ☒ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original non provisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

---OR---

(b) The application is an original non provisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, **or** the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original non provisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature /M. Angela Parsons/	Date /July 10, 2015/
Name (Print/Typed) M. Angela Parsons, Ph.D.	Practitioner Registration Number 44,282

***Note**: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

☐ *Total of _____ forms are submitted.

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The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant *(i.e.,* GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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Docket No. 38767-0010001

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.

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.

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

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.

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,

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).

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

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.

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

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

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

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

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.

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

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.

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,

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

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

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

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.

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).

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.

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, 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

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-

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*

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,

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), 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

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

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

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

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

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.

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 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

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%

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 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

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,

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.

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

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.

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

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%

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

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

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.

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

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

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.

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

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

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.

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,

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

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-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

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

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

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

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.

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-

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

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

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.

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%.

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.

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.

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.

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.

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

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.

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

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

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

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,

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).

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

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

(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

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

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

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

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

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 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.

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

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 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

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.

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-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-

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

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

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).

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 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.

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

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.

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
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

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

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 (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

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

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

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 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,

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.

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,

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.

WHAT IS CLAIMED IS:

1. A method for imparting a beef-like flavor to a consumable product, comprising adding, to the consumable, an heterologous heme-containing protein, wherein, after cooking, a beef-like flavor is imparted to the consumable.

2. The method of claim 1, wherein the heme-containing protein has an amino acid sequence with at least 70% homology to the amino acid sequence set forth in SEQ ID NO: 1.

3. The method of claim 1, wherein the heme-containing protein has a UV-VIS spectrum that is substantially similar to that of myoglobin from an animal.

4. The method of claim 1, wherein the heme-containing protein is selected from the group consisting of 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, a truncated 2/2 globin, a hemoglobin 3, a cytochrome, and a peroxidase.

5. The method of claim 1, wherein the consumable comprises about 0.01% to about 5% of the heterologous heme-containing protein.

6. The method of claim 1, wherein the consumable product comprises fish or chicken.

7. A method for making a white meat composition taste like beef, the method comprising adding a heme-containing protein to the white meat composition, respectively.

8. The method of claim 7, wherein the heme-containing protein has an amino acid sequence with at least 70% homology to the amino acid sequence set forth in SEQ ID NO: 1.

9. The method of claim 7, wherein the heme-containing protein has a UV-VIS spectrum that is substantially similar to that of myoglobin from an animal.

10. The method of claim 7, wherein the heme-containing protein is a heterologous heme-containing protein.

11. The method of claim 7, wherein the heme-containing protein is selected from the group consisting of 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, a truncated 2/2 globin, a hemoglobin 3, a cytochrome, and a peroxidase.

12. The method of claim 7, wherein about 0.01% to about 5% of the heme-containing protein is added to the white meat composition.

13. The method of claim 7, wherein the white meat composition comprises chicken or pork.

14. The method of claim 7, wherein the white meat composition comprises fish.

15. A method for altering the texture of a consumable during or after cooking comprising incorporating one or more plant proteins having a low denaturation temperature into the consumable.

16. The method of claim 15, wherein at least one of the one or more plant proteins is isolated and purified.

17. The method of claim 15, wherein the one or more plant proteins are selected from the group consisting of rubisco, pea proteins, lentil proteins, or other legume proteins.

18. The method of claim 17, wherein the pea proteins comprise pea albumin proteins.

19. The method of claim 15, wherein the one or more plant proteins are present in an amount of between about 1% and about 30%.

20. The method of claim 15, wherein the consumable product is a meat product or a meat substitute product.

21. The method of claim 20, wherein the meat product or the meat substitute product is a pate, a pate analog, a sausage, or a sausage replica.

22. The method of claim 15, wherein the consumable becomes firmer during or after cooking.

23. A tissue replica comprising a freeze-aligned non-animal protein.

24. The tissue replica of claim 23, wherein the non-animal protein is isolated and purified.

25. The tissue replica of claim 23, wherein the non-animal protein is a plant protein.

26. The tissue replica of claim 25, wherein the plant protein is selected from the group consisting of RuBisCo, Moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, legumins, vicilin, a convicilin, and an oleosin.

27. The tissue replica of claim 23, wherein the tissue replica comprises between about 1% and about 80% of the non-animal protein.

28. The tissue replica of claim 23, wherein the tissue replica is a muscle tissue replica.

29. The tissue replica of claim 23, further comprising a heme-containing protein.

30. The tissue replica of claim 29, wherein the heme-containing protein is isolated and purified.

Docket No. 38767-0010001

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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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)

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  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
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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
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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
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1021 syreaavddv vkedngrqvp vkagdrvfvs fvdaardpkh fpdpevvnpr rpakkyihyg
1081 vgphaclgrd asqiaitemf rclfrrrnvr rvpgpqgelk kvprpggfyv ymredwgglf
1141 pfpvtmrvmw dde
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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
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Fusarium graminearum (SEQ ID NO:8)

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  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
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SEQ ID NO: 9 *Chlamydomonas eugametos*

```
MSLFAKLGGREAVEAAVDKFYNKIVADPTVSTYFSNTDMKVQRSKQFAFLAYALGGASE
WKGKDMRTAHKDLVPHLSDVHFQAVARHLSDTLTELGVPPEDITDAMAVVASTRTEVLN
MPQQ
```

SEQ ID NO:10 *Tetrahymena pyriformis*

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MNKPQTIYEKLGGENAMKAAVPLFYKKVLADERVKHFFKNTDMDHQTKQQTDFLTMLLG
GPNHYKGKNMTEAHKGMNLQNLHFDAIIENLAATLKELGVTDAVINEAAKVIEHTRKDM
LGK
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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
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KHPSDFGADAQAAMSKALELFRNDMAAQYKVLGFHG

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

SEQ ID NO: 20 *Equus caballus*

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KH PGDFGADAQGAMTKALELFRNDIAAKYKELGFQG

SEQ ID NO: 21 Nicotiana benthamiana
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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
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FIGURE 2A

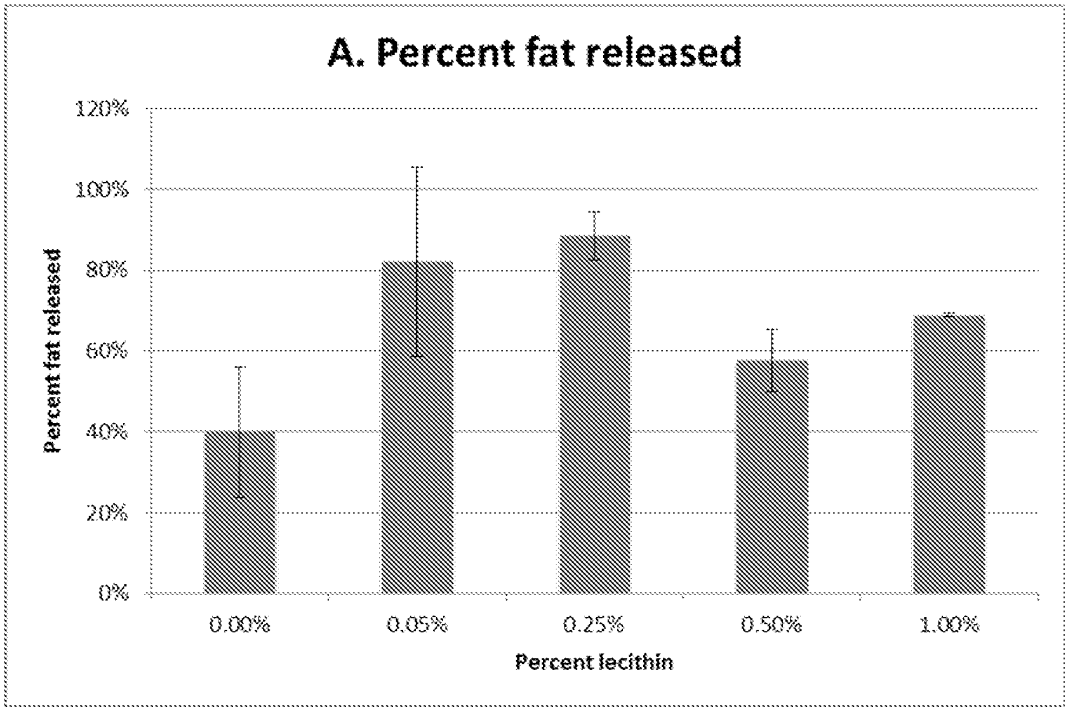


FIGURE 2B

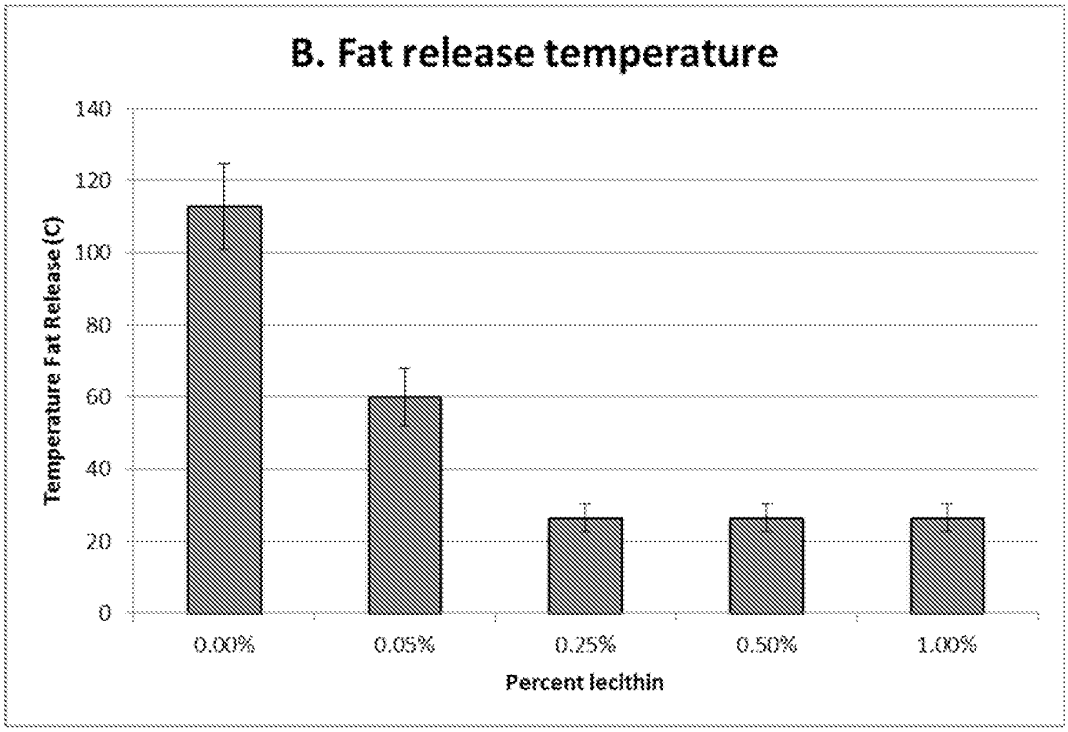


FIGURE 2C

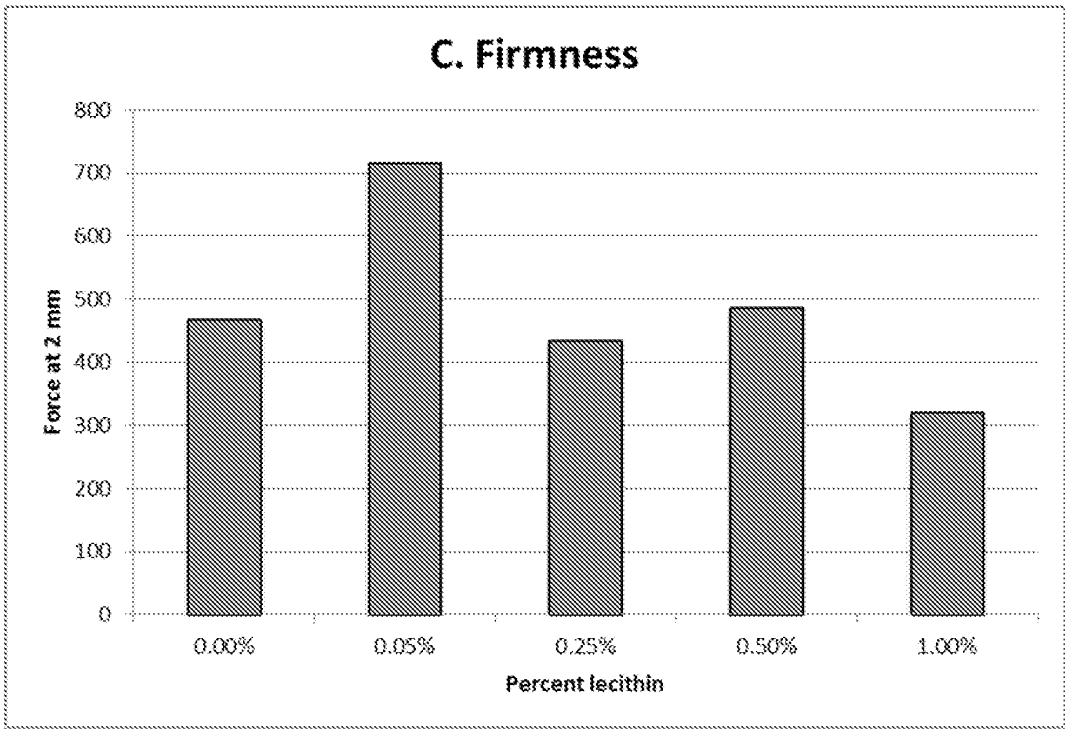

C. Firmness
Force at 2 mm
800
700
600
500
400
300
200
100
0
0.00%
0.05%
0.25%
0.50%
1.00%
Percent lecithin

FIGURE 3

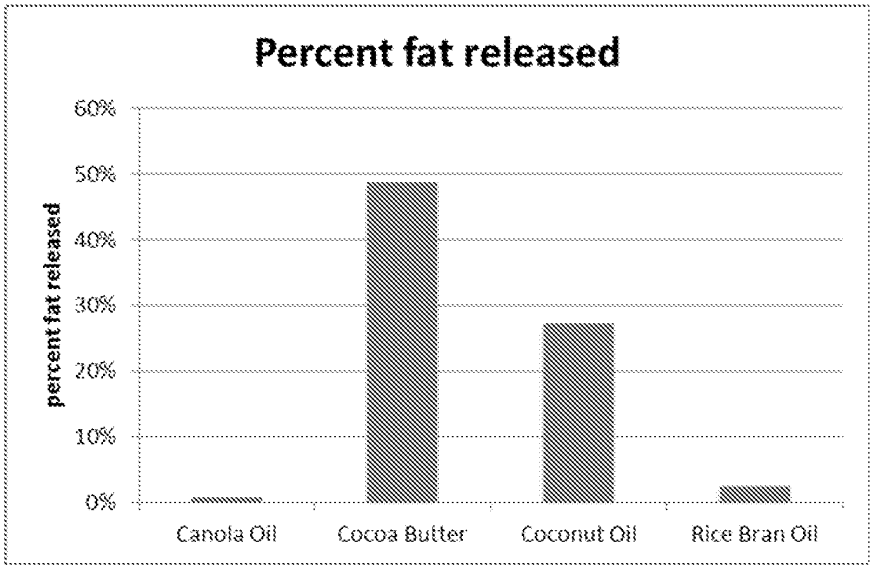
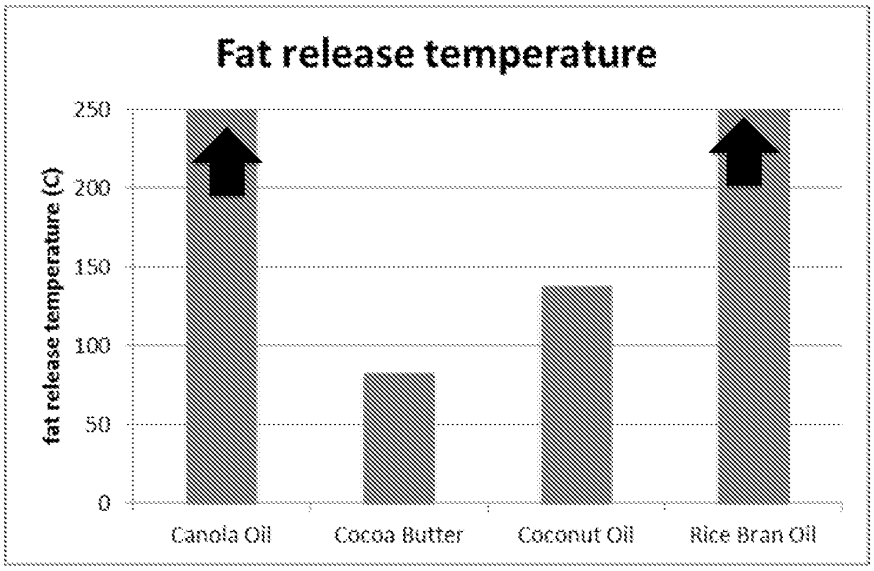

Percent fat released
percent fat released
60%
50%
40%
30%
20%
10%
0%
Canola Oil
Cocoa Butter
Coconut Oil
Rice Bran Oil

FIGURE 4



60902413.doc

DocCode – SEQ.TXT

SCORE Placeholder Sheet for IFW Content

Application Number: 14796970 Document Date: 07/10/2015

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

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- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: August 26, 2013

DocCode – SCORE

SCORE Placeholder Sheet for IFW Content

Application Number: 14796970 Document Date: 07/10/2015

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

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- External customers may access SCORE content via PAIR using the Supplemental Content tab.

Form Revision Date: August 26, 2013

Document code: WFEE

United States Patent and Trademark Office
Sales Receipt for Accounting Date: 07/21/2015

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01 FC : 1051 140.00 DA

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Sequence Listing was accepted.
If you need help call the Patent Electronic Business Center at (866)
217-9197 (toll free).
Reviewer: Douglas, Keisha (ASRC)
Timestamp: [year=2015; month=7; day=14; hr=15; min=50; sec=21; ms=310; ]
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Validated By CRFValidator v 1.0.4

Application No: 14796970 Version No: 1.0

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        35                  40                  45
Leu Phe Ser Phe Leu Arg Asp Ser Thr Val Pro Leu Glu Gln Asn Pro
    50                  55                  60
Lys Leu Lys Pro His Ala Val Ser Val Phe Val Met Thr Cys Asp Ser
65                  70                  75                  80
Ala Val Gln Leu Arg Lys Ala Gly Lys Val Thr Val Arg Glu Ser Asn
                85                  90                  95
Leu Lys Lys Leu Gly Ala Thr His Phe Arg Thr Gly Val Ala Asn Glu
            100                 105                 110
His Phe Glu Val Thr Lys Phe Ala Leu Leu Glu Thr Ile Lys Glu Ala
        115                 120                 125
Val Pro Glu Met Trp Ser Pro Ala Met Lys Asn Ala Trp Gly Glu Ala
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Ser
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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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Val Met Thr Ala Glu
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Phe Ser Lys Tyr Pro Lys Thr Lys Glu Leu Phe Ala Gly Ala Ser Glu
        35   40   45
Glu Gln Pro Lys Lys Leu Ala Asn Ala Ile Ile Ala Tyr Ala Thr Tyr
    50   55   60
Ile Asp Arg Leu Glu Glu Leu Asp Asn Ala Ile Ser Thr Ile Ala Arg
65   70   75   80
Ser His Val Arg Arg Asn Val Lys Pro Glu His Tyr Pro Leu Val Lys
                85   90   95
Glu Cys Leu Leu Gln Ala Ile Glu Glu Val Leu Asn Pro Gly Glu Glu
            100   105   110
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        35   40   45
Ser Asn Gly Val Asp Pro Ser Asn Pro Lys Leu Thr Gly His Ala Glu
    50   55   60
Lys Leu Phe Gly Leu Val Arg Asp Ser Ala Gly Gln Leu Lys Ala Asn
65   70   75   80
Gly Thr Val Val Ala Asp Ala Ala Leu Gly Ser Ile His Ala Gln Lys
                85   90   95
Ala Ile Thr Asp Pro Gln Phe Val Val Val Lys Glu Ala Leu Leu Lys
            100   105   110
Thr Ile Lys Glu Ala Val Gly Asp Lys Trp Ser Asp Glu Leu Ser Ser
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Phe
145

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            20   25   30
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        35   40   45
Gln Met Phe Pro Phe Leu Arg Asp Ser Asp Val Pro Leu Glu Thr Asn
    50   55   60
Pro Lys Leu Lys Thr His Ala Val Ser Val Phe Val Met Thr Cys Glu
65   70   75   80
Ala Ala Ala Gln Leu Arg Lys Ala Gly Lys Ile Thr Val Arg Glu Thr
                85   90   95
Thr Leu Lys Arg Leu Gly Gly Thr His Leu Lys Tyr Gly Val Ala Asp
            100   105   110
Gly His Phe Glu Val Thr Arg Phe Ala Leu Leu Glu Thr Ile Lys Glu
        115   120   125
Ala Leu Pro Ala Asp Met Trp Gly Pro Glu Met Arg Asn Ala Trp Gly
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Ala Glu

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<213> Magnaporthe oryza

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        35                  40                  45
Glu Ser Pro Ser Arg Lys Ser Ser Thr Ile Gly Gln Ser Thr Arg Asn
    50                  55                  60
Gly Ser Cys Gln Ala Asp Thr Gln Lys Gly Gln Leu Pro Pro Val Gly
65                  70                  75                  80
Glu Lys Pro Lys Pro Val Lys Glu Asn Pro Met Lys Lys Leu Lys Glu
                85                  90                  95
Met Ser Gln Arg Pro Leu Pro Thr Gln His Gly Asp Gly Thr Tyr Pro
            100                 105                 110
Thr Glu Lys Lys Leu Thr Gly Ile Gly Glu Asp Leu Lys His Ile Arg
        115                 120                 125
Gly Tyr Asp Val Lys Thr Leu Leu Ala Met Val Lys Ser Lys Leu Lys
    130                 135                 140
Gly Glu Lys Leu Lys Asp Asp Lys Thr Met Leu Met Glu Arg Val Met
145                 150                 155                 160
Gln Leu Val Ala Arg Leu Pro Thr Glu Ser Lys Lys Arg Ala Glu Leu
                165                 170                 175
Thr Asp Ser Leu Ile Asn Glu Leu Trp Glu Ser Leu Asp His Pro Pro
            180                 185                 190
Leu Asn Tyr Leu Gly Pro Glu His Ser Tyr Arg Thr Pro Asp Gly Ser
        195                 200                 205
Tyr Asn His Pro Phe Asn Pro Gln Leu Gly Ala Ala Gly Ser Arg Tyr
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Ala Arg Ser Val Ile Pro Thr Val Thr Pro Pro Gly Ala Leu Pro Asp
225                 230                 235                 240
Pro Gly Leu Ile Phe Asp Ser Ile Met Gly Arg Thr Pro Asn Ser Tyr
                245                 250                 255
Arg Lys His Pro Asn Asn Val Ser Ser Ile Leu Trp Tyr Trp Ala Thr
            260                 265                 270
Ile Ile Ile His Asp Ile Phe Trp Thr Asp Pro Arg Asp Ile Asn Thr
        275                 280                 285
Asn Lys Ser Ser Ser Tyr Leu Asp Leu Ala Pro Leu Tyr Gly Asn Ser
    290                 295                 300
Gln Glu Met Gln Asp Ser Ile Arg Thr Phe Lys Asp Gly Arg Met Lys
305                 310                 315                 320
Pro Asp Cys Tyr Ala Asp Lys Arg Leu Ala Gly Met Pro Pro Gly Val
                325                 330                 335
Ser Val Leu Leu Ile Met Phe Asn Arg Phe His Asn His Val Ala Glu
            340                 345                 350
Asn Leu Ala Leu Ile Asn Glu Gly Gly Arg Phe Asn Lys Pro Ser Asp
        355                 360                 365
Leu Leu Glu Gly Glu Ala Arg Glu Ala Ala Trp Lys Lys Tyr Asp Asn
    370                 375                 380
Asp Leu Phe Gln Val Ala Arg Leu Val Thr Ser Gly Leu Tyr Ile Asn
385                 390                 395                 400
Ile Thr Leu Val Asp Tyr Val Arg Asn Ile Val Asn Leu Asn Arg Val
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Glu Phe Asn Leu Cys Tyr Arg Trp His Ser Cys Ile Ser Glu Lys Asp
    450                 455                 460
Ser Lys Phe Val Glu Ala Gln Phe Gln Asn Ile Phe Gly Lys Pro Ala
465                 470                 475                 480
Ser Glu Val Arg Pro Asp Glu Met Trp Lys Gly Phe Ala Lys Met Glu
                485                 490                 495
Gln Asn Thr Pro Ala Asp Pro Gly Gln Arg Thr Phe Gly Gly Phe Lys
            500                 505                 510
Arg Gly Pro Asp Gly Lys Phe Asp Asp Asp Asp Leu Val Arg Cys Ile
        515                 520                 525
Ser Glu Ala Val Glu Asp Val Ala Gly Ala Phe Gly Ala Arg Asn Val
    530                 535                 540
Pro Gln Ala Met Lys Val Val Glu Thr Met Gly Ile Ile Gln Gly Arg
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Lys Trp Asn Val Ala Gly Leu Asn Glu Phe Arg Lys His Phe His Leu
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Lys Pro Tyr Ser Thr Phe Glu Asp Ile Asn Ser Asp Pro Gly Val Ala
            580                 585                 590
Glu Ala Leu Arg Arg Leu Tyr Asp His Pro Asp Asn Val Glu Leu Tyr
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Pro Gly Leu Val Ala Glu Glu Asp Lys Gln Pro Met Val Pro Gly Val
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Gly Ile Ala Pro Thr Tyr Thr Ile Ser Arg Val Val Leu Ser Asp Ala
625                 630                 635                 640
Val Cys Leu Val Arg Gly Asp Arg Phe Tyr Thr Thr Asp Phe Thr Pro
                645                 650                 655
Arg Asn Leu Thr Asn Trp Gly Tyr Lys Glu Val Asp Tyr Asp Leu Ser
            660                 665                 670
Val Asn His Gly Cys Val Phe Tyr Lys Leu Phe Ile Arg Ala Phe Pro
        675                 680                 685
Asn His Phe Lys Gln Asn Ser Val Tyr Ala His Tyr Pro Met Val Val
    690                 695                 700
Pro Ser Glu Asn Lys Arg Ile Leu Glu Ala Leu Gly Arg Ala Asp Leu
705                 710                 715                 720
Phe Asp Phe Glu Ala Pro Lys Tyr Ile Pro Pro Arg Val Asn Ile Thr
                725                 730                 735
Ser Tyr Gly Gly Ala Glu Tyr Ile Leu Glu Thr Gln Glu Lys Tyr Lys
            740                 745                 750
Val Thr Trp His Glu Gly Leu Gly Phe Leu Met Gly Glu Gly Gly Leu
        755                 760                 765
Lys Phe Met Leu Ser Gly Asp Asp Pro Leu His Ala Gln Gln Arg Lys
    770                 775                 780
Cys Met Ala Ala Gln Leu Tyr Lys Asp Gly Trp Thr Glu Ala Val Lys
785                 790                 795                 800
Ala Phe Tyr Ala Gly Met Met Glu Glu Leu Leu Val Ser Lys Ser Tyr
                805                 810                 815
Phe Leu Gly Asn Asn Lys His Arg His Val Asp Ile Ile Arg Asp Val
            820                 825                 830
Gly Asn Met Val His Val His Phe Ala Ser Gln Val Phe Gly Leu Pro
        835                 840                 845
Leu Lys Thr Ala Lys Asn Pro Thr Gly Val Phe Thr Glu Gln Glu Met
    850                 855                 860
Tyr Gly Ile Leu Ala Ala Ile Phe Thr Thr Ile Phe Phe Asp Leu Asp
865                 870                 875                 880
Pro Ser Lys Ser Phe Pro Leu Arg Thr Lys Thr Arg Glu Val Cys Gln
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                885                 890                 895
Lys Leu Ala Lys Leu Val Glu Ala Asn Val Lys Leu Ile Asn Lys Ile
            900                 905                 910
Pro Trp Ser Arg Gly Met Phe Val Gly Lys Pro Ala Lys Asp Glu Pro
        915                 920                 925
Leu Ser Ile Tyr Gly Lys Thr Met Ile Lys Gly Leu Lys Ala His Gly
    930                 935                 940
Leu Ser Asp Tyr Asp Ile Ala Trp Ser His Val Val Pro Thr Ser Gly
945                 950                 955                 960
Ala Met Val Pro Asn Gln Ala Gln Val Phe Ala Gln Ala Val Asp Tyr
                965                 970                 975
Tyr Leu Ser Pro Ala Gly Met His Tyr Ile Pro Glu Ile His Met Val
            980                 985                 990
Ala Leu Gln Pro Ser Thr Pro Glu Thr Asp Ala Leu Leu Leu Gly Tyr
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Ala Met Glu Gly Ile Arg Leu Ala Gly Thr Phe Gly Ser Tyr Arg Glu
    1010                1015                1020
Ala Ala Val Asp Asp Val Val Lys Glu Asp Asn Gly Arg Gln Val Pro
1025                1030                1035                1040
Val Lys Ala Gly Asp Arg Val Phe Val Ser Phe Val Asp Ala Ala Arg
                1045                1050                1055
Asp Pro Lys His Phe Pro Asp Pro Glu Val Val Asn Pro Arg Arg Pro
            1060                1065                1070
Ala Lys Lys Tyr Ile His Tyr Gly Val Gly Pro His Ala Cys Leu Gly
        1075                1080                1085
Arg Asp Ala Ser Gln Ile Ala Ile Thr Glu Met Phe Arg Cys Leu Phe
    1090                1095                1100
Arg Arg Arg Asn Val Arg Arg Val Pro Gly Pro Gln Gly Glu Leu Lys
1105                1110                1115                1120
Lys Val Pro Arg Pro Gly Gly Phe Tyr Val Tyr Met Arg Glu Asp Trp
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Gly Gly Leu Phe Pro Phe Pro Val Thr Met Arg Val Met Trp Asp Asp
            1140                1145                1150
Glu
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<400> 7

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Ala Ser Gln Leu Val Trp Pro Ser Lys Trp Asp Glu Val Glu Asp Leu
            20                  25                  30
Leu Tyr Met Gln Gly Gly Phe Asn Lys Arg Gly Phe Ala Asp Ala Leu
        35                  40                  45
Arg Thr Cys Glu Phe Gly Ser Asn Val Pro Gly Thr Gln Asn Thr Ala
    50                  55                  60
Glu Trp Leu Arg Thr Ala Phe His Asp Ala Ile Thr His Asp Ala Lys
65                  70                  75                  80
Ala Gly Thr Gly Gly Leu Asp Ala Ser Ile Tyr Trp Glu Ser Ser Arg
                85                  90                  95
Pro Glu Asn Pro Gly Lys Ala Phe Asn Asn Thr Phe Gly Phe Phe Ser
            100                 105                 110
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Gly Phe His Asn Pro Arg Ala Thr Ala Ser Asp Leu Thr Ala Leu Gly
        115                 120                 125
Thr Val Leu Ala Val Gly Ala Cys Asn Gly Pro Arg Ile Pro Phe Arg
    130                 135                 140
Ala Gly Arg Ile Asp Ala Tyr Lys Ala Gly Pro Ala Gly Val Pro Glu
145                 150                 155                 160
Pro Ser Thr Asn Leu Lys Asp Thr Phe Ala Ala Phe Thr Lys Ala Gly
                165
```

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/796,970	07/10/2015	Marija Vrljic	38767-0010001

CONFIRMATION NO. 8501

26191
FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

INFORMAL NOTICE

OC000000076433993

Date Mailed: 07/23/2015

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  Marija Vrljic
  Sergey Solomatin
  Rachel Fraser
  Patrick O'Reilly Brown
  Jessica Karr
  Celeste Holz-Schietinger
  Michael Eisen
  Ranjani Varadan

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/tle/

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 14/796,970

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	280
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	600
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	720
TOTAL CLAIMS (37 CFR 1.16(i))	30 minus 20 =	* 10			OR	x 80 =	800
INDEPENDENT CLAIMS (37 CFR 1.16(h))	4 minus 3 =	* 1				x 420 =	420
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 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).						400
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	3220

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
14/796,970	07/10/2015	1791	3360	38767-0010001	30	4

CONFIRMATION NO. 8501

26191
FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

FILING RECEIPT

OC000000078433992

Date Mailed: 07/23/2015

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Marija Vrljic, San Mateo, CA;
Sergey Solomatin, Mountain View, CA;
Rachel Fraser, San Francisco, CA;
Patrick O'Reilly Brown, Stanford, CA;
Jessica Karr, San Francisco, CA;
Celeste Holz-Schietinger, East Palo Alto, CA;
Michael Eisen, Berkeley, CA;
Ranjani Varadan, Fremont, CA;

Applicant(s)

Impossible Foods Inc., Redwood City, CA

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a CON of PCT/US2014/011361 01/13/2014
which claims benefit of 61/908,634 11/25/2013
and claims benefit of 61/751,816 01/11/2013

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

If Required, Foreign Filing License Granted: 07/21/2015

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/796,970**

Projected Publication Date: 10/29/2015

Non-Publication Request: No

Early Publication Request: No
Title

METHODS AND COMPOSITIONS FOR CONSUMABLES

Preliminary Class

426

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

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www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
14/796,970	07/10/2015	Marija Vrljic	38767-0010001

CONFIRMATION NO. 8501

26191
FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

IMPROPER CFR REQUEST

OC000000076433873

Date Mailed: 07/23/2015

RESPONSE TO REQUEST FOR CORRECTED FILING RECEIPT

Continuity, Priority Claims, Petitions, and Non-Publication Requests

In response to your request for a corrected Filing Receipt, the Office is unable to comply with your request because:

Improper Benefit Claim(s) to Prior-Filed Nonprovisional Application(s)

The benefit claim(s) to prior-filed nonprovisional application(s) is improper because there is no specific reference for each prior-filed application that includes: (1) the identification of the prior-filed application by application number, or international application number and international filing date; and (2) a clear indication of the relationship (i.e., continuation, divisional, or continuation-in-part) of the nonprovisional applications. Applicant must submit a corrected application data sheet (ADS) to include a specific reference. Applicant should review each benefit claim submitted and, if appropriate, provide the proper reference to the prior-filed application(s) as required by 37 CFR 1.78. A proper relationship includes an identification of each nonprovisional application as a continuation, divisional, or continuation-in-part application of the immediate prior-filed nonprovisional application for which a benefit is claimed under 37 CFR 1.78 in order to establish copendency throughout the entire chain of prior-filed applications. The specific reference must be included in the domestic benefit information section of an application data sheet (37 CFR 1.76).

Timeliness: The required reference for each benefit claim must be filed during the pendency of the instant application and within the later of: (1) four months from the actual filing date of the instant application, or the national stage commencement date if the instant application is a national stage application under 35 U.S.C. 371; or (2) sixteen months from the filing date of the prior-filed application. Failure to timely file the required reference is considered a waiver of any benefit claim, unless a grantable petition to accept an unintentionally delayed claim under 37 CFR 1.78(a), the surcharge set forth in 37 CFR 1.17(t), and the required reference are filed. See 37 CFR 1.78(a).

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/tha/

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PO BOX 1022
MINNEAPOLIS MN 55440-1022

MAILED
JUL 28 2015
OFFICE OF PETITIONS

Doc Code: TRACK1.GRANT

Decision Granting Request for Prioritized Examination (Track I or After RCE)	Application No.: 14/796,970

1. THE REQUEST FILED July 10, 2015 IS **GRANTED.**

The above-identified application has met the requirements for prioritized examination

A. ☒ for an original nonprovisional application (Track I).
B. ☐ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination.** The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

A. filing a **petition for extension of time** to extend the time period for filing a reply;
B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims**, or a multiple dependent claim;
C. filing a **request for continued examination**;
D. filing a notice of appeal;
E. filing a request for suspension of action;
F. mailing of a notice of allowance;
G. mailing of a final Office action;
H. completion of examination as defined in 37 CFR 41.102; or
I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to **Michelle R. Eason** at (571) 272-4231. In his/her absence, calls may be directed to Brian W. Brown at (571) 272-5338.

/Michelle R. Eason/
(*Signature*)

Paralegal Specialist, Office of Petitions
(Title)

U.S. Patent and Trademark Office
PTO-2298 (Rev. 02-2012)

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www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
14/796,970	07/10/2015	Marija Vrljic	38767-0010001	8501

26191 7590 09/17/2015
FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

EXAMINER	
GWARTNEY, ELIZABETH A	

ART UNIT	PAPER NUMBER
1791	

NOTIFICATION DATE	DELIVERY MODE
09/17/2015	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PATDOCTC@fr.com

Office Action Summary	Application No. 14/796,970	Applicant(s) VRLJIC ET AL.	
	Examiner ELIZABETH GWARTNEY	Art Unit 1791	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-30* is/are pending in the application.

5a) Of the above claim(s) *15-30* is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-14* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☒ The specification is objected to by the Examiner.

11)☒ The drawing(s) filed on *7/10/2015* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

DETAILED ACTION

1. The present application is being examined under the pre-AIA first to invent provisions.

Election/Restrictions

2. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. **Claims 1-14**, drawn to a method of imparting beef-like flavor to a consumable product by adding a heterologous heme-containing protein to the consumable product, classified in A23L1/31427.

II. **Claims 15-22**, drawn to a method for altering texture of a consumable by incorporating one or more plant proteins into the consumable, classified in A23L1/31436.

III. **Claims 23-30**, drawn to a tissue replica comprising a freeze-aligned non-animal protein, classified in A23J3/14.

The inventions are distinct, each from the other because of the following reasons:

3. Inventions I, II and III are ***unrelated.*** Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different designs, modes of operation, and effects (MPEP § 802.01 and § 806.06). In the instant case, the different inventions are distinct and not related because they are not disclosed as capable of use together. Invention I operates by adding a heterologous heme-containing protein to a consumable product, Invention II operates by adding incorporating a plant protein into a consumable product and Invention III is designed as a tissue replica comprising freeze-aligned non-animal protein.

4. Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above and there would be a

serious search and/or examination burden if restriction were not required because one or more of the following reasons apply:

(a) the inventions have acquired a separate status in the art in view of their different classification;

(b) the inventions have acquired a separate status in the art due to their recognized divergent subject matter;

(c) the inventions require a different field of search (for example, searching different classes/subclasses or electronic resources, or employing different search queries);

(d) the prior art applicable to one invention would not likely be applicable to another invention;

(e) the inventions are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a invention to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected invention.**

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103 or pre-AIA 35 U.S.C. 103(a) of the other invention.

5. During a telephone conversation with Angela Parsons on August 27, 2015 a provisional election was made without traverse to prosecute the invention of Group I, claims 1-14. Affirmation of this election must be made by applicant in replying to this Office action. Claims 15-30 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

6. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be corrected in compliance with 37 CFR 1.48(a) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. A request to correct inventorship under 37 CFR 1.48(a) must be accompanied by an application data sheet in accordance with 37 CFR 1.76 that identifies each inventor by his or her legal name and by the processing fee required under 37 CFR 1.17(i).

Specification

7. The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code. Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code; references to websites should be limited to the top-level

domain name without any prefix such as http:// or other browser-executable code. See MPEP § 608.01.

Claim Rejections - 35 USC § 102

8. The following is a quotation of the appropriate paragraphs of pre-AIA 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

9. **Claim 7-9, 11 and 13 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Jamieson ("Iroquois Stew With Beef, Chicken and Pork" – Bon Appétit, November 1995).**

Regarding **claims 7-9, 11 and 13**, Jamieson discloses a method for making a Iroquois stew with beef comprising adding beef to a composition of pork, chicken, flour, oil, onion, chicken broth, carrots, bay leaves, all spice and potatoes (Ingredients, Preparation). Given Jamieson discloses beef, inherently the beef comprises a heme-containing protein including myoglobin. Moreover, given the composition would comprise myoglobin from beef, inherently the myoglobin would exhibit a VIS spectrum that would be identical to that of myoglobin from an animal and an amino acid sequence at least 70% homologous to that set forth in SEQ ID NO:1.

10. **Claims 7-9, 11 and 14 are rejected under pre-AIA 35 U.S.C. 102(b) as being anticipated by Foo ("Beef and Scallop Stir Fry" – Food & Wine, July 2001).**

Regarding **claims 7-9, 11 and 13**, Foo discloses a method for making Beef and Scallop Stir-Fry comprising adding beef to a composition of scallops (i.e. fish), chicken stock, soy sauce, rice vinegar, cornstarch, sugar, crushed red pepper, vegetable oil, cloves, red bell pepper, corn, scallions and fresh basil. Given Foo discloses beef, inherently the beef comprises a heme-containing protein including myoglobin. Moreover, given the composition would comprise myoglobin from beef, inherently the myoglobin would exhibit a VIS spectrum that would be identical to that of myoglobin from an animal and an amino acid sequence at least 70% homologous to that set forth in SEQ ID NO: 1.

Claim Rejections - 35 USC § 102/ 35 USC § 103

11. The following is a quotation of the appropriate paragraphs of pre-AIA 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –

12. The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

13. The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

14. This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

15. **Claims 1-6, 10 and 12 are rejected under pre-AIA 35 U.S.C. 102(b) as anticipated by or, in the alternative, under pre-AIA 35 U.S.C. 103(a) as obvious over Jamieson ("Iroquois Stew With Beef, Chicken and Pork"– Bon Appétit, November 1995) as evidenced by Merriam-Webster ("Definition of HETEROLOGOUS - www.meriam-webster.com/dictionary/heterologous, downloaded September 10, 2015).**

Regarding **claims 1, 2, 3, 4 and 6**, Jamieson discloses a method for making a Iroquois stew with beef comprising adding beef to a composition of pork, chicken, flour, oil, onion, chicken broth, carrots, bay leaves, all spice and potatoes (Ingredients, Preparation). Given Jamieson discloses beef, intrinsically the beef comprises a heme-containing protein including myoglobin. Moreover, given the composition would comprise myoglobin from beef, intrinsically the myoglobin would exhibit a VIS spectrum that would be identical to that of

myoglobin from an animal and an amino acid sequence at least 70% homologous to that set forth in SEQ ID NO: 1.

While Jamieson discloses the addition of a heme-containing protein (in the form of beef), the reference is silent with respect to a heterologous heme-containing protein. Given broadly, the term heterologous means "derived from another species" (see Merriam-Webster), since Jamieson discloses the combination of meat species (i.e. pork, chicken and beef), it is clear the limitation requiring a heterologous (e.g. different than from beef) heme-containing protein has been satisfied.

In the alternative, absent evidence to the contrary, it would have been obvious to one of ordinary skill in the art to have added a heme-protein from any source, including a heterologous heme-protein and have arrived at the present invention. Although the heme-protein is obtained from a different source (i.e. beef), the burden shifts to establish an unobvious difference between the claimed product and the prior art product.

Regarding **claim 5**, Jamieson discloses all of the claim limitations as set forth above. While Jamieson discloses a composition comprising about 16% beef (*see* Ingredients wherein there is 1 lb. of beef in approximately 6 lb. total ingredients), the reference is silent with respect to the precise amount of heme-containing protein or myoglobin.

Given the “beef” flavor of the Iroquois stew is a variable that can be adjusted by varying the amount of beef in the composition, one of ordinary skill in the art would have been motivated to adjust, in routine processing, the amount of beef added to the stew, to obtain the desired flavor profile.

Regarding **claim 10**, Jamieson discloses all of the claim limitations as set forth above. While Jamieson discloses the addition of a heme-containing protein (in the form of beef), the reference is silent with respect to a heterologous heme-containing protein. Given broadly, the term heterologous means "derived from another species" (see Merriam-Webster), since Jamieson discloses the combination of meat species (i.e. pork, chicken and beef), it is clear the limitation requiring a heterologous (e.g. different than from beef) heme-containing protein has been satisfied.

In the alternative, absent evidence to the contrary, it would have been obvious to one of ordinary skill in the art to have added a heme-protein from any source, including a heterologous heme-protein and have arrived at the present invention. Although the heme-protein is obtained from a different source (i.e. beef), the burden shifts to establish an unobvious difference between the claimed product and the prior art product.

Regarding **claim 12**, Jamieson discloses all of the claim limitations as set forth above. While Jamieson discloses a composition comprising about 16% beef (*see* Ingredients wherein there is 1 lb. of beef in approximately 6 lb. total ingredients), the reference is silent with respect to the precise amount of heme-containing protein or myoglobin.

Given the “beef” flavor of the Iroquois stew is a variable that can be adjusted by varying the amount of beef in the composition, one of ordinary skill in the art would have been motivated to adjust, in routine processing, the amount of beef added to the stew, to obtain the desired flavor profile.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ELIZABETH GWARTNEY whose telephone number is (571)270-3874. The examiner can normally be reached on M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, D. Lawrence Tarazano can be reached on (571) 272-1515. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ELIZABETH GWARTNEY/
Primary Examiner, Art Unit 1791

Notice of References Cited	Application/Control No. 14/796,970	Applicant(s)/Patent Under Reexamination VRLJIC ET AL.	
	Examiner ELIZABETH GWARTNEY	Art Unit 1791	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
	A	US-				
	B	US-				
	C	US-				
	D	US-				
	E	US-				
	F	US-				
	G	US-				
	H	US-				
	I	US-				
	J	US-				
	K	US-				
	L	US-				
	M	US-				

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Jamieson, J., "Iroquois Stew With Beef, Chicken and Pork" – Bon Appétit, November 1995.
	V	Foo, S., "Beef and Scallop Stir Fry" – Food & Wine, July 2001.
	W	Merriam-Webster, "Definition of HETEROLOGOUS - www.meriam-webster.com/dictionary/heterologous, downloaded September 10, 2015.
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
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www.uspto.gov

BIB DATA SHEET

CONFIRMATION NO. 8501

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
14/796,970	07/10/2015 RULE	426	1791	38767-0010001

APPLICANTS

Impossible Foods Inc., Redwood City, CA

INVENTORS

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Patrick O'Reilly Brown, Stanford, CA;
Jessica Karr, San Francisco, CA;
Celeste Holz-Schietinger, East Palo Alto, CA;
Michael Eisen, Berkeley, CA;
Ranjani Varadan, Fremont, CA;

**** CONTINUING DATA ***************************

This application is a CON of PCT/US2014/011361 01/13/2014
which claims benefit of 61/908,634 11/25/2013
and claims benefit of 61/751,816 01/11/2013

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

07/21/2015

Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☑ No Verified and Acknowledged / / Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		CA	9	30	4

ADDRESS

FISH & RICHARDSON P.C. (TC)
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MINNEAPOLIS, MN 55440-1022
UNITED STATES

TITLE

METHODS AND COMPOSITIONS FOR CONSUMABLES

FILING FEE RECEIVED		
3360	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

BIB (Rev. 05/07).

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14796970	VRLJIC ET AL.
	Examiner	**Art Unit**
	ELIZABETH GWARTNEY	1791

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	09/10/2015								
	1	✓								
	2	✓								
	3	✓								
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	24	N								
	25	N								
	26	N								
	27	N								
	28	N								
	29	N								
	30	N								

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	705	((heme ADJ protein) OR myoglobin OR hemoglobin) WITH meat	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/09/10 10:54
S2	128	((heme ADJ protein) OR myoglobin OR hemoglobin) WITH heterologous	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/09/10 10:54
S3	6	"8927490"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/09/10 12:05
S4	1131	A23L1/31427	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/09/10 12:18
S5	0	"14796970"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/09/10 12:25

9/10/2015 4:07:11 PM
C:\Users\egwartney\Documents\EAST\Workspaces\14796970.wsp

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	14796970	VRLJIC ET AL.
	Examiner	Art Unit
	ELIZABETH GWARTNEY	1791

CPC- SEARCHED		
Symbol	Date	Examiner
A23L1/31427	9/10/2015	EG

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
PALM inventor search completed	9/9/2015	EG
EAST text search - see search history	9/9/2015	EG
Google search / Google Scholar search - beef, chicken, pork and fish stew; heme-containing proteins from bacteria/ and in general.	9/10/2015	EG

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

Attorney's Docket No.: 38767-0010001 / M2CON-Track 1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Marija Vrljic	Art Unit	:	Unknown
Serial No.	:	14/796,970	Examiner	:	Unknown
Filed	:	July 10, 2015	Conf. No.	:	8501
Title	:	METHODS AND COMPOSITIONS FOR CONSUMABLES			

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

INFORMATION DISCLOSURE STATEMENT

Please consider the references listed on the enclosed PTO-1449 Form. Foreign patent documents and non-patent literature are enclosed; U.S. patents and patent application publications will be provided upon request.

This statement is being filed after the receipt of a first Office Action but before the receipt of a Final Office Action or a Notice of Allowance. Therefore, please apply the fees required under 37 CFR § 1.17(p) and any other charges, or credits, to Deposit Account No. 06-1050, referencing the above-indicated Attorney Docket Number.

Please contact the undersigned if there are any questions regarding this Statement.

Respectfully submitted,

/September 21, 2015/ /M. Angela Parsons/

Date:_____ _____

M. Angela Parsons, Ph.D.
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61211968.doc

Substitute Disclosure Form (37 CFR §1.98(b))	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 38767-0010001	Application No. 14/796,970
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Impossible Foods Inc.	
		Filing Date July 10, 2015	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
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Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form

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		Filing Date July 10, 2015	Group Art Unit

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Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
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Examiner Signature	Date Considered

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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		Filing Date July 10, 2015	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
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Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 38767-0010001	Application No. 14/796,970
Information Disclosure Statement by Applicant (Use several sheets if necessary) (37 CFR §1.98(b))		Applicant Impossible Foods Inc.	
		Filing Date July 10, 2015	Group Art Unit

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Examiner Signature	Date Considered

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 38767-0010001	Application No. 14/796,970
Information Disclosure Statement by Applicant (Use several sheets if necessary) (37 CFR §1.98(b))		Applicant Impossible Foods Inc.	
		Filing Date July 10, 2015	Group Art Unit

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Examiner Initial	Desig. ID	Document
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EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form

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Bibliographic data: CN1252231 (A) — 2000-05-10

Hemoglobin food series and its production process

Inventor(s): YU GUOHUA [CN] ± (GUOHUA YU)

Applicant(s): YU GUOHUA [CN] ± (YU GUOHUA)

Classification: **- international:** ***A23J1/06; A23L1/30;*** (IPC1-7): A23J1/06; A23L1/30

- cooperative:

Application number: CN1999117139 19991014

Priority number(s): CN1999117139 19991014

Abstract of CN1252231 (A)

Hemoglobin food series is produced with animal hemoglobin and food material. High-purity hemolobin is extracted by using animal blood as raw material and through traditional separation process. During food production, hemoglobin as additive is added into food raw material. The present invention makes it possible to replace pharmacotherapy with dietotherapy. With high effect and without toxic side-effect, the hemoglobin food series may be used to cure iron-deficiency anemia radically.

[19]中华人民共和国国家知识产权局

[51]Int. Cl[7]
A23J 1/06
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权利要求书1页 说明书3页 附图页数0页

[54]发明名称 血红蛋白系列食品及其制造方法

[57]摘要

本发明涉及一种血红球蛋白系列食品及其制造方法。其特点是:由动物血液提取的血红蛋白和食品原料组成;以动物血为原料,用经典分离血红蛋白的方法提取分离高纯度的血红蛋白,在系列食品的加工生产过程中,将已分离提纯的动物血红蛋白以食品添加剂的形式加入生产中的食品原料中,按照各种食品的生产工艺流程和工艺要求生产出系列食品。本发明以食疗代替药疗,不但可提高服用者特别是儿童患者的兴趣,而且没有服食其他药物的毒性副作用,可以根据缺铁性贫血,安全高效。

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权 利 要 求 书

1. 血红蛋白系列食品，其特征是：由动物血液提取的血红蛋白和食品原料组成。

2. 如权利要求 1 所述的血红蛋白系列食品的制造方法，其特征是：以动物血为原料，用经典分离血红蛋白的方法提取分离高纯度的血红蛋白，在系列食品的加工生产过程中，将已分离提纯的动物血红蛋白以食品添加剂的形式加入生产中的食品原料中，按照各种食品的生产工艺流程和工艺要求生产出系列食品。

3. 如权利要求 1 或 2 所述的血红蛋白系列食品及其制造方法，其特征是：含血红蛋白的系列食品，包括：血红蛋白饮料、血红蛋白饮品、血红蛋白口服液、血红蛋白浸膏、血红蛋白冲剂、血红蛋白胶囊、血红蛋白糖果、血红蛋白饼干、血红蛋白蛋糕、血红蛋白雪羔、血红蛋白雪批（雪条）、血红蛋白甜筒，所述血红蛋白食品的制造方法是：在各种食品的常规工艺条件下，将适量已提纯的动物血红蛋白加入在加工的食品原料中加工而成。

说　明　书

血红蛋白系列食品及其制造方法

本发明涉及一种系列食品及其制造方法，特别是一种血红蛋白系列食品及其制造方法。属于食品技术领域。

贫血是一种常见病，轻症贫血患者面唇及粘膜均乏血色，主要症状为面色苍白；重症贫血患者除具有轻症患者的症状外，还出现毛发干燥，指甲呈匙反甲，并会出现肝、脾淋巴结肿大，食欲减退，心悸、乏力、烦躁、精神不振、注意力不集中、记忆力差、头晕、耳鸣等症状。现有技术中，对贫血的常规治疗方法有两种，一种是服用富含铁质的食物及维生素 C，另一种是药物治疗，药物治疗分为口服硫酸亚铁、葡萄糖亚铁和静肪注射含糖氧化铁或葡萄糖铁。其中口服硫酸亚铁或枸橼酸铁铵对胃粘膜刺激较大，服后会出现恶心、呕吐、腹泻等症状，虽然饭后服食可减少铁剂和刺激，但由于食物冲淡胃酸减低铁的游子化，食物中的磷化物与铁结合生成不溶解的磷酸盐，因而影响人体对铁的吸收，疗效不佳；静脉注射疗法只适用以下几种情况：（1）口服各种铁剂均发生严重反应，改变剂量及服药时间仍然无效，（2）胃肠道疾病影响铁的吸收，（3）胃肠道手术后继发缺铁性贫血，（4）应用口服铁剂治疗血象无改变者。虽然输血能使患者起死回生，但输血是在的血的红蛋白低于 3 克%时的危重患者身上才使用，而且输血只能解决暂时性贫血，不能彻底根治贫血。

本发明的目的是为了克服现有治疗缺铁性贫血技术的缺点，提供一种既无治疗贫血化学药品的毒性副作用，又能根治缺铁性贫血症的血红蛋白系列食品及其制造方法。

本发明的目的可以通过采取如下措施达到：血红蛋白系列食品，其特点是：由动物血液提取的血红蛋白和食品原料组成。

血红蛋白的系列食品的制造方法，其特点是：以动物血为原料，用经典分离血红蛋白的方法提取分离高纯度的血红蛋白，在系列食品的加工生产过程中，将已分离提纯的动物血红蛋白以食品添加剂的形式加入生产中的食品原料中，按照各种食品的生产工艺流程和工艺要求生产出系列食品。

本发明的目的还可通过采取如下措施达到：

血红蛋白系列食品，包括：血红蛋白饮料、血红蛋白饮品、血红

蛋白口服液、血红蛋白浸膏、血红蛋白冲剂、血红蛋白胶囊、血红蛋白糖果、血红蛋白饼干、血红蛋白蛋糕、血红蛋白雪羔、血红蛋白雪批（雪条）、血红蛋白甜筒，所述血红蛋白食品的制造方法是：在各种食品的常规工艺条件下，将适量已提纯的动物血红蛋白加入在加工的食品原料中加工而成。

本发明具有如下突出优点：

1．动物如猪、牛、羊 、马、鸡、鸭、鹅、狗等的血液资源丰富，从动物血液中提取血红蛋白的技术条件和工艺成熟，因此本发明涉及的系列食品具有品质保证，成本低廉。

2．以食品的形式给病患者服用，以食疗代替药疗，不但可提高服用者特别是儿童患者的兴趣，而且没有服食其他药物的毒性副作用，无须考虑服药过量的危险，可以根治缺铁性贫血，增加营养、安全高效。

3．从动物血液中提取高纯度的血红蛋白是一种纯天然的蛋白，用这种血红蛋白制制造的食品是纯天然食品，易于被患者吸收，因此疗效显著，而且生产工艺简单、成本低廉，能减轻患者的经济负担，易于推广应用，特别适合在儿童中推广应用。

众所周知，血红蛋白是一种含铁蛋白质，这种含铁蛋白质在血液中占有重要位置，因此在生命的活动中对含铁蛋白质的补给非常重要。幼儿时铁的摄入量一定要超过排泄量，否则儿童易于缺铁，引起贫血症。一般婴儿体内含铁量为 0.5 克，成人 3～5 克，故小儿在生长发育过程中必须从体外至少获得 2.5～4.5 克铁，即日平均需要 6～16 毫克铁，女性需要量又比男性多，如果正常的红细胞缺铁可影响红细胞在血液循环中正常生存，且易患缺铁性细胞性贫血。正常人体内储存铁，如果原储存的血量正常，当体内大约相当于全血内总量的三分之一时，就算不额外补充铁剂，血象亦可迅速恢复正常，不致于发生贫血。但如果长期慢性失血，每失血 4 毫升约合铁 1 毫克，，虽然每天失血不多，但实际消耗的铁血量已超过正常消耗量的数倍，日积月累，自然造成贫血。此时必须从体外吸收补充铁。

前述分析可知，口服铁剂药物易引起毒性副作用，而静脉注射只能消除暂时的症状，两者都不是理想的治疗方法。只有在食物中添加血红蛋白，长期服用而没有毒性副作用，才能根治缺铁性贫血。

说 明 书

本发明从动物血液中提取高纯度的血红蛋白是一种纯天然的蛋白，用这种血红蛋白制制造的食品是纯天然食品，易于被患者吸收，以食品的形式给病患者服用，以食疗代替药疗，不但可提高服用者特别是儿童患者的兴趣，而且没有服食其他药物的毒性副作用，无须考虑服药过量的危险，可以根治缺铁性贫血，增加营养、安全高效。

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Bibliographic data: CN1301811 (A) — 2001-07-04

Transgenosis ferritin enzyme capable of compensating iron for human and its producing method

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Applicant(s): WU GUANGYAO [CN] ± (WU GUANGYAO)

Classification: **- international:** ***C12N1/19; C12N15/81;*** (IPC1-7): A61K35/72; C12N1/19; C12N15/81

- cooperative:

Application number: CN1999127335 19991229

Priority number (s): CN1999127335 19991229

Abstract of CN1301811 (A)

A production method of transgenic ferritin yeast includes the preparation of engineering bacterium yeast and the high density culture and the culture medium for high density culture consists of cooked germinated soy beam 10 g in water 100g, cane sugar 8 g, yeast extract 1 g, and tryptone 1 g, trace elements Cu, Co, Ca, Zn, Mo and Mn 10 to the power -5 - 10 to the power -4 mole/L each, and saturated fumaric acid solution of iron 6 ml. The present invention has simple production process and high ferritin expression amount in ferritin yeast, each liter of which may be used to obtain dry iron-riched yeast with iron content of 5% in 15g. The transgenic ferritin yeast may be used in compensation iron for both healthy people and iron deficiency patient.

[19]中华人民共和国国家知识产权局

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权利要求书1页 说明书3页 附图页数4页

[54]发明名称 一种可用于人体补铁的转基因铁蛋白酵母及其生产方法

[57]摘要

一种可用于人体补铁的转基因铁蛋白酵母及其生产方法，方法包括：工程菌 酵母的制备及其高密度培养，所述的高密度培养的培养基配方为发芽黄豆10克，水100ml，煮后，溶液中加入蔗糖8克、酵母提取物1克、胰蛋白胨1克，配方中还 含微量元素（铜、钴、钙、锌、钼、锰）各10^{-5}—10^{-4}克分子/升，6ml 饱和延胡索酸铁溶液。本发明生产工艺简单，铁蛋白酵母的铁蛋白表达量高、每升可得15克干富铁酵母产品含铁量达5%，可用于正常人体铁的补充，也可用 于缺铁病人的补铁。

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权 利 要 求 书

1、一种可用于人体补铁的转基因铁蛋白酵母，其特征在于：它含有铁蛋白，每分子 8.9KD 的蛋白含有 8 个铁原子。

2、一种生产权利要求 1 所述的用于人体补铁的转基因铁蛋白酵母的方法，其特征在于：它包括以下步骤：

① 工程菌酵母的制备，它包括：重组质粒的构建，得到 pYES2-psaC 表达载体；酵母的转化，经电击法将表达载体进入酵母；

② 转基固铁蛋白酵母的生产，它包括：转基因酵母在高密度的液体培养基上培养，再经破壁，干燥、粉碎得到粉末产品。

3、按权利要求2所述铁蛋白酵母生产方法其特征在于所用的高密度培养基配方为： 发芽黄豆10克，水100ml，煮后，溶液中加入蔗糖8克、酵母提取物1克、胰蛋白胨1克，配方中还含微量元素(铜、钴、钙、锌、钼、锰)各 10^{-5}-10^{-4}克分子/升，6ml 饱和延胡索酸铁溶液。

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说　　明　　书

一种可用于人体补铁的转基因铁蛋白酵母及其生产方法

本发明涉及一种可用于人体补铁的转基因铁蛋白酵母及其制备方法。

人体容易缺铁，特别是儿童和孕妇，目前医药上用无机铁(如硫酸亚铁)补充人体需要，以减少因缺铁而引起贫血症，但效果很差，因无机铁不易吸收，且口感不好，易引起恶心和降低食欲，国际上都正在以铁蛋白补铁为研究方向。我们曾用植物中的铁氧还蛋白(Ferredoxin)基因转化到大肠杆菌中用于提取补铁蛋白(申请号：98125609.0)。

本发明目的是提供一种可用于人体补铁的转基因铁蛋白酵母及其生产方法，该方法中获得的工程菌酵母的铁蛋白表达量高，含铁量多。

为达到上述的目的，本发明采用以下技术方案：这种可用于人体补铁的转基因铁蛋白酵母，它含有铁蛋白，每分子8.9KD的蛋白含有8个铁原子。

用于人体补铁的转基因铁蛋白酵母的方法包括以下步骤：

① 工程菌酵母的制备，它包括：重组质粒的构建，得到pYES2-psaC 表达载体；酵母的转化，经电击法将表达载体进入酵母；

② 转基因铁蛋白酵母的生产，它包括：转基因酵母在高密度的液体培养基上培养，再经破壁，干燥、粉碎得到粉末产品。

现在我们使用的铁蛋白 (PsaC)是植物中广泛存在的一种蛋白质，例如，聚球兰藻中的铁蛋白,在它的每分子中含有8个铁原子，其分子量是8900道尔顿，也是一种非血晶质的铁。

以下结合实施例及附图对本发明作详细说明

图1.重组质粒pYES2-psaC构建图

图2.聚球兰藻psaC的基因序列和蛋白序列

图3.重组质粒pYES2-psaC的酶切鉴定图谱

图4.转PsaC基因酵母的Southern鉴定

图5.psaC基因在酵母中高效表达鉴定图谱

图2中，蛋白中的氨基酸用一字英文字母表示法。图3中，M为标准DNA(pBR322/BstNI Marker)，I为质粒的酶切(EcoRI+BamHI)结果。证明重组质粒构建正确。

图4中，1.质粒酶切(EcoRI+BamHI)；2.pYES2-psaC质粒；3.转基因酵母总DNA酶切(EcoRI+BamHI)；4.未转基因酵母总DNA对照。0.45kb为含有psaC基因片段，结

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果证明psaC基因已转化进入酵母。

图5中，1.标准蛋白分子量；2.pET3a-psaC在大肠杆菌中被表达的全蛋白对照；3.含pYES2空载酵母全蛋白对照；4.含pYES2-psaC的酵母全蛋白。证明psaC基因已在酵母中高效表达。

实施例1.工程菌酵母的制备

1.重组质粒的构建

首先将载有psaC基因的载体pUC19用XbaI和NdeI双酶切，获得的psaC DNA片段被克隆在pET-3a原核表达载体上。再将pET3a-psaC表达载体用BamHI和EcoRI双酶切，所得psaC 片段克隆在pYES2载体上，从而获得pYES2-psaC表达载体。构建策略见图1。

以重组质粒pYES2-psaC为模板，用T7启动子引物进行测序，经ABI PRISM™ 777DNA测序仪测定，结果表明psaC序列完全正确，其核苷酸序列和氨基酸序列见图2，全序列由81个氨基酸残基组成。

2. 重组质粒的酶切鉴定

pYES2-psaC表达载体的酶切鉴定图谱见图3。经BamHI和EcoRI 双酶切后，得到两条各为5.9kb和0.45kb 的预期片断，0.45kb片段中含有psaC基因，证明构建完全正确。

3.酵母的转化

酵母在增殖培养基(1%酵母提取物（市场有售），2%蛋白胨和2%葡萄糖)中30℃震荡培养至对数生长期，4000×g离心收集菌体。菌体用重蒸水洗涤一次，并用预冷山梨醇溶液(1mol/L)洗涤两次，得酵母悬液(OD值为200)。取40ul酵母悬液与5ul质粒DNA(100ug)混合，在150V，25uF和200Ω下电击4.2-4.9ms。电击后加入1ml预冷山梨醇溶液(1mol/L)，在选择性培养基(1mol/L山梨醇，0.17%YNB-AA/As，0.5%$(NH4)_2SO4$，2%葡萄糖，0.01%L-His，0.01%L-Leu，0.01%L-Trp和2%琼脂)上，30℃培养，2-3天后出现克隆斑。

4.转化酵母的鉴定

经电击法将表达载体转化进入酵母后，对酵母的 PsaC进行Southern杂交分析，分析结果如图4。由图4表明psaC基因已被整合到酵母中。

实施例2.转基因铁蛋白酵母的生产

转基因酵母在高密度的液体培养基上30℃通气培养12小时内即可达到对数生长期高峰，每升培养液可获得15克酵母干粉，产品含铁量达5%。培养基配方为：发芽黄豆10克，沸水100ml，煮30分后，溶液中加入蔗糖8克、酵母提取物1克、胰蛋

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白胨1克，配方中还含微量元素(铜、钴、钙、锌、钼、锰)各 10^{-5}-10^{-4}克分子/升，6ml饱和延胡索酸铁溶液。

上述培养的酵母经高压细胞破碎仪破壁，上清液进行SDS-PAGE电泳测定，表明psaC基因被表达，电泳结果见图5。

大量生产上可经板框过滤得湿酵母，再加热干燥粉碎即可得富铁酵母粉。

本发明优点：

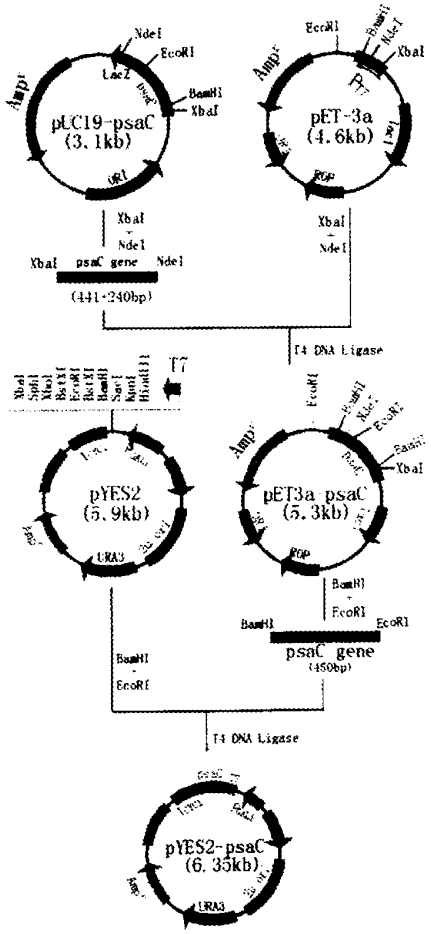
① 首次用转植物铁蛋白基因到酵母中，并用于给人补铁。

② 工程菌酵母的产物表达量高，不用纯化即可为人补铁，有利于工业化生产。

③ 面包酵母中除含有大量铁外，还有其它丰富的营养有助于人体健康。

④ 由于使用高密度培养基，酵母产量高，每升培养基可产生15克酵母干粉，产品含铁量达5%。

说 明 书 附 图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ori
XbaI
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XbaI psaC gene NdeI
(441+240bp)
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URA3
ori

图 1

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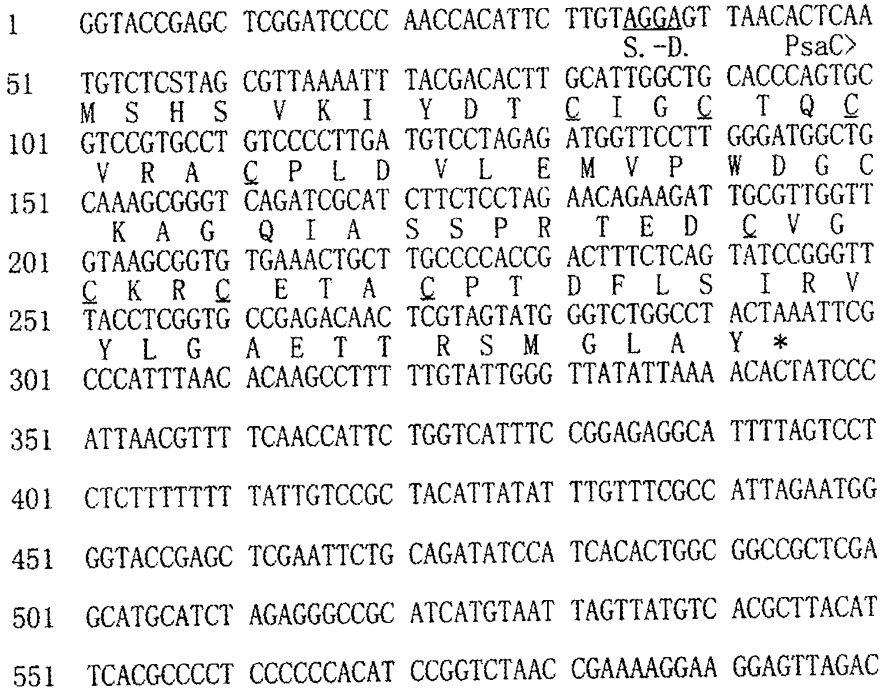
1 GGTACCGAGC TCGGATCCCC AACCACATTC TTGTAGGAGT TAACACTCAA
S.-D. PsaC>
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M S H S V K I Y D T C I G C T Q C
101 GTCCGTGCCT GTCCCCTTGA TGTCCTAGAG ATGGTTCCTT GGGATGGCTG
V R A C P L D V L E M V P W D G C
151 CAAAGCGGGT CAGATCGCAT CTTCTCCTAG AACAGAAGAT TGCGTTGGTT
K A G Q I A S S P R T E D C V G
201 GTAAGCGGTG TGAAACTGCT TGCCCCACCG ACTTTCTCAG TATCCGGGTT
C K R C E T A C P T D F L S I R V
251 TACCTCGGTG CCGAGACAAC TCGTAGTATG GGTCTGGCCT ACTAAATTCG
Y L G A E T T R S M G L A Y *
301 CCCATTTAAC ACAAGCCTTT TTGTATTGGG TTATATTAAA ACACTATCCC
351 ATTAACGTTT TCAACCATTC TGGTCATTTC CGGAGAGGCA TTTTAGTCCT
401 CTCTTTTTTT TATTGTCCGC TACATTATAT TTGTTTCGCC ATTAGAATGG
451 GGTACCGAGC TCGAATTCTG CAGATATCCA TCACACTGGC GGCCGCTCGA
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551 TCACGCCCCT CCCCCCACAT CCGGTCTAAC CGAAAAGGAA GGAGTTAGAC

图 2

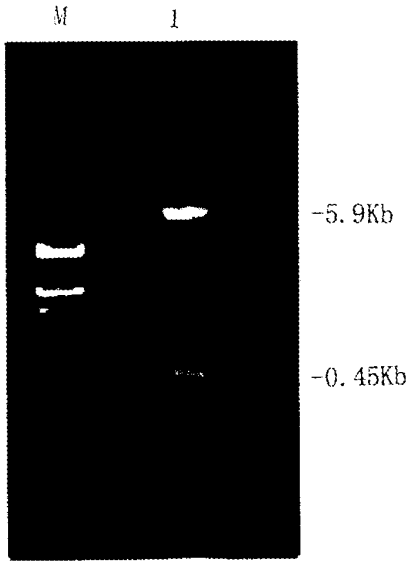

图 3

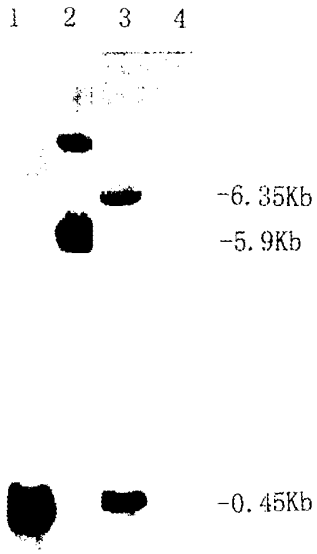

图 4

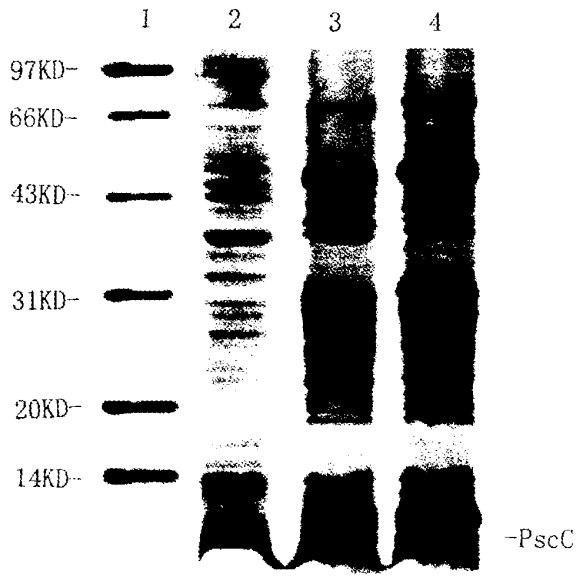

图 5

Espacenet

Bibliographic data: CN1407108 (A) — 2003-04-02

Genetic engineering recombination strain expression of lactoferritin and use thereof

Inventor(s): HU FANG [CN]; WANG CHUNXIANG [CN] ± (FANG HU, ; CHUNXIANG WANG)

Applicant(s): HU FANG [CN] ± (HU FANG)

Classification: **- international:** ***A61K35/74; C12N1/19; C12N1/21; C12N15/12; C12N15/31; C12N15/54; C12N15/74; C12N15/81; C12P21/00;*** (IPC1-7): A61K35/74; C12N1/19; C12N1/21; C12N15/12; C12N15/31; C12N15/54; C12N15/74; C12N15/81; C12P21/00

- cooperative:

Application number: CN2001142001 20010906

Priority number(s): CN2001142001 20010906

Abstract of CN1407108 (A)

A molecular biologic technique is used to configure the expression carrier containing lactoferritin gene, which is then transferred into useful bacteria by electric transfer method to obtain the recombinant useful bacterial strains which can stably exprss human endostatin gene. After fermented, one or more recombinant strains can be used as oral-applied preparation to treat or prevent the infective diseases caused by viruses, suppress the growth of tumor cells and improve immunity.

[19] 中华人民共和国国家知识产权局

[12] 发明专利申请公开说明书

[21] 申请号 01142001.4

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C12N 15/74
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C12N 15/31 C12N 15/54
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A61K 35/72

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权利要求书 2 页 说明书 7 页 附图 4 页

[54] 发明名称 表达乳铁蛋白的基因工程重组菌株及其应用

[57] 摘要

本发明阐述用分子生物学技术构建含有乳铁蛋白基因的表达载体，并通过电转化法转入益生菌中，得到稳定表达人内皮细胞抑制素基因的重组益生菌菌株。一种或多种重组菌株经发酵处理后作为口服制剂，通过口服，在体内表达有活性的乳铁蛋白及其多肽片段。所表达的乳铁蛋白及其多肽片段能够治疗和预防病毒引起的传染病，抑制肿瘤细胞的生长，缓解肿瘤病人经放化疗后引起的消化道功能紊乱、促进患者胃肠道内益生菌群的生长和提高机体免疫能力；并可用于食品、保健品。

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1. 一种新的表达乳铁蛋白多肽的基因工程重组益生菌菌株,其特征为带有乳铁蛋白基因的表达元件整合到其染色体上或存在于游离质粒中。

2. 权利要求 1 中所述的乳铁蛋白来自人、牛或猪的乳铁蛋白,包括天然的全长的乳铁蛋白、乳铁蛋白多肽片段.

3 权利要求 1 中所述的益生菌为原核革兰氏阳性细菌和真核酵母菌。

4. 权利要求 3 中所述的原核革兰氏阳性细菌为乳酸菌，包括双歧杆菌属（*Bifidobacterium*）、乳杆菌属(*Lactobacillus*)、乳球菌属(*Lactococcus*)、链球菌属(*Streptococcus*)、肠球菌属(*Enterococcus*)、明串珠菌属（*Leuconostoc*）、利斯特氏菌属（*Listeria*）、或芽胞杆菌属（*Bacillus*）。

5 权利要求 4 中所述的双歧杆菌为青春双歧杆菌（*B.adolescentis*）或长双歧杆菌（*B.longum*）。

6 权利要求 4 中所述的乳杆菌为干酪乳杆菌（*L.casei*）或米酒乳杆菌（*L.sake*）或植物乳杆菌（*L.plantarum*）。

7 权利要求 4 中所述的乳球菌为乳酸乳球菌（*L.lactis*）。

8 权利要求 4 中所述的链球菌为格氏链球菌（*S.gordoni*）。

9 权利要求 4 中所述的肠球菌为粪肠球菌（*E.faecalis*）。

10 权利要求 4 中所述的利斯特氏菌为无害利斯特氏菌（*L.innocua*）。

11 权利要求 4 中所述的芽胞杆菌为枯草芽孢杆菌（*B.subtilis*）或蜡样芽孢杆菌（*B.cereus*）。

12 权利要求 4 中所述的真核酵母菌为酿酒酵母（*Saccharomyces serevisiae*）。

13 一种用于转化益生菌的质粒，其特征在于含有下列从 5’ 到 3’连接的调控因子：启动子、编码乳铁蛋白基因序列、转录终止子。

14 权利要求 13 中所述启动子，是适用于相应重组益生菌菌株的启动子，包括选自乳球菌 NisA 基因、短乳杆菌 SlpA 基因、乳球菌 P59、乳球菌 P32 启动子、乳球菌 P23 启动子或酵母磷酸甘油酸激酶(Phosphoglycerate Kinase,PGK)基因启动子。

15 权利要求 13 中所述转录终止子，是适用于相应重组益生菌菌株的转录终止

Case No.: IPR2023-00208
U.S. Patent No. 10,039,306

子，包括选自人乳头瘤病毒 E7 基因、乳球菌 Usp45 基因、短乳杆菌 SlpA 基因或酵母磷酸甘油酸激酶(Phosphoglycerate Kinase,PGK)基因转录终止信号。

16 权利要求 13 中所述的质粒，可进一步包含分泌信号肽。

17 权利要求 16 中所述分泌信号肽，是适用于相应重组益生菌菌株的分泌信号肽，包括选自短乳杆菌 SlpA 基因、乳球菌 Usp45 基因或酵母 MFα1 信号肽编码序列。

18 权利要求 13 中所述的质粒，可进一步包含适用于相应重组益生菌菌株的复制起始子，包括选自乳酸菌 pWV01、pAMß1 复制子或大肠杆菌 colE1 复制子。

19 含有权利要求 13 所述的重组质粒的益生菌菌株，包括：重组乳酸乳球菌 CGMCC －0622、CGMCC－0623。

20 一种新的传递乳铁蛋白多肽的方法，其特征是利用可接受药用载体在消化道、女性生殖道、或其它粘膜处及体表施用权利要求 1 或权利要求 19 所述的表达乳铁蛋白多肽的重组益生菌菌株。

21 权利要求 1 中所述重组益生菌菌株经发酵后，一种或多种菌株混合制成口服液、喷雾剂、胶囊、或片剂，用于治疗或预防病毒或细菌引起的传染病，抑制肿瘤细胞生长、缓解肿瘤病人经放化疗后引起的消化道功能紊乱、促进患者胃肠道内益生菌群的生长和提高机体免疫能力；并可用于食品、保健品。

Motif Exhibit 1148, Page 239 of 3341

表达乳铁蛋白的基因工程重组菌株及其应用

本发明涉及一种全新的产品，它能在体内传递有活性的乳铁蛋白多肽，并用于预防和治疗多种疾病。本发明的方法是将乳铁蛋白基因克隆到益生菌，通常是乳酸菌如乳球菌和乳杆菌的表达载体上，用电转化方法获得转乳铁蛋白基因的重组菌株。通过蛋白杂交及生物活性测定，证明得到的重组菌株确实能够产生有生物活性的乳铁蛋白多肽。然后用一种或多种重组益生菌菌株制成不同的制剂如口服液、冻干粉压片、胶囊或喷雾剂，提供给患者，使其在消化道和阴道或其它粘膜处及体表能表达乳铁蛋白，由于所使用的益生菌不仅不生产任何对人体有害的毒素，而且对人体健康还有不同程度的促进作用，益生菌在人体内能够不断地繁殖，因而我们能够利用这种重组的益生菌成为在体内制造这种生物活性蛋白的“工厂”，这也是在体内传递有生物活性的乳铁蛋白的一种全新的方法，从而达到预防和治疗多种疾病的目的。

乳铁蛋白是一种天然的非血红素铁结合糖蛋白，属于转铁蛋白家族的一员。上个世纪六十年代首次在牛初乳中发现的。其分布很广，主要存在于母乳中，尤其于初乳中含量最高。它是母乳中含量第二多的蛋白质，占普通母乳蛋白质的 10-20%。除乳汁外，其他的消化、呼吸和生殖系统的外分泌液中也含有一定量的乳铁蛋白，如泪液、精液、唾液、滑液等。血液中也能检测到乳铁蛋白的存在。血液中的乳铁蛋白来自于嗜中性粒细胞，嗜中性粒细胞在炎症处被激活后会合成并释放出乳铁蛋白。

人乳铁蛋白是分子量为 78 kDa 的单体糖蛋白，其在不同的种属中分别含 1-4 个多聚糖。牛与人的乳铁蛋白分别含 689 和 691 个氨基酸，其同源性为 69%。两者的三维结构非常相似。每个乳铁蛋白由两个相同的叶组成，分别命名为 N-和 C-叶。每个叶又可分为两个亚叶，亚叶之间形成一个裂缝。此裂缝通过一个双碳酸根离子与 Fe^{3+}的协同作用牢固结合 Fe^{3+}，每分子可结合两个 Fe^{3+}。乳铁蛋白具有促铁吸收、免疫调节、抑菌、杀菌、抗病毒、阻断氧自由基、调节骨随细胞的生长和促进肠道内益生菌生长等功能。最新研究显示乳铁蛋白还有预防和抑制大肠癌发生和扩散的作用。此外，它还能抑制丙型肝炎病毒的增殖，而且没有副作用。现已发现去糖基化蛋白保留了天然乳铁蛋白的所有生物功能。乳铁蛋白的稳定性是随 PH、盐离子浓度和环境其它因素而改变。在酸性条件下，乳铁蛋白非常稳定，对体内一些蛋白酶不敏感。大量试验证明被蛋白酶水解的一些乳铁蛋白多肽仍具有抗菌活性，如 lactoferricin H (包含 N 端 1-47 氨基酸)，其抑菌和杀菌活性比完整的乳铁蛋白还高。乳铁蛋白的 N1 端参与与 LPS 的结合，乳铁蛋白还与溶菌酶，肝素、和 DNA 结合。

由于乳铁蛋白的重要生理功能，自其发现的近四十年来，吸引了众多科学家的兴趣，目前已对其结构特征和生理功能有相当的了解，对其进行商业化开发的时机已经成熟。由于乳铁蛋白具有以上的生理功能，所以其在国外得到广泛的应用。多年来乳铁蛋白主要用作乳制

品添加剂和婴幼儿食品添加剂，模仿母乳，提高抗病菌能力。在乳制品中乳铁蛋白与益生菌混用，作为运动员营养的一部分。生产片剂或速溶饲料，用作宠物食品，抗病毒。作为功能食品，增加铁的可溶性和帮助对铁的吸收，用于儿童和孕妇的补铁制品。添加到化妆品中，用于抗氧化，抗衰老，甚至用于口腔保护剂和口香糖，改善口腔卫口等。市售多种奶粉、鲜奶，多不含乳铁蛋白， 这是因为其产品制造过程中，加热、杀菌等程序，破坏了乳铁蛋白。目前商业化的乳铁蛋白来源主要有两种：1）从牛奶中直接提取，用作保健品、饮料、婴儿食品、口腔护理以及化妆品等。2）基因工程表达的重组人乳铁蛋白。表达系统有用曲霉菌表达和转基因牛，表达后经提纯为蛋白质纯品，用作临床药物的开发。

目前临床实验表明，大剂量的乳铁蛋白才有较好的预防和治疗效果，由于以上两种方法产量低，生产成本高，不能满足人们对乳铁蛋白的需求。因而，需要发展一种更有效和更经济的方法生产乳铁蛋白。我们发明的用表达乳铁蛋白多肽的基因工程重组益生菌菌株在体内传递乳铁蛋白的方法，具有生产工艺简单、成本低、疗效好的巨大优势，将取代其它几种方法。

本发明提供了获得表达乳铁蛋白多肽的重组益生菌的方法，并提供了生产这种重组益生菌菌株的方法和其在动物和人体内的应用。本发明也提供了新的益生菌表达载体的构建，特别是乳酸菌中乳球菌和乳杆菌质粒的构建，它使乳铁蛋白多肽能够表达，并具有一定的生物学活性。

本发明方法包括以下步骤：

1. 本发明构建了带有乳铁蛋白基因的各种不同的益生菌表达载体。

益生菌主要包括原核革兰氏阳性细菌和真核酵母菌，而原核革兰氏阳性细菌主要是指一些有利于人和畜牧身体健康的乳酸菌，如双歧杆菌、乳杆菌、乳球菌和链球菌。基因工程乳酸菌的研究起步较晚，由于乳酸菌自身遗传特性比较复杂性，其技术难点主要集中在质粒的构建和蛋白的表达。真核蛋白表达的难度大，与大肠杆菌相比，表达量极低。不同质粒、不同启动子及不同信号肽都对外源蛋白的表达影响很大。

本发明采用几种乳酸菌能识别的强启动子及信号肽等元件，构建了多种诱导和非诱导型胞内和胞外表达的乳酸菌表达质粒，这些质粒可游离地存在于乳酸菌中。

另外，将这些质粒中包括乳铁蛋白基因在内的表达元件部分通过酶切、连接等方法克隆到乳酸菌整合质粒，如 pG+host、pJIM2481 或 Tn916、Tn917、Tn919 等转座子上，从而整合到乳酸菌染色体中，构建了可持续、稳定表达乳铁蛋白基因的重组乳酸菌菌株。

酵母表达体系最大的优点在于它属于真核生物表达系统，能够进行蛋白质修饰作用，而且酵母为单细胞生物，生长速度快，便于进行分子生物学操作。

在酵母中表达外源基因和分泌外源蛋白必须具备下列条件：酵母识别的强启动子，信号

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肽序列，转录终止信号，翻译终止密码子，合适的表达框架等。本发明采用带有酵母磷酸甘油酸激酶(Phosphoglycerate Kinase,PGK)基因启动子和转录终止信号的表达载体，在启动子后插入酵母天然信号肽——MFα1 信号肽编码序列和乳铁蛋白基因序列，从而构建了可在酵母菌中分泌表达重组乳铁蛋白或多肽片段的表达质粒。

2. 本发明构建了能稳定表达具有生物学活性的乳铁蛋白多肽的益生菌菌株。

上述含有乳铁蛋白基因或基因片段的相应表达载体，通过电转化的方法，分别转入酵母菌、双歧杆菌、乳杆菌、乳球菌、链球菌、肠球菌、明串珠菌、利斯特氏菌、和芽胞杆菌中，游离存在或整合到染色体上，得到能稳定表达具有生物学活性的乳铁蛋白多肽的重组益生菌菌株。

重组菌株经发酵诱导后，提纯所表达的乳铁蛋白，用标准品作对照，用已建立好的乳铁蛋白生物活性的检测方法证明重组益生菌分泌出的乳铁蛋白本身具有生物活性。

3. 提供了利用上述重组益生菌在动物或人体内生产和传递乳铁蛋白多肽的方法及应用。

重组菌株分别用适宜培养基培养，得到的发酵液可制备成各种剂型，如口服液喷雾剂、胶囊、或片剂，施用于消化道、阴道或其它粘膜处，用于治疗或预防病毒或细菌引起的传染病，抑制肿瘤细胞生长、缓解肿瘤病人经放化疗后引起的消化道功能紊乱、促进患者胃肠道内益菌群如双歧杆菌的生长和提高机体免疫能力。

本发明具有以下优点：

1. 采用益生菌自身表达载体，可以稳定高效表达外源基因。

2. 所用的益生菌本身对人和畜牧是安全的，不产生内外毒素，同时对人和畜牧的身体健康还有促进作用，如某些益生菌能够维持人体肠道内固有菌群的平衡，对肠道致病菌有拮抗和抑制作用，对调整肠道菌群失调和治疗急慢性腹泻有良好的效果，同时能降低血内毒素水平，提高人体免疫力。

3. 直接使用能够表达人乳铁蛋白基因的重组益生菌，不需经过蛋白纯化，此产品生产工艺简单，成本低。

4. 重组益生菌可以在人和动物体内不断繁殖，在一定时期内持续表达并提供给机体乳铁蛋白，具有很好的特异性和有效性。

以下通过实施例和附图对本发明作进一步详细说明。这些实施例和附图说明本发明优选的实施方案，因此不被认为限制其范围。此发明应包括其它等效的实施方案。

图 1 表示乳酸菌表达载体 pLA311 的构建过程。其中，PnisA 为启动子，SPusp 为信号肽序列。

图 2 表示乳酸菌表达载体 pLA313 的构建过程。其中，PslpA 为启动子，SPslpA 为信号

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肽序列。

图 3 表示乳酸菌表达载体 pLA315 的构建过程。其中，P59 为启动子，SPusp 为信号肽序列。

图 4 表示乳酸菌表达载体 pLA317 的构建过程。其中，P32 为启动子，SPusp 为信号肽序列。

实施例 1：乳酸菌表达载体 pLA311 和 pLA319 的构建

质粒 pLA141 来源于乳酸菌质粒 pGK，有 colE1 和 pWV01 复制子，可以在大肠杆菌和乳酸菌中复制，带有乳球菌 NisA 启动子和乳球菌 Usp45 信号肽，在 Nisin 诱导下可启动其后外源基因的表达，表达的重组蛋白在信号肽的牵引下分泌至胞外。

根据已发表的人乳铁蛋白 cDNA 序列，设计合成两段引物

上游：agcgatgcatcaggccgtaggagaaggagtgtt

下游：agcgactagtttacttcctgaggaattcacaggcttc

在两段引物中分别引入 NsiI 位点和 SpeI 位点，以人乳铁蛋白 cDNA 为模板，PCR 扩增人乳铁蛋白基因。

反应条件为 94℃变性 5 分钟，以下循环 94℃变性 30 秒，65℃退火 30 秒，72℃延伸 3 分钟，共 30 个循环。

将 PCR 产物用限制性内切酶 NsiI 和 SpeI 消化，将得到的人乳铁蛋白基因连接到 pLA141 质粒的 NsiI-SpeI 位点，电转化乳酸乳球菌 NZ9000，筛选氯霉素抗性的转化子，提取质粒，经酶切鉴定，PCR 扩增及测序，证明人乳铁蛋白基因已插入 pLA141，序列正确，重组质粒命名为 pLA311。

具体过程见图 1

将重组质粒 pLA311 用限制性内切酶 BamHI 和 NsiI 消化，切除质粒上的 usp45 信号肽序列，再加入 klenow 片段补平酶切后的粘性末端，质粒自连后转化乳酸乳球菌 NZ9000，筛选氯霉素抗性的转化子，提取质粒，经酶切鉴定，PCR 扩增及测序，证明序列正确，得到的重组质粒命名为 pLA319

实施例 2：乳酸菌表达载体 pLA313 和 pLA321 的构建

质粒 pLA178 来源于乳酸菌质粒 pGK,有 colE1 和 pWV01 复制子，可以在大肠杆菌和乳酸菌中复制，带有乳球菌 slpA 启动子和 slpA 信号肽，可高效启动外源基因的表达。将 PCR 得到的人乳铁蛋白基因用限制性内切酶 NsiI 和 SpeI 消化，得到的片段连接到 pLA178 质粒启动子和信号肽后的的 NsiI-SpeI 位点，构建出可在乳酸菌中持续高效表达乳铁蛋白基因的

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重组表达载体，电转化乳酸乳球菌 MG1363，筛选氯霉素抗性的转化子，提取质粒，经酶切鉴定，PCR 扩增及测序，证明人乳铁蛋白基因已插入 pLA178，序列正确，重组质粒命名为 pLA313。pLA313 在乳酸菌中表达的乳铁蛋白可分泌至胞外。

具体过程见图 2

将重组质粒 pLA313 用限制性内切酶 BamHI 和 NsiI 消化，切除质粒上的 slpA 信号肽序列，再加入 klenow 片段补平酶切后的粘性末端，质粒自连后转化乳酸乳球菌 MG1363，筛选氯霉素抗性的转化子，提取质粒，经酶切鉴定，PCR 扩增及测序，证明序列正确，得到的重组质粒命名为 pLA321

实施例 3：乳酸菌表达载体 pLA315 和 pLA323 的构建

质粒 pLA136 来源于乳酸菌质粒 pIL252，有 colE1 和 p AMß1 复制子，在大肠杆菌和乳酸菌中都能复制，带有乳球菌 P59 启动子和乳球菌 Usp45 信号肽，将 PCR 得到的人乳铁蛋白基因用限制性内切酶 NsiI 和 SpeI 消化，得到的片段接入启动子和信号肽后的 NsiI-SpeI 位点，构建带有乳铁蛋白基因的重组表达载体，电转化乳酸乳球菌 MG1363，筛选氯霉素抗性的转化子，提取质粒，经酶切鉴定，PCR 扩增及测序，证明人乳铁蛋白基因已插入 pLA136，序列正确，重组质粒命名为 pLA315。。该载体在乳酸菌中可持续表达乳铁蛋白，生成的乳铁蛋白能够分泌到胞外。

具体过程见图 3

将重组质粒用限制性内切酶 BamHI 和 NsiI 消化，切除质粒上的 Usp45 信号肽序列，再加入 klenow 片段补平酶切后的粘性末端，质粒自连后转化乳酸乳球菌 MG1363，筛选氯霉素抗性的转化子，提取质粒，经酶切鉴定，PCR 扩增及测序，证明序列正确，得到的重组质粒命名为 pLA323

实施例 4：乳酸菌表达载体 pLA317 和 pLA325 的构建

质粒 pLA176 来源于乳酸菌质粒 pGK，有 colE1 和 pWV01 复制子，可以在大肠杆菌和乳酸菌中复制，带有乳球菌 P32 启动子和乳球菌 Usp45 信号肽，可启动外源基因的表达。将 PCR 得到的人乳铁蛋白基因用限制性内切酶 NsiI 和 SpeI 消化，得到的片段接入该质粒启动子和信号肽后的 NsiI-SpeI 位点，构建带有乳铁蛋白基因的重组表达载体，电转化乳酸乳球菌 MG1363，筛选氯霉素抗性的转化子，提取质粒，经酶切鉴定，PCR 扩增及测序，证明人乳铁蛋白基因已插入 pLA176，序列正确，重组质粒命名为 pLA317。该载体在乳酸菌中可持续表达乳铁蛋白基因，生成的乳铁蛋白能够分泌到胞外。

具体过程见图 4

将重组质粒用限制性内切酶 BamHI 和 NsiI 消化，切除质粒上的 Usp45 信号肽序列，再加入 klenow 片段补平酶切后的粘性末端，质粒自连后转化乳酸乳球菌 MG1363，筛选氯霉素抗性的转化子，提取质粒，经酶切鉴定，PCR 扩增及测序，证明序列正确，得到的重组质

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粒命名为 pLA325

实施例 5：重组表达载体 pLA312 和 pLA320 的构建

根据已发表的人乳铁蛋白 cDNA 序列，设计合成两段引物

上游：agcgatgcatcaggccgtaggagaaggagtgtt

下游：agcgaggttcttagatacactggatgggggagtctct

在两段引物中分别引入 NsiI 位点和 EcoRI 位点，以人乳铁蛋白 cDNA 为模板，PCR 扩增人乳铁蛋白基因编码前 47 个氨基酸的基因片段。

反应条件为 94℃变性 5 分钟，以下循环 94℃变性 30 秒，65℃退火 30 秒，72℃延伸 30 秒，共 30 个循环。

将 PCR 产物用限制性内切酶 NsiI 和 EcoRI 消化，将得到的基因片段连接到 pLA141 质粒的 NsiI-EcoRI 位点，电转化乳酸乳球菌 NZ9000，筛选氯霉素抗性的转化子，提取质粒，经酶切鉴定，PCR 扩增及测序，证明人乳铁蛋白基因片段已插入 pLA141，序列正确，重组质粒命名为 pLA312。

将重组质粒用限制性内切酶BamHI和NsiI消化，切除质粒上的信号肽序列，再加入klenow片段补平酶切后的粘性末端，质粒自连后转化乳酸乳球菌 NZ9000，筛选氯霉素抗性的转化子，提取质粒，经酶切鉴定，PCR 扩增及测序，证明序列正确，得到的重组质粒命名为 pLA320。

实施例 6：重组表达载体 pLA314 和 pLA322 的构建

将 PCR 得到的人乳铁蛋白前 47 个氨基酸的基因片段用限制性内切酶 NsiI 和 EcoRI 消化，得到的片段连接到 pLA178 质粒的 NsiI-EcoRI 位点，电转化乳酸乳球菌 MG1363，筛选氯霉素抗性的转化子，提取质粒，经酶切鉴定，PCR 扩增及测序，证明基因片段已插入 pLA178，序列正确，重组质粒命名为 pLA314。

将重组质粒用限制性内切酶BamHI和NsiI消化，切除质粒上的信号肽序列，再加入klenow片段补平酶切后的粘性末端，质粒自连后转化乳酸乳球菌 MG1363，筛选氯霉素抗性的转化子，提取质粒，经酶切鉴定，PCR 扩增及测序，证明序列正确，得到的重组质粒命名为 pLA322。

实施例 7：采用电转化法，将上述几种重组质粒分别转入乳球菌中。

将重组表达载体 pLA313、pLA314、pLA315、pLA317、pLA321、pLA322、pLA323、pLA325 分别与乳酸乳球菌 MG1363 感受态细胞在电转杯中混匀，电击，涂到含氯霉素的培养基上，筛选转入重组质粒的菌株。

乳酸乳球菌 NZ9000 是将 Nisin 抗性基因转入 MG1363 染色体中得到的工程菌，可在 Nisin 存在的条件下正常生长，将带有 PnisA 启动子的重组质粒转入 NZ9000 中，可加入 Nisin 诱导质粒表达外源基因。将重组表达载体 pLA311、pLA312、pLA319、pLA320 与乳酸乳球菌 NZ9000 感受态细胞在电转杯中混匀，电击，涂到含氯霉素的培养基上，筛选转入重组质粒

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的菌株。

实施例 8：采用电转化法，将上述几种重组质粒分别转入乳杆菌中。

将重组表达载体 pLA313、pLA314、pLA315、pLA317、pLA321、pLA322、pLA323、pLA325 分别与干酪乳杆菌感受态细胞在电转杯中混匀，电击，涂到含氯霉素的 MRS 培养基上，在厌氧箱中培养，筛选转入重组质粒的菌株。

干酪乳杆菌 NY1000 是将 Nisin 抗性基因转入干酪乳杆菌染色体中得到的工程菌，可在 Nisin 存在的条件下正常生长，将带有 PnisA 启动子的重组质粒转入 NY1000 中，可加入 Nisin 诱导质粒表达外源基因。将重组表达载体 pLA311、pLA312、pLA319、pLA320 与干酪乳杆菌 NY1000 感受态细胞在电转杯中混匀，电击，涂到含氯霉素的培养基上，在厌氧箱中培养，筛选转入重组质粒的菌株。

实施例 9：重组菌株表达乳铁蛋白

将得到的重组乳球菌、乳杆菌分别在含有对应抗生素的 GM17 和 MRS 培养基中静置培养过夜，对带有 NisA 启动子的重组菌株，待其达到生长对数期 O.D.值为 0.5 左右时，加入诱导剂 Nisin，诱导 2-3 个小时，收获菌体。对非诱导的重组菌株，待其生长到达平台期时收获。收获的菌株经常规处理后，进行 Western 杂交验证蛋白表达：首先进行 SDS 聚丙烯酰胺凝胶电泳，然后转膜，与兔抗人乳铁蛋白抗体杂交。

结果证明，在转入分泌型重组质粒 pLA311、pLA313、pLA315、pLA317、pLA312、pLA314 的菌株培养液上清中有重组乳铁蛋白，蛋白大小与从 Sigma 购买的人乳铁蛋白标准品大小相同。在转入非分泌型重组质粒 pLA319、pLA321、pLA323、pLA325、pLA320、pLA322 的菌株细胞内有重组乳铁蛋白，蛋白大小与从 Sigma 购买的人乳铁蛋白标准品大小相同。

实施例 10：重组益生菌菌株表达的乳铁蛋白多肽的生物活性测定

采用从 Sigma 购买的人乳铁蛋白作为标准品，通过体外分析测定标准品及重组乳铁蛋白对大肠杆菌及弗氏志贺菌的抗微生物作用。

将大肠杆菌和弗氏志贺菌在适宜培养基上培养至对数生长期，分别加入不同浓度的标准品及重组乳铁蛋白，37℃震荡培养，在各个时间段等份取出，连续稀释并铺板培养过夜用于计数。结果发现重组乳铁蛋白和标准品的抗微生物作用基本相同。

实施例 11：重组菌株的应用。

重组乳球菌、乳杆菌分别用 GM17 和 MRS 培养基静置培养，口服液、或将菌体冻干制成胶囊和片剂。得到的发酵液可直接制备成口服液、喷雾剂、或加入保护剂制成冻干粉，然后制成胶囊或片剂，施用于消化道、阴道或其它粘膜处，用于治疗或预防病毒或细菌引起的传染病，抑制肿瘤细胞生长、缓解肿瘤病人经放化疗后引起的消化道功能紊乱、促进患者胃肠道内益菌群如双歧杆菌的生长和提高机体免疫能力。

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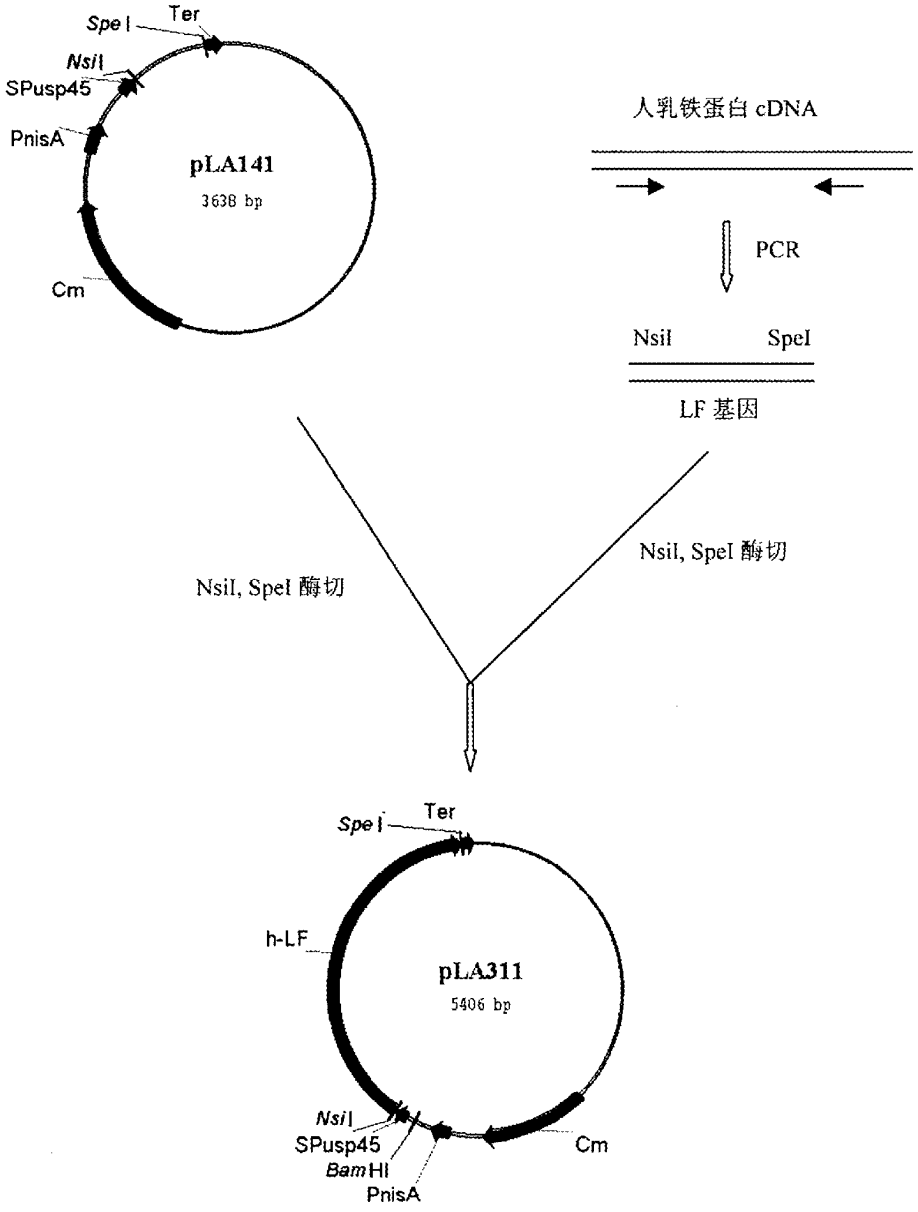
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NsiI, SpeI 酶切
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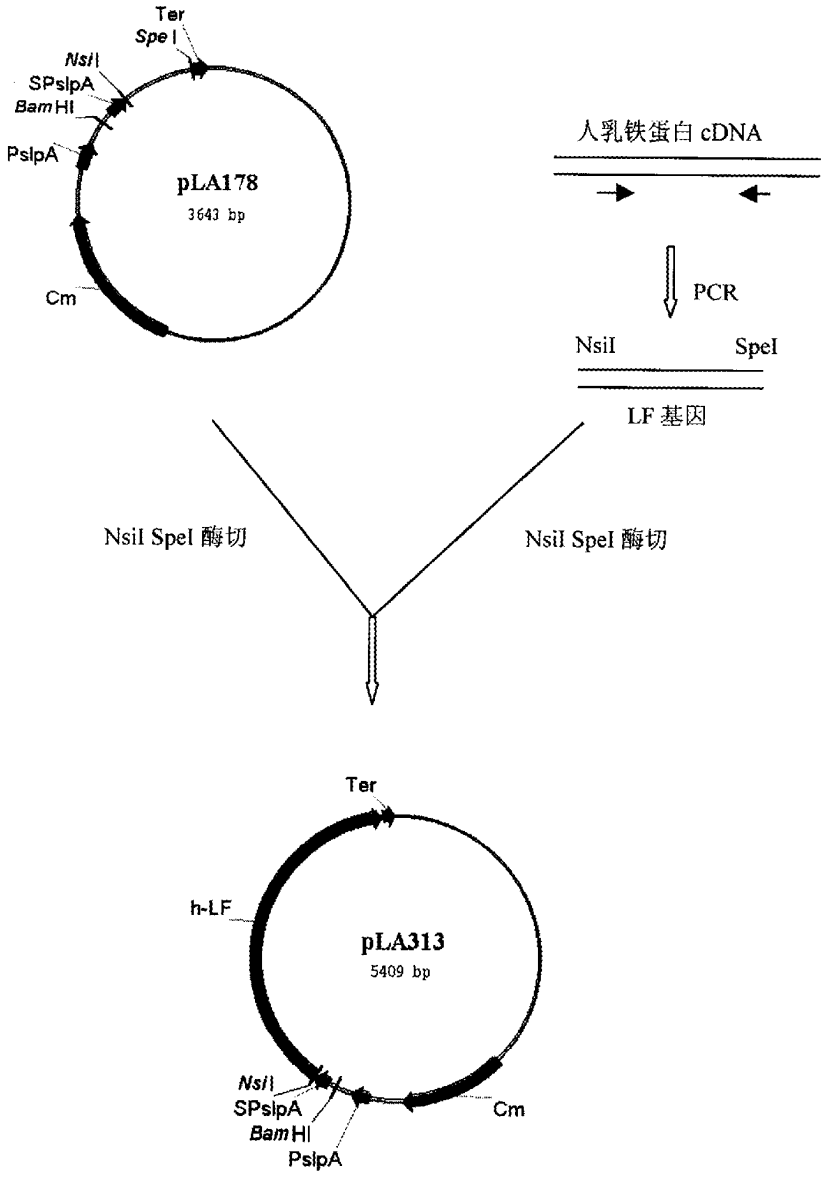
图 1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SpeI
LF 基因
NsiI SpeI 酶切
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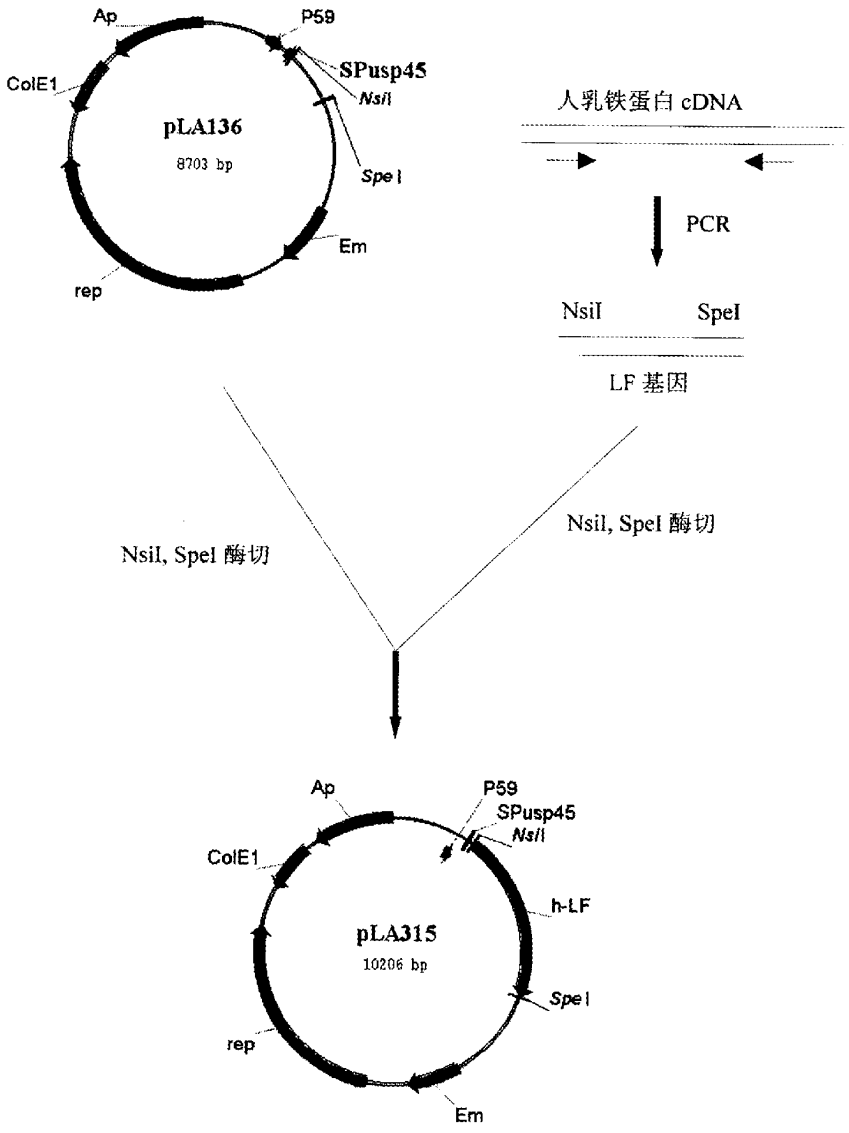
PslpA
Cm

图 2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Em
rep
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SpeI
LF 基因
NsiI, SpeI 酶切
NsiI, SpeI 酶切
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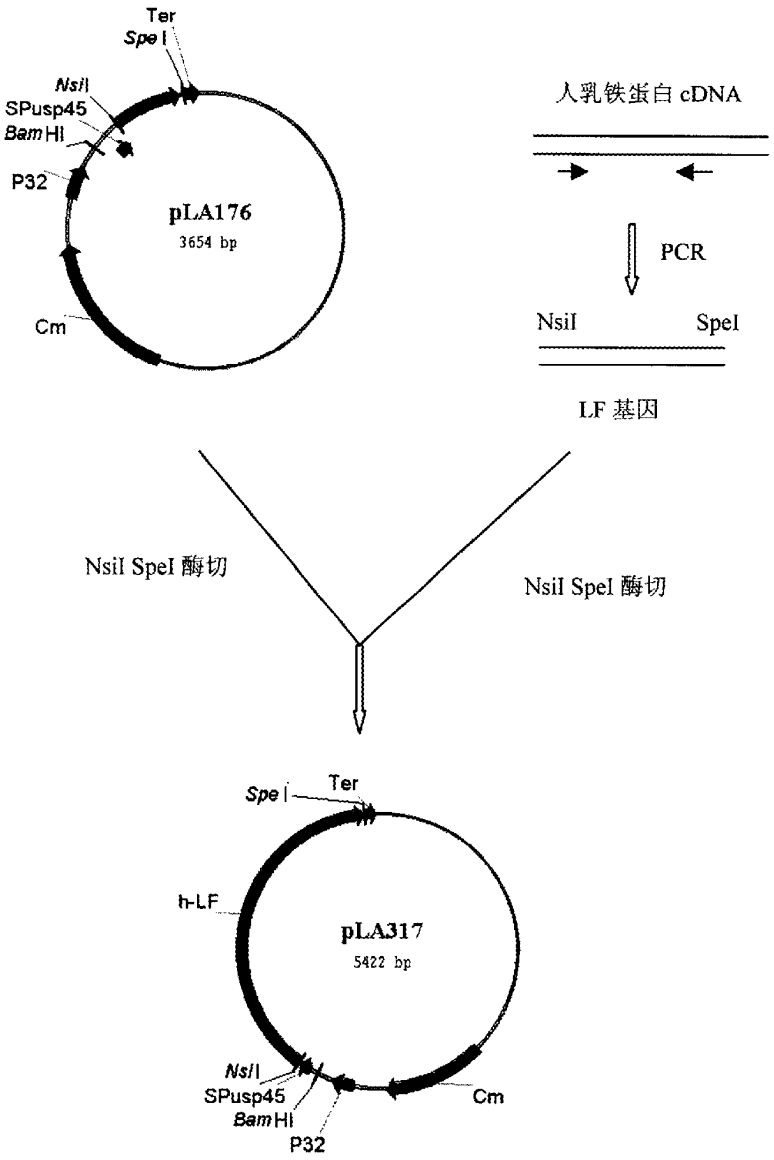
SpeI
rep
Em

图 3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LF 基因
NsiI SpeI 酶切
NsiI SpeI 酶切
Spe I
Ter
h-LF
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NsiI
SPusp45
BamHI
P32
Cm

图 4

Espacenet

Bibliographic data: CN1593223 (A) — 2005-03-16

Active nutritious health oral liquid of donkey-hide gelatin

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Classification: **- international:** ***A23L1/29; A23L1/302; A61K35/36; A61K38/01;*** (IPC1-7): A23L1/29; A23L1/302; A61K35/36; A61K38/01

- cooperative:

Application number: CN2004124211 20040526

Priority number (s): CN2004124211 20040526

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Abstract of CN1593223 (A)

The donkey-hide gelatin nourishing health oral liquid is prepared from donkey-hide gelatin and donkey-hide gelatin collagen as main materials, as well as auxiliary materials including haemachrome, vitamin B12, folic acid and sweetening materials, its preparing process consists of adding pure water, mixing homogeneously, heating, bottling, packing and sterilizing.

[19] 中华人民共和国国家知识产权局

[51] Int. Cl[7]
A23L 1/29
A23L 1/302 A61K 35/36
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权利要求书 2 页 说明书 3 页

[54] 发明名称 一种活性阿胶营养保健口服液浆

[57] 摘要

一种阿胶营养保健口服液浆，以阿胶和阿胶胶原蛋白为主料，然后配以其它的原辅料：血红素、维生素 B_{12}、叶酸、甜味料等，加纯净水后，经过混合均匀、加热、装瓶、密封、杀菌，从而制成具有营养保健补血作用的阿胶口服液浆产品。

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1. 一种阿胶营养保健口服液浆，其特征在于由以下原料：
阿胶、水解阿胶胶原蛋白肽、血红素（或氯化血红素）、维生素 B_{12} 组成其主要成分。

2. 根据权利要求 1 所述，一种阿胶营养保健口服液浆，其特征在于由以下原料配成：
阿胶：1～15%，水解阿胶胶原蛋白肽：1～15%；维生素 B_{12}：1~100μg/kg，
血红素（氯化血红素）：0.01～1‰，叶酸：0.1mg~10mg/kg ；

3. 根据权利要求 1 所述，一种阿胶营养保健口服液浆，其特征在于由以下原料配成：
阿胶：1～15%， 水解阿胶胶原蛋白肽：1～15%；维生素 B_{12}：1~100μg/kg ，
血红素（氯化血红素）：0.01～1‰， 人参皂苷：0.01～1%；

4. 根据权利要求 1 所述，一种阿胶营养保健口服液浆，其特征在于由以下原料配成：
阿胶：1～15% ，水解阿胶胶原蛋白肽：1～15%；维生素 B_{12}：1~100μg/kg ，
血红素(氯化血红素):0.01～1‰，免疫球蛋白 lgG（来自奶、蛋）：0.01～1%；

5. 根据权利要求 1 所述，一种阿胶营养保健口服液浆，其特征在于由以下原料配成：
阿胶：1～15%， 水解阿胶胶原蛋白肽：1～15%； 维生素 B_{12}：1~100μg/kg，
血红素（氯化血红素）：0.01～1‰， 红花提取物：0.01～1%；

6. 根据权利要求 1 所述，一种阿胶营养保健口服液浆，其特征在于由以下原料配成：
阿胶：1～15%， 水解阿胶胶原蛋白肽：1～15%；维生素 B_{12}：1~100μg/kg ，
血红素（氯化血红素）：0.01～1‰， 红花提取物：0.01～1%；
免疫球蛋白 lgG（来自奶、蛋）：0.01～1%；人参皂苷：0.01～1%；

7. 根据权利要求 1 所述，一种阿胶营养保健口服液浆，其特征在于由以下原料配成：
阿胶：1～15%， 水解阿胶胶原蛋白肽：1～15%； 维生素 B_{12}：1~100μg/kg ，
血红素（氯化血红素）：0.01～1‰， 红花提取物：0.01～1%；
免疫球蛋白 lgG（来自奶、蛋）：0.01～1%，叶酸：0.1mg~10mg/kg ；

8. 根据权利要求 1 所述，一种阿胶营养保健口服液浆，其特征在于由以下原料配成：
阿胶：1～15%， 水解阿胶胶原蛋白肽：1～15%； 维生素 B_{12}：1~100μg/kg ，
血红素（氯化血红素）：0.01～1‰， 叶酸：0.1mg~10mg/kg ，
甘氨酸亚铁或柠檬酸铁胺：0.01～1%， 乳酸锌：0.01～1‰；

9. 根据权利要求 1 所述，一种阿胶营养保健口服液浆，其特征在于由以下原料配成：
阿胶：1～15%， 水解阿胶胶原蛋白肽：1～15%；维生素 B_{12}：1~100μg/kg，
血红素（氯化血红素）：0.01～1‰，免疫球蛋白 lgG（来自奶、蛋）：0.01～1%；
红花提取物：0.01～1%，甘氨酸亚铁或柠檬酸铁胺：0.01～1%；乳酸锌：0.01～1‰；

10. 如权利要求 2、3、4、5、6、7、8、9 所述，分别将各组阿胶粉碎并与组内其它原料混合加入甜味料、纯净水混合均匀，经加热、装瓶、密封、杀菌制得成品。

一种活性阿胶营养保健口服液浆

本发明涉及一种活性阿胶营养保健口服液浆的制造。

阿胶是一种传统的补血营养佳品，最早始载于《神农本草经》，列为上品；曰：阿胶以阿井之水入黑驴皮练成胶也；阿井在山东兖州府阳谷县东北六十里；此清济之水伏行地中历千里而发于此井；以之制胶正与血脉相宜也。据《本草纲目》：除驴皮外，水牛、猪、马等皮亦作煎制阿胶用，而后世则以驴皮为贵；用阿井之水煎乌驴皮制成的阿胶品种最优，故名阿胶；李时珍认为：阿胶可补血补液，凡阴虚诸症都可用之治疗。现代医学证明，阿胶能够提高红细胞和血红蛋白的数量，具有促进造血的功能。

阿胶溶于水后，经过蛋白酶（如木瓜蛋白酶）的水解作用，可使胶原蛋白的分子量大大降低，成为低分子的胶原蛋白肽结构，从而更容易被人体吸收，且具有生理生物活性。阿胶水解后，经喷雾干燥制成水解阿胶胶原蛋白肽。

经分析阿胶如果完全水解后可产生多种氨基酸：甘氨酸、脯氨酸、谷氨酸、丙氨酸、精氨酸、天门冬氨酸、赖氨酸、苯丙氨酸、丝氨酸、组氨酸、半胱氨酸、缬氨酸、异亮氨酸、亮氨酸、色氨酸、羟脯氨酸、苏氨酸、酪氨酸等；并有多种微量元素，钾、钙、镁、铁、锌、铜、锶、钡、钛、锆等；阿胶不仅可以补血，还能改善人体内钙的平衡，使低钙血症趋于正常。

血红素是从动物（猪、牛、羊）的血液中提取出的一种补铁物质，一般以氯化血红素的形式存在，是人体补血补铁的理想物质。

本阿胶营养保健口服液浆，以阿胶、水解阿胶胶原蛋白为主料，然后配以其它的原辅料：血红素、维生素 B12、叶酸、甜味料等，加纯净水后，经过混合均匀、加热、装瓶、密封、杀菌，从而制成具有营养保健补血作用的阿胶口服液浆。

下面以实际制作为例进一步说明该营养保健口服液浆的制作过程。

实施方案之一：

原辅材料的基本组成为：

阿胶：1～15%，水解阿胶胶原蛋白肽 1～15%，维生素 B_{12}：1~100μg/kg，血红素（或氯化血红素，以下同）：0.01～1‰ ,，叶酸：0.1mg~10mg/kg，甜味料适量， 纯净水足量；

首先，将干阿胶粉碎后与水解阿胶胶原蛋白肽共同溶解于水，同时把血红素、维生素 B_{12}、叶酸、甜味料溶解于水；然后将上述两种液体混合均匀，经过加热、装瓶、密封，杀菌制得成品。

实施方案之二：

原辅材料的基本组成为：

阿胶 1～15%，水解阿胶胶原蛋白肽 1～15%， 维生素 B_{12}：1~100μg/kg，血红素：0.01～1‰，人参提取物皂苷：0.01～1%，甜味料适量， 纯净水足量；

首先，将干阿胶粉碎后与水解阿胶胶原蛋白肽共同溶解于水，同时把血红素、维生素 B_{12}、人参提取物皂苷、甜味料溶解于水；然后将上述两种液体混合均匀，经过加热、装瓶、密封，杀菌制得成品。

实施方案之三：

原辅材料的基本组成为：

阿胶 1～15%，水解阿胶胶原蛋白肽 1～15%，维生素 B_{12}：1~100μg/kg，血红素：0.01～1‰， 叶酸：0.1mg~10mg/kg， 红花提取物：0.01～1%；免疫球蛋白 lgG（来自奶、蛋）：0.01～1%；甜味料适量， 纯净水足量；

首先，将干阿胶粉碎后与水解阿胶胶原蛋白肽共同溶解于水，同时把血红素、维生素 B_{12}、叶酸、红花提取物、免疫球蛋白 lgG、甜味料、溶解于水；然后将上述两种液体混合均匀，经过加热、装瓶、密封、杀菌制得成品。

实施方案之四：

原辅材料的基本组成为：

阿胶 1～15%，水解阿胶胶原蛋白肽 1～15%， 维生素 B_{12}：1~100μg/kg ，血红素：0.01～1‰， 免疫球蛋白 lgG（来自奶、蛋）：0.01～1%；红花提取物：0.01～1%；人参提取物皂苷：0.01～1%，甜味料适量,纯净水足量。

首先，将干阿胶粉碎后与水解阿胶胶原蛋白肽共同溶解于水，同时把血红素、维生素 B_{12}、红花提取物、免疫球蛋白 lgG、人参提取物皂苷、甜味料、溶解于水；然后将上述两种液体混合均匀，经过加热、装瓶、密封、杀菌制得成品。

实施方案之五：

原辅材料的基本组成为：

阿胶 1～15%，水解阿胶胶原蛋白肽 1～15%， 维生素 B_{12}：1~100μg/kg ，血红素：0.01～1‰， 叶酸：0.1mg~10mg/kg ， 甘氨酸亚铁：0.01～1% ，乳酸锌：0.01～1‰，甜味料适量， 纯净水足量；

首先，将干阿胶粉碎后与水解阿胶胶原蛋白肽共同溶解于水，同时把血红素、维生素 B_{12}、叶酸、甘氨酸亚铁、乳酸锌、甜味料、溶解于水；然后将上述两种液体混合均匀，经过加热、装瓶、密封、杀菌制得成品。

下面以实际生产为例进一步说明本产品的制作过程。

实施例 1：分别称取阿胶块 10kg；水解阿胶胶原蛋白肽 10kg，血红素 100g；维生素 B_{12}：10mg ；叶酸：1000mg。将阿胶粉碎后与水解阿胶胶原蛋白肽共同溶解于水，另外将其它原料也溶解于水，加入适量甜味料，上述物料用纯净水定量至 200kg 后混合均匀，经过加热、装瓶、杀菌、密封，制得成品。

实施例 2：分别称取阿胶块 10kg，水解阿胶胶原蛋白肽 20kg， 血红素 45g；维生素 B_{12}：20mg，人参提取物皂苷：800g；将阿胶粉碎后与水解阿胶胶原蛋白肽共同溶解于水，另外将其它原料也溶解于水，加入适量甜味料，上述物料用纯净水定量至 400kg 后混合均匀，经过加热、装瓶、杀菌、密封，制得成品。

实施例 3：分别称取阿胶块 30kg，水解阿胶胶原蛋白肽 10kg，血红素 30g；维生素 B_{12}：60mg ，叶酸 1200mg，红花提取物 400g，免疫球蛋白 IgG：300g；将阿胶粉碎后与水解阿胶胶原蛋白肽共同溶解于水，另外将其它原料也溶解于水，加入适量甜味料，上述物料用纯净水定量至 1000kg 混合均匀，经过加热、装瓶、杀菌、密封，制得成品。

实施例 4：分别称取阿胶块 15kg，水解阿胶胶原蛋白肽 25kg，血红素 20g；维生素 B_{12}：30mg，红花提取物 100g；免疫球蛋白 IgG：420g；人参提取物皂苷：500g；将阿胶粉碎后与水解阿胶胶原蛋白肽共同溶解于水，另外将其它原料也溶解于水，加入适量甜味料，上述物料用纯净水定量至 500kg 混合均匀后，经过加热、装瓶、杀菌、密封，制得成品。

实施例 5：分别称取阿胶块 25kg， 水解阿胶胶原蛋白肽 5kg， 血红素 10g；维生素 B_{12}：20mg ，叶酸 1200mg，甘氨酸亚铁 500g、乳酸锌：50g；将阿胶粉碎与水解阿胶胶原蛋白肽共同后溶解于水，另外将其它原料也溶解于水，加入适量甜味料，上述物料用纯净水定量至 600kg 混合均匀后，经过加热、装瓶、杀菌、密封，制得成品。

Espacenet

Bibliographic data: CN1634524 (A) — 2005-07-06

Health-care food for improving nutritional anemia

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Classification: **- international:** ***A61K9/48; A61P7/06;*** (IPC1-7): A61K35/80; A61K9/48; A61P7/06

- cooperative:

Application number: CN2004190268 20041031

Priority number (s): CN2004190268 20041031

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Abstract of CN1634524 (A)

The invention provides a health-care food for improving nutritional anemia, which is prepared from (by weight ratio) spirulina azurin 1-50%, matrimony vine extract 5-80%, ferrous lactate 1-60%, the preparation of the food comprises the steps of raw material preparation, batching, capsule filling, capsule polishing, bottling, examining, boxing, warehouse-in.

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权利要求书 1 页 说明书 4 页

[54] 发明名称 改善营养性贫血保健食品

[57] 摘要

本发明涉及一种保健食品，特别是涉及一种改善营养性贫血保健食品。本发明改善营养性贫血保健食品由下述重量百分含量的原料制成：螺旋藻藻蓝蛋白 1－50%；枸杞提取物 5－80%；乳酸亚铁 1－60%。该改善营养性贫血保健食品由下述方法制成：原料准备→配料→混合→胶囊填充→胶囊抛光→装瓶→检验→装盒装箱→入库制得产品。藻蓝蛋白能够显著提高血红蛋白、血清铁蛋白和红细胞压积，具有显著的改善营养性贫血的作用，同时藻蓝蛋白具有提高机体免疫功能，抗疲劳等多重保健功效。乳酸亚铁具有显著的补血和补铁的作用，枸杞是补血强体的传统中药，本发明保健食品同时具有了所选原料的优点和功效，共同发挥了改善营养性贫血的保健功效。

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1、一种改善营养性贫血保健食品，其特征在于：所述保健食品由下述重量百分含量的原料制成：

螺旋藻藻蓝蛋白 1－50%；枸杞提取物 5－80%；乳酸亚铁 1－60%。

2、如权利要求 1 所述的改善营养性贫血保健食品，其特征在于所述的保健食品原料最佳重量百分含量为：

螺旋藻藻蓝蛋白 20－30%；枸杞提取物 40－60%；乳酸亚铁 20－40%。

3、如权利要求 1 或 2 所述的改善营养性贫血保健食品由如下方法制备而成：该螺旋藻补铁补血保健食品由下述方法制成

原料准备→配料→混合→胶囊填充→胶囊抛光→装瓶→检验→装盒装箱→入库制得产品。

4、如权利要求 3 所述的改善营养性贫血保健食品的制备方法，其特征在于：所述的制备工序流程中配料→混合→胶囊填充→胶囊抛光→装瓶工序是在洁净区无菌条件下进行。

5、如权利要求 3 所述的改善营养性贫血保健食品的制备方法，其特征在于：所述原料准备中螺旋藻藻蓝蛋白是将螺旋藻干粉进行细胞破碎，然后用水溶液抽取，所得成品冻干后得粗藻蓝蛋白晶体。

6、如权利要求 4 所述的改善营养性贫血保健食品的制备方法，其特征在于：将上述方法制得的粗藻蓝蛋白晶体采用凝胶过滤和羟基磷灰石柱层析进行藻蓝蛋白的分离纯化，得到精品藻蓝蛋白晶体备用。

7、如权利要求 3 所述的改善营养性贫血保健食品的制备方法，其特征在于：所述原料准备中的枸杞提取物是将枸杞经水煮提取，分离除去果核、果皮，浓缩后喷雾干燥，得到枸杞提取物备用。

改善营养性贫血保健食品

技术领域

本发明涉及一种保健食品，特别是涉及一种以螺旋藻或其提取物为主要原料的改善营养性贫血保健食品。

背景技术

据调查显示，营养性贫血是人类四大营养性疾病之一，由贫血引起的一系列疾病降低了患者的生活质量，给人们的生活带来了痛苦。为解决这一问题，人们通过纠正偏食的饮食习惯，改善饮食结构，多食富含铁的食物等措施来减轻贫血症状。近年来又研制开发的改善营养性贫血的保健食品多以既是食品又是药品的物品为基础，根据生产原料资源特点开发出的药食两用补铁补血保健食品等，至 2001 年在国家卫生部申请批准的 34 个改善营养性贫血的保健食品中，口服液和饮料类有 20 种，阿胶类产品有 6 种，档次较高的胶囊剂有 7 种。如朴雪乳酸亚铁口服液，红桃 K 牌养血活力片、阿牌阿胶软胶囊、娃哈哈牌铁锌钙奶饮料等。但以补血作用突出的螺旋藻及其深加工原料为主，添加改善营养性贫血功能突出的一些传统药食两用物质的提取物以强化补血作用的螺旋藻补铁补血保健食品及其制备方法，还未见记载。

发明内容

本发明的目的是提供一种以螺旋藻及其深加工提取物为主要原料，添加改善营养性贫血功能突出的一些传统药食两用物质的提取物来制取改善营养性贫血的保健食品。

本发明的技术方案是这样实现的：本发明改善营养性贫血保健食品由下述

Case No.: IPR2023-00208
U.S. Patent No. 10,039,306

重量百分含量的原料制成：螺旋藻藻蓝蛋白 1－50%；枸杞提取物 5－80%；乳酸亚铁 1－60%。该螺旋藻补铁补血保健食品由下述方法制成：原料准备→配料→混合→胶囊填充→胶囊抛光→装瓶→检验→装盒装箱→入库制得产品；其中配料→混合→胶囊填充→胶囊抛光→装瓶工序是在洁净区无菌条件下进行。

本发明保健食品由螺旋藻的深加工提取物藻蓝蛋白、枸杞提取物、乳酸亚铁组成，藻蓝蛋白能够显著提高血红蛋白、血清铁蛋白和红细胞压积，具有显著的改善营养性贫血的作用，同时藻蓝蛋白具有提高机体免疫功能，抗疲劳等多重保健功效。乳酸亚铁具有显著的补血和补铁的作用，可明显提高血液中铁的含量，继而提高血红蛋白、血清铁蛋白的含量，有效改善贫血症状。枸杞是补血强体的传统中药，其提取物具有明显的补血和提高免疫功能的作用。本发明保健食品同时具有了所选原料的优点和功效，共同发挥了改善营养性贫血的保健功效。本发明保健食品适用于青藏高原等高海拔低氧地区和各类人群。

具体实施方式

下面结合实施例进一步说明本发明技术方案是如何实现的：

改善营养性贫血保健食品由下述重量百分含量的原料制成：

螺旋藻藻蓝蛋白 1－50%；枸杞提取物 5－80%；乳酸亚铁 1－60%。

所述的保健食品原料最佳重量百分含量为：螺旋藻藻蓝蛋白 20－30%；枸杞提取物 40－60%；乳酸亚铁 20－40%。

该改善营养性贫血保健食品由下述方法制成

原料准备→配料→混合→胶囊填充→胶囊抛光→装瓶→检验→装盒装箱→入库制得产品。

所述原料准备中螺旋藻藻蓝蛋白是将螺旋藻干粉进行细胞破碎，然后用水溶液抽取，所得成品冻干后得粗藻蓝蛋白晶体。

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将上述方法制得的粗藻蓝蛋白晶体采用凝胶过滤和羟基磷灰石柱层析进行藻蓝蛋白的分离纯化，得到精品藻蓝蛋白晶体备用。

所述原料准备中的枸杞提取物是将枸杞经水煮提取，分离除去果核、果皮，浓缩后喷雾干燥，得到枸杞提取物备用。

所述的乳酸亚铁由乳酸钙或乳酸钠溶液与硫酸亚铁或氧化亚铁反应而得，也可以在乳酸溶液中添加蔗糖及精制铁粉，直接反应后结晶而得。也可以由专业生产企业生产后购得。

实施例 1。

取已制得的藻蓝蛋白重量百分含量 20%，枸杞提取物重量百分含量 40%，乳酸亚铁 30%，枸杞提取物过 80 目筛备用；将上述原料配料置于混料机中，充分混合均匀；混合后的配料在胶囊装填机上填充胶囊；用医用塑料瓶作包装瓶包装，外包装为纸盒；灭菌、检验装箱入库制得产品。生产过程中从配料至包装工序均在无菌条件下进行，当菌检超标时，可选用紫外线照射或辐照灭菌。

实施例 2。

取已制得的粗藻蓝蛋白重量百分含量 25%；枸杞提取物重量百分含量 50%；乳酸亚铁 25%，枸杞提取物过 80 目筛备用；将上述原料配料置于混料机中充分混合均匀；混合后的配料在胶囊装填机上填充胶囊；用医用塑料瓶作包装瓶包装，外包装为纸盒；灭菌、检验装箱入库制得产品。生产过程中从配料至包装工序均在无菌条件下进行，当菌检超标时，可选用紫外线照射或辐照灭菌。

实施例 3。

取已制得的粗藻蓝蛋白重量百分含量 30%；枸杞提取物重量百分含量 50%；乳酸亚铁 20%；枸杞提取物过 80 目筛备用；将上述原料配料置于混料机中充分混合均匀；混合后的配料在胶囊装填机上填充胶囊；用医用塑料瓶作包装瓶包

装，外包装为纸盒；灭菌、检验装箱入库制得产品。生产过程中从配料至包装工序均在无菌条件下进行，当菌检超标时，可选用紫外线照射或辐照灭菌。

Espacenet

Bibliographic data: CN101138405 (A) — 2008-03-12

Lazy soybean curd nutrition instant dish and making method thereof

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Applicant(s): BOGEN ZHOU [CN] ± (ZHOU BOGEN)

Classification: **- international:** *A23L1/20; A23L1/212; A61K36/00*
- cooperative:

Application number: CN20061122061 20060908

Priority number(s): CN20061122061 20060908

Abstract of CN101138405 (A)

The present invention relates to a bean curd nutritious convenient dish and the preparation method; vegetable is taken as the main material and nut seed kernel is taken as the auxiliary material; in addition, medicine-food product with pharmaceutical action is used for matching the materials to prepare the dish; the preparation process comprises collection, purchase, cleaning, screening, cutting, mixing, cooking, forming, drying, sterilization and packing; the dish is easy for storage and convenient for eating; the dish is palatable and rich in nutrition; in addition, the dish is provided with functions of enhancing stomach and regulating qi, nourishing liver and preventing cancer and body building. Therefore, the dish is a delicious food, which is loved by people and can be used for improving health and strengthening body.

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权利要求书 1 页 说明书 3 页

[54] 发明名称

懒豆腐营养方便菜及其制做方法

[57] 摘要

本发明涉及一种懒豆腐营养方便菜及其制做方法，它由蔬菜为主料，副以坚果种仁为辅料，配以具有药理作用的药食品组成，经采集、商购、清洗、淋干、切碎、混合、煮制、成型、烘干、灭菌、包装而成，它易于保存，食用方便，味美可口，营养丰富，有健胃理气，养肝明目，防癌健身的功效，是一种深受人们喜爱的能强身健体的美食佳品。

1、一种懒豆腐营养方便菜，它由蔬菜为主料，副以坚果种仁为辅料，配以具有药理作用的药食品组成，经采集、商购、清洗、淋干、切碎、混合、粉碎、煮制、成型、烘干、灭菌、包装而成，其特征在于它是由下述重量百分比的原料制做的懒豆J膏营养方便菜

主料： 蔬 菜 75-85%

辅料： 坚果种仁 10-15%

配料： 药食品 8—12% 。

2、根据权利要求1所述的懒豆腐营养方便菜，其特征还在于：所述的蔬菜为干鲜时令菜，如大白菜，黄心菜，芹菜，红、白萝卜，萝卜叶，笋叶，菠菜，茄子，冬瓜，蒲公英，黄花菜，海带，木耳、香菇，只要是无毒的能食用的菜即可，可是单独一种菜配用，也可是几种菜同时配用。

3、根据权利要求1所述的懒豆腐营养方便菜，其特征还在于：所述的坚果种仁可为大豆，黑豆，扁豆，蚕豆，碗豆，绿豆，青豆，松子，黑、白芝麻。

4、根据权利要求1所述的懒豆腐营养方便菜，其特征还在于：所述的药食品为陈皮，砂仁，紫苏，薏苡仁，山渣，肉桂，山药，大枣和枸杞果。

5、懒豆腐营养方便菜的制做方法，其特征在于：

①商购并称重上述重量百分比的优质原料，

②将坚果种仁用冷水浸泡，泡透后，淋去水份，磨碎，成粗末浆，

③将干鲜时令菜洗净，去杂质烂叶，淋去水分，切成碎块状，

④将陈皮、砂仁、紫苏、山渣、肉桂、山药、大枣分别干燥、粉碎成粗末状，薏苡仁用冷水浸泡2小时，淋去水分，枸杞果做用，

⑤将切成碎块的蔬菜同坚果种仁的粗末浆和配料混合，加水煮制成熟成半成品，

⑥将煮熟后的半成品淋干水份，挤压成型，制成长方形状，低温烘干，

⑦配以调料品，

⑧灭菌，检验，包装，即得1本发明的懒豆腐营养方便菜。

6、根据权利要求5所述的制做方法，其特征还在于：所述的调料品为食盐，辛香料粉，鸡精粉，味精，葱，姜，香菜，也可加配香油调制成各种口味的调料品。

懒豆腐营养方便菜及其制做方法

技术领域

本发明涉及一种懒豆腐营养方便菜及其制做方法，具体的说是涉及一种由纯天然蔬菜、坚果种仁与具有药理作用的药食品加工制做而成的，易于保管、营养丰富的方便菜及其制做方法。

技术背景

目前，随着国民经济的迅猛发展和人民生活水平的日益提高，市场上涌现出各种味美可口的方便食品，如：方便面、方便米等，以方便人们的生活，满足人们快节奏生活的需要。然而，在这琳琅满目的方便食品中，人们只能见到方便面、方便米等主食，却见不到方便菜的供应，在这众多的方便食品中方便菜仍属空白，不能满足人们食用方便食品时全面营养的需求。故人们希望能有一种方便菜问世，以弥补现有方便食品的不足。

发明内容

本发明的目的在于提供一种由纯天然蔬菜、坚果种仁与具有药理作用的药食品加工制做而成的懒豆腐营养方便菜及其制做方法。以弥补现有方便食品缺少方便菜的不足。

本发明的目的是这样实现的：它由蔬菜为主料，副以坚果种仁为辅料，配以具有药理作用的药食品组成，经采集、商购、清洗、淋干、切碎、混合、粉碎、煮制、成型、烘干、灭菌、包装而成，其特征在于它是由下述重量百分比的原料制做的懒豆腐营养方便菜

主料： 蔬 菜 75．-85%

辅料： 坚果种仁 10．15%

配料： 药食品 8—12%。

其特征还在于：所述的蔬菜为干鲜时令菜，如大白菜，黄心菜，芹菜，红、白萝卜，萝卜叶，笋叶，菠菜，茄子，冬瓜，蒲公英，黄花菜，海带，木耳、香菇等，只要是无毒的能食用的菜即可，可是单独一种菜配用，也可是几种菜同时配用。

其特征还在于：①所述的坚果种仁可为大豆，黑豆，扁豆，蚕豆，豌豆，绿

豌豆

②将大豆用冷水浸泡 4 小时，泡透后，淋去水份，磨碎，成粗昧浆，

③将大白菜洗净，去杂质烂叶，淋去水分，切成碎块状，

④将陈皮、砂仁、紫苏、山渣、肉桂、山药、大枣分剐干燥、粉碎成粗末状，薏苡仁用冷水浸泡 2 小时，淋去水分，枸杞果做用，

⑤将切成碎块的大白菜、黄花菜、黑木耳、香菇同大豆的粗末浆和粉碎成粗末的陈皮、砂仁、紫苏、山渣、肉桂、山药、大枣，用冷水浸泡的薏苡仁，枸杞果混合，加水煮制成熟，成半成品，

⑥将煮热后的半成品淋干水份，挤压成型，制成长方形状，低温烘干，

⑦佐以辛香料、精制食盐，配以用香油调拌好的葱、姜、香菜的调料品，

⑧灭菌，检验，包装，即得 2 块本发明懒豆腐营养方便菜。

具体实施方式

实施例 1

①商购并称重(9)下述符合国家卫生标准的优质原料，

黄心菜 30 芹菜 30 黄花菜 5 药 1

大豆 6 花生 2 海带 5 大枣 2

核桃仁 4 紫苏 1 黑豆 3 青豆 2

杞果 1 意苡仁 2 山渣 1 绿豆 2

陈皮 1 砂仁 0.5 肉桂 o.5

②将大豆、花生、黑豆、青豆、绿豆、海带、黄花菜分别用冷水浸泡 4 小时，泡透后，淋去水份，磨碎，成粗末浆，

③将黄心菜和芹菜洗净，去杂质烂叶，淋去水分，切成碎块状，

④将陈皮、砂仁、紫苏、山渣、肉桂、山药、大枣分别干燥、粉碎成粗末状，薏苡仁用冷水浸泡 2 小时，淋去水分，枸杞果做用，

(5)将切成碎块的黄心菜和芹菜同大豆、花生、黑豆、青豆、绿豆的粗末浆和粉碎成粗末的陈皮、砂仁、紫苏、山渣、肉桂、山药、大枣，用冷水浸泡的薏苡仁，枸杞果混合，加水煮制成熟，成半成品，

⑥将煮熟后的半成品淋干水份，挤压成型，制成长方形状，低温烘干，

⑦佐以味精、精制食盐，配以葱、姜、香菜的调料品，

⑧灭菌，检验，包装，即得 2 块本发明懒豆腐营养方便菜。

Espacenet

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Meat substitute food product and preparation method thereof

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Abstract of CN101541187 (A)

The invention relates to a method for preparing meat substitute food products, including phases comprising: the hydration under vacuum of at least one vegetable protein mixed with water and at least one colouring agent; the mixing of at least one meat product with the hydrated and coloured vegetable protein mixture; the mixing of flavouring agents and texturing agents into the mixture comprising the meat products and the hydrated and coloured vegetable protein, such as to form a paste; the cooking of the paste; and the forming of said paste to produce an appearance similar to a meat product. The resulting meat substitute food product can have the appearance, for example, of steaks, breaded meat fillets, flank steaks, dried meats, breaded flank steaks, escalopes, shredded meat, ground meat, sirloin tips, meat strips or meatballs.

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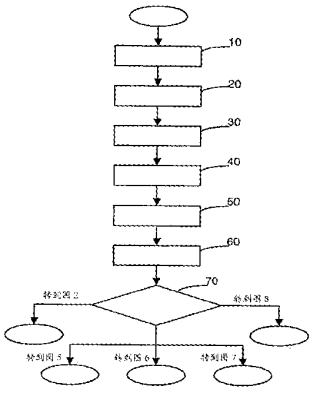
权利要求书 13 页 说明书 18 页 附图 7 页

[54] 发明名称

肉类替代食品及其制备方法

[57] 摘要

本发明涉及一种用于制备肉类替代食品的方法，包括以下步骤：在真空下水合至少一种与水和至少一种着色剂混合的植物蛋白；将至少一种肉类产品与经水合和着色的植物蛋白混合物混合；将调味单位和质感单位混合到肉类产品和经水合和着色的植物蛋白的混合物中，因此获得制备好的糊；烹调所述制备好的糊；以及成形所述经烹调的糊，以产生类似于肉类产品的外观。 根据本发明获得的替代肉类食品可以具有例如牛排、肉排、侧腹横肌牛排、薰腌牛排、蘸面牛肉肉排、蘸面横隔膜牛排、小牛肉薄片、肉丝、碎肉、整肉、肉条或肉丸的外观。



1. 一种用于制备肉类替代食品的方法，所述方法的特征在于包括以下步骤：

在真空下水合至少一种与水和至少一种着色剂混合的植物蛋白；

使至少一种肉类产品与所述经水合和着色的植物蛋白的混合物混合；

使所述肉类产品与所述经水合和着色的植物蛋白的混合物的组织改性单位混合，以获得制备好的糊；

烹调所述制备好的糊；以及

形成所述经烹调的糊以赋予它与肉类产品类似的表观。

2. 根据权利要求 1 所述的方法，其特征在于，不管是挤压的或水合的，所述植物蛋白是纤维的或组织改性的。

3. 根据权利要求 1 所述的方法，其特征在于，所述植物蛋白选自由浓缩蛋白、分离物、面粉、颗粒、以及它们的组合组成的组。

4. 根据权利要求 1 所述的方法，其特征在于，所述着色剂为焦糖色素。

5. 根据权利要求 1 所述的方法，其特征在于，所述在真空下水合至少一种与水和至少一种着色剂混合的植物蛋白的步骤包括以下步骤：

在混合器中混合所述植物蛋白、水以及着色剂，其中搅棒在 10 min^{-1} 至 20 min^{-1} 的范围内顺时针旋转，并且每在 2 分

钟至 10 分钟的范围内各发生一次逆时针旋转；其中在真空下在 40 分钟至 80 分钟范围内的期间内并在-100 kPa 至-50 kPa 的范围内的压力下获得所述混合物。

6. 根据权利要求 1 所述的方法，其特征在于，所述肉类产品选自由牛肉、猪肉、火鸡肉、鸡肉、牛杂碎、猪杂碎、火鸡杂碎、鸡杂碎、机械去骨牛肉、机械去骨猪肉、机械去骨火鸡肉、机械去骨鸡肉、以及它们的组合组成的组。

7. 根据权利要求 1 所述的方法，其特征在于，所述使至少一种肉类产品与所述经水合和着色的植物蛋白混合的步骤包括以下步骤：

在混合器中使所述经水合和着色的植物蛋白与以剂量方式加入的所述肉类产品混合，其中搅棒在 10 min^{-1} 至 20 min^{-1} 的范围内顺时针旋转，并且每在 2 分钟至 10 分钟的范围内各发生一次逆时针旋转；其中在 15 分钟至 45 分钟范围内的期间内获得所述混合物；以及

在混合器中使所述肉类产品与经水合和着色的植物蛋白混合，其中搅棒在 20 min^{-1} 至 40 min^{-1} 的范围内顺时针旋转，并且每在 2 分钟至 10 分钟的范围内各发生一次逆时针旋转，其中在真空下在 10 分钟至 20 分钟范围内的期间内并在-100 kPa 至-50 kPa 的压力下获得所述混合物。

8. 根据权利要求 7 所述的方法，其特征在于，使所述经水合和着色的植物蛋白与以剂量方式加入的所述肉类产品在混合器中混合，其中搅棒在 10 min^{-1} 至 20 min^{-1} 的范围内顺时针旋转，并且每在 2 分钟至 10 分钟的范围内各发生一次逆时针旋转；其中，在 15 分钟至 45 分钟范围内的期间内获得所述混合物的步骤包括与防腐剂、盐以及亚硝酸钠进行混合的步骤。

9. 根据权利要求 1 所述的方法，其特征在于，使组织改性单位与所述肉类产品和经水合和着色的植物蛋白混合，以获得制备好的糊的步骤包括以下步骤：

在混合器中使所述肉类产品以及经水合和着色的植物蛋白与所述组织改性单位混合，其中搅棒在 10 min^{-1} 至 20 min^{-1} 的范围内顺时针旋转，并且每在 2 分钟至 10 分钟的范围内各发生一次逆时针旋转；其中在 5 分钟至 20 分钟范围内的期间内获得所述混合物；

在混合器中混合所述肉类产品、经水合和着色的植物蛋白、以及所述组织改性单位，其中搅棒在 20 min^{-1} 至 40 min^{-1} 的范围内顺时针旋转，并且每在 2 分钟至 10 分钟的范围内各发生一次逆时针旋转；其中在 3 分钟至 10 分钟范围内的期间内获得所述混合物；以及

在混合器中混合所述肉类产品、经水合和着色的植物蛋白以及所述组织改性单位，其中搅棒在 20 min^{-1} 至 40 min^{-1} 的范围内顺时针旋转，并且每在 2 分钟至 10 分钟的范围内各发生一次逆时针旋转；其中，在真空下在 10 分钟至 20 分钟范围内的期间内并在-100 kPa 至-50 kPa 范围内的压力下获得所述混合物。

10. 根据权利要求 9 所述的方法，其进一步的特征在于，肉类产品、经水合和着色的植物蛋白、以及组织改性单位的所述混合物与调味单位进行混合。

11. 根据权利要求 10 所述的方法，其特征在于，所述调味单位选自由肉风味、猪肉风味、火鸡肉风味、鸡肉风味、以及它们的组合组成的组。

12. 根据权利要求 1 所述的方法，其进一步的特征在于，包括在约 0°C 至约 4°C 的温度下挤压所述制备好的糊的步骤。

13. 根据权利要求 1 所述的方法，其特征在于，烹调所述制备好的糊的所述步骤包括以下步骤：

定量给予所述制备好的糊；以及

在烤箱中以基本同时和均匀的方式碾平和烹调所述定量给予的糊并且同样地对所述糊的上表面和下表面进行碾平和烹调，其中所述烤箱包括至少一个上部加热板和至少一个下部加热板，所述两个加热板均与所述糊接触以获得肉的颜色和质地。

14. 根据权利要求 13 所述的方法，其特征在于，在烤箱中以基本同时和均匀的方式碾平和烹调所述定量给予的糊并且同样对所述糊的上表面和下表面进行碾平和烹调，其中所述烤箱包括至少一个上部加热板和至少一个下部加热板，所述两个加热板均与所述糊接触以获得肉的颜色和质地的所述步骤是在所述上部加热板和下部加热板为约 100°C 至约 150°C 的温度下进行的。

15. 根据权利要求 13 所述的方法，其特征在于，在烤箱中以基本同时和均匀的方式碾平和烹调所述定量给予的糊并且同样对所述糊的上表面和下表面进行碾平和烹调，其中所述烤箱包括至少一个上部加热板和至少一个下部加热板，所述两个加热板均与所述糊接触以获得肉的颜色和质地的所述步骤的特征在于所述烹调是在所述定量给予的糊在所述烤箱中的停留时间为约 10 秒至约 120 秒的情况下进行的。

16. 根据权利要求 13 所述的方法，其特征在于，在烤箱中以基本同时和均匀的方式碾平和烹调所述定量给予的糊并且同样对所述糊的上表面和下表面进行碾平和烹调，其中所述烤箱包括至少一个上部加热板和至少一个下部加热板，所述两个加热板均与所述糊接触以获得肉的颜色和质地的所述步骤的特征在于所述定量给予的糊的所述烹调是在所述上部加热板与下部加热板之间的间距为约 6 mm 至约 15 mm 的情况下进行的。

17. 根据权利要求 13 所述的方法，其特征在于，获得具有牛排、肉排、侧腹横肌牛排、或薰腌牛排的外观的肉类替代食品。

18. 根据权利要求 17 所述的方法，其进一步的特征在于，包括在所述具有肉排或侧腹横肌牛排的外观的所述肉类替代食品上洒面包屑，以获得具有蘸面牛肉肉排或蘸面横隔膜牛排的外观的肉类替代食品的步骤。

19. 根据权利要求 18 所述的方法，其进一步的特征在于，包括油炸所述具有蘸面牛肉肉排或蘸面横隔膜牛排的外观的肉类替代食品，以获得具有小牛肉薄片的外观的肉类替代食品的步骤。

20. 根据权利要求 1 所述的方法，其特征在于，烹调所述制备好的糊的所述步骤包括以下步骤：

挤压或模塑所述制备好的糊，使得所述制备好的糊的纤维以与肉的纤维取向类似的方式被取向；以及

油炸、或油炸和烹调经挤压或模塑的糊，以获得肉的颜色和质地。

21. 根据权利要求 20 所述的方法，其特征在于，油炸所述经挤压或模塑的糊的所述步骤是在油炸锅中在约 150°C 至约 250°C 的温度下进行的。

22. 根据权利要求 21 所述的方法，其特征在于，所述经挤压或模塑的糊在所述油炸锅中的停留时间为约 10 秒至约 200 秒。

23. 根据权利要求 21 所述的方法，其特征在于，在所述油炸锅中，所述经挤压或模塑的糊达到约 70°C 至约 80°C 的内部温度。

24. 根据权利要求 20 所述的方法，其特征在于，油炸和烹调所述经挤压或模塑的糊的所述步骤是在对流烤箱中在约 130°C 至约 250°C 的温度下进行的。

25. 根据权利要求 24 所述的方法，其特征在于，在所述对流烤箱中，所述经挤压或模塑的糊达到约 70°C 至约 80°C 的内部温度。

26. 根据权利要求 1 所述的方法，其特征在于，烹调所述制备好的糊的所述步骤包括以下步骤：

填充所述制备好的糊；以及

在烤箱或烹调锅中烹调所述填充的糊。

27. 根据权利要求 26 所述的方法，其特征在于，所述制备好的糊被装入天然或人造的、可食用的或不可食用的包装。

28. 根据权利要求 26 所述的方法，其特征在于，在烤箱或烹调锅中烹调所述经填充的糊的所述步骤中，所述经填充的糊达到约 70°C 至约 80°C 的内部温度。

29. 根据权利要求 26 所述的方法，其进一步的特征在于，包括在形成所述经烹调的糊以后油炸所述经烹调的糊用于生产类似于肉类产品的表观的步骤。

30. 根据权利要求 1 所述的方法，其特征在于，烹调所述制备好的糊的所述步骤包括在搅拌所述糊的同时在烹调罐中烹调所述制备好的糊的步骤。

31. 根据权利要求 30 所述的方法，其特征在于，在搅拌所述制备好的糊的同时在烹调罐中烹调所述制备好的糊的所述步骤中，所述制备好的糊达到约 70°C 至约 80°C 的内部温度。

32. 根据权利要求 1 所述的方法，其特征在于，形成所述经烹调的糊以赋予它类似于肉类产品的表观的所述步骤包括以下步骤：

冷却所述经烹调的糊；以及

在切碎机中切碎所述经烹调的糊以赋予它肉丝形状；或

在食品切块机中切割所述经烹调的糊以赋予它整肉或肉条型的肉形状，或

在肉碾磨机中磨碎所述经烹调的糊以赋予它碎肉形状。

33. 根据权利要求 32 所述的方法，其特征在于，在冷却所述经烹调的糊的所述步骤中，所述经烹调的糊达到约 0°C 至约 15°C 的温度。

34. 根据权利要求 32 所述的方法，其特征在于，在肉碾磨机中磨碎所述经烹调的糊以赋予它碎肉形状的所述步骤进一步包括形成肉丸的步骤。

35. 根据权利要求 1 所述的方法，其进一步的特征在于，包括加入香肠用于对所述形成的糊进行调味的步骤。

36. 根据权利要求 1 所述的方法，其特征在于，烹调所述制备好的糊的所述步骤可以在烹调的不同阶段进行，所述阶段选自由在包括至少一个上部加热板和至少一个下部加热板的烤箱中碾平和烹调、在油炸锅中油炸、在对流烤箱中油炸和烹调、以及它们的组合组成的组。

37. 一种用于制备肉类替代食品的糊，所述糊的特征在于包含：

至少一种经水合的植物蛋白；

至少一种着色剂；

至少一种肉类产品；以及

调味单位和组织改性单位。

38. 根据权利要求 37 所述的糊，其特征在于，不管是被挤压或被水合，所述植物蛋白是纤维的或经组织改性的。

39. 根据权利要求 37 所述的糊，其特征在于，所述植物蛋白选自由浓缩蛋白、分离物、面粉、颗粒、以及它们的组合组成的组。

40. 根据权利要求 37 所述的糊，其特征在于，所述着色剂为焦糖色素。

41. 根据权利要求 37 所述的糊，其特征在于，所述肉类产品选自由牛肉、猪肉、火鸡肉、鸡肉、牛杂碎、猪杂碎、火鸡杂碎、鸡杂碎、机械去骨牛肉、机械去骨猪肉、机械去骨火鸡肉、机械去骨鸡肉、以及它们的组合组成的组。

42. 根据权利要求 37 所述的糊，其特征在于，所述调味单位选自由肉风味、猪肉风味、火鸡肉风味、鸡肉风味、以及它们的组合组成的组。

43. 根据权利要求 37 所述的糊，其进一步的特征在于，包含防腐剂、盐以及亚硝酸钠。

44. 一种制备用于制备肉类替代食品的糊的方法，所述方法的特征在于包括以下步骤：

在真空下水合至少一种与水和至少一种着色剂混合的植物蛋白；

使至少一种肉类产品与经水合和着色的植物蛋白的所述混合物混合；以及

使组织改性单位与肉类产品以及经水合和着色的植物蛋白的所述混合物混合，以获得制备好的糊。

45. 根据权利要求 44 所述的方法，其特征在于，不管是被挤压或被水合，所述植物蛋白是纤维的或组织改性的。

46. 根据权利要求 44 所述的方法，其特征在于，所述植物蛋白选自由浓缩蛋白、分离物、面粉、颗粒、以及它们的组合组成的组。

47. 根据权利要求 44 所述的方法，其特征在于，所述着色剂为焦糖色素。

48. 根据权利要求 44 所述的方法，其特征在于，在真空下水合至少一种与水和至少一种着色剂混合的植物蛋白的所述步骤包括以下步骤：

在混合器中混合所述植物蛋白、水、以及着色剂，其中搅棒在 10 min^{-1} 至 20 min^{-1} 的范围内顺时针旋转，并且每在 2 分钟至 10 分钟的范围内各发生一次逆时针旋转；其中在真空下在 40 分钟至 80 分钟范围内的期间内以及在-100 kPa 至-50 kPa 的范围内的压力下获得所述混合物。

49. 根据权利要求 44 所述的方法，其特征在于，所述肉类产品选自由牛肉、猪肉、火鸡肉、鸡肉、牛杂碎、猪杂碎、火鸡杂碎、鸡杂碎、机械去骨牛肉、机械去骨猪肉、机械去骨火鸡肉、机械去骨鸡肉、以及它们的组合组成的组。

50. 根据权利要求 44 所述的方法，其特征在于，使至少一种肉类产品与所述经水合和着色的植物蛋白混合的所述步骤包括以下步骤：

在混合器中使所述经水合和着色的植物蛋白与以剂量方式加入的所述肉类产品混合，其中搅棒在 10 min^{-1} 至 20 min^{-1} 的范围内顺时针旋转，并且每在 2 分钟至 10 分钟的范围内各发生一次逆时针旋转；其中在 15 分钟至 45 分钟范围内的期间内获得所述混合物；以及

在混合器中使肉类产品的所述混合物与经水合和着色的植物蛋白混合，其中搅棒在 20 min^{-1} 至 40 min^{-1} 的范围内顺时针旋转，并且每在 2 分钟至 10 分钟的范围内各发生一次逆时针旋转；其中在真空下在 10 分钟至 20 分钟范围内的期间内以及在-100 kPa 至-50 kPa 范围内的压力下获得所述混合物。

51. 根据权利要求 50 所述的方法，其特征在于，在混合器中使所述经水合和着色的植物蛋白与以剂量方式加入的所述肉类产品混合的所述步骤包括与防腐剂、盐、以及亚硝酸钠混合的步骤，其中搅棒在 10 min^{-1} 至 20 min^{-1} 的范围内顺时针旋转，并

且每在 2 分钟至 10 分钟的范围内各发生一次逆时针旋转；其中在 15 分钟至 45 分钟范围内的期间内获得所述混合物。

52. 根据权利要求 44 所述的方法，其特征在于，使组织改性单位与所述肉类产品以及经水合和着色的植物蛋白混合以获得制备好的糊的所述步骤包括以下步骤：

在混合器中使肉类产品以及经水合和着色的植物蛋白的所述混合物与所述组织改性单位混合，其中搅棒在 10 min^{-1} 至 20 min^{-1} 的范围内顺时针旋转，并且每在 2 分钟至 10 分钟的范围内各发生一次逆时针旋转；其中在 5 分钟至 20 分钟范围内的期间内获得所述混合物；

在混合器中混合肉类产品、经水合和着色的植物蛋白以及所述组织改性单位的所述混合物，其中搅棒在 20 min^{-1} 至 40 min^{-1} 的范围内顺时针旋转，并且每在 2 分钟至 10 分钟的范围内各发生一次逆时针旋转；其中在 3 分钟至 10 分钟范围内的期间内获得所述混合物；以及

在混合器中混合肉类产品、经水合和着色的植物蛋白以及所述组织改性单位的所述混合物，其中搅棒在 20 min^{-1} 至 40 min^{-1} 的范围内顺时针旋转，并且每在 2 分钟至 10 分钟的范围内各发生一次逆时针旋转；其中在真空下在 10 分钟至 20 分钟范围内的期间内并在-100 kPa 至-50 kPa 范围内的压力下获得所述混合物。

53. 根据权利要求 52 所述的方法，其进一步的特征在于，使肉类产品、经水合和着色的植物蛋白、以及组织改性单位的所述混合物与调味单位进行混合。

54. 根据权利要求 53 所述的方法，其特征在于，所述调味单位选自由肉风味、猪肉风味、火鸡肉风味、鸡肉风味、以及它们的组合组成的组。

55. 一种经烹调的肉类替代食品，其特征在于包含:

至少一种经水合的植物蛋白;

至少一种着色剂;

至少一种肉类产品；以及

调味单位和组织改性单位。

56. 根据权利要求 55 所述的食品，其特征在于，不管是挤压的或水合的，所述植物蛋白是纤维的或组织改性的。

57. 根据权利要求 55 所述的食品，其特征在于，所述植物蛋白选自由浓缩蛋白、分离物、面粉、颗粒、以及它们的组合组成的组。

58. 根据权利要求 55 所述的食品，其特征在于，所述着色剂为焦糖色素。

59. 根据权利要求 55 所述的食品，其特征在于，所述肉类产品选自由牛肉、猪肉、火鸡肉、鸡肉、牛杂碎、猪杂碎、火鸡杂碎、鸡杂碎、机械去骨牛肉、机械去骨猪肉、机械去骨火鸡肉、机械去骨鸡肉、以及它们的组合组成的组。

60. 根据权利要求 55 所述的食品，其特征在于，所述调味单位选自由肉风味、猪肉风味、火鸡肉风味、鸡肉风味、以及它们的组合组成的组。

61. 根据权利要求 55 所述的食品，其进一步的特征在于包含防腐剂、盐、以及亚硝酸钠。

62. 根据权利要求 55 所述的食品，其特征在于，所述食品具有牛排、肉排、侧腹横肌牛排、或腌制牛肉的外观。

63. 根据权利要求 55 所述的食品，其进一步的特征在于，包括用于涂布面包屑的制剂。

64. 根据权利要求 63 所述的食品，其特征在于，所述食品具有蘸面牛肉肉排或蘸面横隔膜牛排的外观。

65. 根据权利要求 63 所述的食品，其进一步的特征在于，所述食品被油炸。

66. 根据权利要求 65 所述的食品，其特征在于，所述食品具有小牛肉薄片的外观。

67. 根据权利要求 55 所述的食品，其特征在于，所述食品被碾平、模塑、油炸、或油炸和烹调。

68. 根据权利要求 55 所述的食品，其特征在于，所述食品具有肉丝、碎肉、整肉、肉条、或肉丸的外观。

肉类替代食品及其制备方法

技术领域

本发明涉及食品，尤其是肉类替代食品的精心制作（elaboration）及其制备方法，使得获得的食品具有类似于例如牛排、肉排、侧腹横肌牛排（裙带牛排，skirt steak）、薰腌牛排（cured steak）、蘸面牛肉肉排（面包牛肉肉排，breaded veal cutlet）、蘸面横隔膜牛排（面包裙带牛排，breaded skirt steak）、小牛肉薄片（veal escalope）、肉丝、碎肉、整肉、肉条或肉丸的质地（质感，质构，texture）和外观。

背景技术

目前，肉类替代食品通常由动物蛋白和植物蛋白的组合制备。消费者对这些产品的接受直接涉及感官感觉品质如质地、味道、口感以及外观。

术语质地描述食品的广泛的物理性能。可接受质地的食品通常与食品的质量同义。质地已被定义为“物质的属性，其来自物理性能的组合，并且其是通过触觉、味觉、视觉和听觉来感知”。国际标准化组织将术语质地定义为“食品的所有神学和结构属性（几何结构和表面），其是通过机械感受器、接触以及，在合适的情况下，通过视觉和听觉感受器来感知。

在制备和制造仿制产品、肉类似物或替代物时，质地具有接受和加速的重要性，其中进行了很大的努力来复制原来食品物质的性

能。非传统原料、合成香料、填料以及延伸剂（stretcher）的使用均倾向于改变成品的某些质地特性。经常地，在复制味道、气味、以及颜色方面，质地性能的仿制具有很大的困难。已开发了许多处理方法（包括挤压和组织改性(质构化，质地化，texturization)）以模拟天然质地的性能，从而使类似于肉的产品获得更大的市场接受。已考虑到，质地相关属性涉及外观，触觉和口感，并且甚至类似于肉的产品与口腔之间的相互作用（作为消费者的适当咀嚼感觉）直接涉及肉替代产品是否被接受。

肉替代品的微观结构决定了替代品在质地、水分、香味、口味以及柔嫩方面是否具有与肉类似的质量。

在宏观水平上，某些天然肉具有视觉上可感觉到的肌纤维，它们具有圆柱形状并且是平行的。这些纤维的微观结构包含直径在微米范围的微原纤维并且它们也是圆柱形和平行的。

通过目前的方法获得的肉类产品的替代品在许多重要质量方面不同于自然牛肉。这些产品与肉是不同的，因为它们缺少肉的纤维和微原纤维结构。它们与肉的相似性仅是表面上的，并且它们还未被公众广泛接受。

在下面的专利文献中描述了目前用来提供与原先肉类产品具有类似质地的仿制产品、肉替代品或类似品的解决方案的实例。

Albert Spiel在美国专利US-4,057,656中描述了一种用于制备快速烹饪食品的方法，当被水合时，快速烹饪食品是美味咀嚼大块、软的、颜色淡的并且具有肉型质地。该方法包括在足以将水分转化成蒸汽的温度下压制植物蛋白材料，其包含30%以上的蛋白质、5%至10%的水分含量以及氮溶解指数为30至70。结果，蛋白质材料是部分或基本上软的。

Edward M. McCabe 在墨西哥专利 MX-165467 中描述了一种用于加工整个大豆的方法，以产生具有不规则形状的组织改性（texturised）蛋白质材料的离散块或离散片，其没有气味和香味并且具有外观和肉型质地。该方法包括酸化整个大豆，在水介质中将它们磨碎以提供含水悬浮液或大量的大豆颗粒，在高温下并在使组织改性的大豆蛋白具有离散片或块的形式的条件下使其通过加压水蒸气。对经组织改性的片进行干燥和再水合，以用于各种食品。可以在经组织改性的大豆蛋白的片中加入一些添加剂如开胃品、色素、脂肪、调味品以及其它蛋白质材料。

Albert Monferrer Ballester 在西班牙专利申请 ES-2,102,974 中描述了一种用于获得类似于肉或鱼的食品的方法。该方法是基于通过混合肉或鱼与成分和食品添加剂如水、盐、淀粉、面粉、蛋白质、稳定剂等所形成的块，其中通过在制得的块状产品中结合海藻酸的可溶性盐、可溶性钙盐以及 pH 值调节剂来引起内部胶凝。

Paul Hargarten 在墨西哥专利申请 MX PA04006552 中描述了一种用于制备基于植物的肉类似品的方法。所述肉类似品可以用于各种素食食品如炒绞细牛肉三明治和填充肉（stuffed meats），该方法涉及在水和冰的混合物中混合甲基纤维素以形成乳剂，然后在改性的面筋中混合植物蛋白产品（其在水中具有高溶解度并且在温和的热处理下能够形成凝胶）、油（以获得乳液基质）、以及改性的食物淀粉和调味成分（以形成经调味的乳液的基质）；可以将经调味的乳液的基质装入包装中，然后加以烹调；基于经烹调乳液的肉类仿制品与经加工的肉类产品具有高相似性，并具有改善的处理性能；在素食食品中加入肉类似品乳液有助于改善质地、口感以及汁液性。

Matthew K. McMindes、Mitchell A. Kaestner 以及 Michael W. Finfrock 在美国专利申请公开 US-2006/0035003 中描述了一种基于大豆蛋白的食品，其由以下物质形成：大豆蛋白材料，该大豆蛋白材料选自大豆蛋白面粉、大豆蛋白浓缩物、来自大豆蛋白的分离物以及它们的混合物；湿润剂，其由着色剂和任何以下物质中的至少一种形成：风味剂、甘油三酸酯、食品级的酸或酸式盐、食品级的碱或碱式盐、以及食品级乳液；以及水。可以将肉块加入到基于大豆蛋白的食品中。

Matthew K. McMindes 和 Eduardo Godinez 在美国专利申请公开 US-2006/0035005 中描述了一种重组肉的产品，其包含：纤维材料，该纤维材料包含大豆蛋白和子叶大豆的纤维，其中在没有水分的基质中按重量计子叶大豆纤维的含量为 1%至 8%；肉块以及水。制备方法包括：水合纤维材料，其包含大豆蛋白和子叶大豆纤维；加入温度低于 10°C 的肉块；以及混合纤维材料和肉块以生产均匀的和质地化的肉类产品，其具有至少 50%的水分含量。

以上描述的现有技术涉及食品工业中的限制，其中产品的质地以及其成本非常重要，因此，需要提供肉类替代食品以及其制备方法，使得获得的食品具有肉形式的长纤维，以及还优选类似于肉的微原纤维，并且涉及质地的属性，其涉及外观、触觉和口感、并且甚至类似品与口腔的相互作用、以及类似于肉的味道、气味以及颜色。

发明内容

参照上述以及为了发现对所遇到限制的解决方案，本发明的目的在于提供一种用于制备肉的食物替代品的方法，该方法包括以下步骤：在真空下水合至少一种与水和至少一种着色剂混合的植物蛋白；使至少一种肉类产品与经水合和着色的植物蛋白的混合物混

合；使组织改性材料的单位与肉类产品以及经水合和着色的植物蛋白的混合物混合，从而获得制好的食品糊；烹调制备的糊；以及形成所述烹调的糊，从而给出类似于肉类产品的表观。

可以通过以下来实施一种用于烹调制备的糊的可替换实施方式，在烤箱中以基本同时和均匀的方式碾平（滚压，滚动，roll）和烹调所述定量给予（定量给料）的糊，并且同样地对糊的上表面和下表面进行碾平和烹调，所述烤箱包括至少一个上部加热板和至少一个下部加热板，两个加热板与所述糊接触以获得肉的颜色和质地。

用于烹调制备的糊的另一种可替换实施方式是挤压或模塑所述制备的糊，使得所述制备的糊的纤维以与肉纤维的取向（定向）类似的方式加以取向（定向）；以及油炸、或油炸和烹调经挤压或模塑的糊，以获得肉的颜色和质地。

用于烹调制备的糊的另一种可替换实施方式是填充（stuffing）所述制备的糊；在烤箱或烹调锅中烹调所述经填充的糊；形成经烹调的糊；以及油炸形成的糊。

用于烹调制备的糊的又一种可替换实施方式是在烹调罐中通过搅拌来烹调制备的糊。

此外，本发明的目的是一种用于制备代替肉类食品的糊，其中所述糊包含至少一种水合植物蛋白、至少一种着色剂、至少一种肉类产品、以及调味单位和组织改性单位（质构单位，texturising unit）。

本发明的另一目的是提供一种制备用于制备肉的食物替代品的糊的方法，该方法包括以下步骤：在真空下水合至少一种与水混合的植物蛋白和至少一种着色剂；使至少一种肉类产品与经水合和

着色的植物蛋白的混合物混合；以及使肉类产品与经水合和着色的植物蛋白的混合物的组织改性单位混合，因此获得制备好的糊。

最后，本发明的目的还是一种经烹调的肉替代食物产品，其中所述产品包含至少一种水合植物蛋白、至少一种着色剂、至少一种肉类产品、以及调味单位（flavoring units）和组织改性单位。

根据本发明获得的代替肉类食品可以呈现例如，牛排、肉排、侧腹横肌牛排、薰腌牛排、蘸面牛肉肉排、蘸面横隔膜牛排、小牛肉薄片、肉丝、碎肉、整肉、肉条、或肉丸的外观。

附图说明

下文连同涉及其的附图描述本发明的特性细节，以便说明本发明，但并不限制本发明的范围。

图 1 是示出了用于制备根据本发明的肉类替代食品的糊的方法的框图。

图 2 是示出了根据本发明的用于烹调在图 1 的方法中制备好的糊的方法的第一种实施方式的框图。

图 3 示出了按照图 2 的方法的根据本发明的肉类替代食品的经烹调部分的照片。

图 4 示出了根据本发明的肉类替代食品在电子显微镜下放大 100 倍的照片。

图 5 是示出了根据本发明的用于烹调在图 1 的方法中制备好的糊的方法的第二种实施方式的框图。

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图 6 是示出了根据本发明的用于烹调在图 1 的方法中制备好的糊的方法的第三种实施方式的框图。

图 7 是示出了根据本发明的用于烹调在图 1 的方法中制备好的糊的方法的第四种实施方式的框图。

图 8 是示出了根据本发明的用于烹调在图 1 的方法中制备好的糊的方法的第五种实施方式的框图。

图 9 是示出了根据本发明的用于形成制备好的糊的方法的可替换实施方式的框图，其中制备好的糊用于在图 2、图 5、以及图 8 中描述的方法，以便产生肉类产品的类似外观。

图 10 是示出了根据本发明的用于形成制备好的糊的方法的可替换实施方式的框图，其中制备好的糊用于在图 6 中描述的方法，以便产生肉类产品的类似表观。

图 11 是示出了根据本发明的用于形成经烹调的糊的方法的可替换实施方式的框图，其中经烹调的糊用于在图 7 中描述的方法，以便产生肉类产品的类似表观。

具体实施方式

参照图 1，其示出了用来制备用于肉替代产品的糊的方法，其中该方法开始于步骤 **10**，其中纤维或组织改性型的植物蛋白（挤压的或水合的，具有浓缩蛋白质、分离物面粉(isolate flour)、颗粒、或它们组合的形式）与着色剂（优选焦糖色素）以及水混合。在真空下，以 40 至 80 分钟的间隔并在-100 kPa 至-50 kPa 的压力下，在混合器中进行混合和水合作用，其中混合器具有在 10 min^{-1} 至 20 min^{-1} 的范围顺时针旋转并且每在 2 至 10 分钟的范围内各发生一次

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逆时针旋转的搅棒（桨叶，paddle）。这种类型的在真空下的混合和水合作用减少了混合器的搅棒的转数，从而允许保护所用植物蛋白的纤维或筋（sinew）。

其后，将经水合和着色的植物蛋白的混合物与至少一种肉类产品进行混合。该肉类产品可以是，例如，肉块或碎牛肉、碎猪肉、碎火鸡肉或碎鸡肉，块状杂碎或碎牛肉（chopped beef）、猪肉、火鸡肉或鸡肉，机械去骨肉，如牛肉、猪肉、火鸡肉或鸡肉，以及它们的混合物。在混合期间，以一剂量加入肉类产品以及防腐剂、盐以及亚硝酸钠。以两个阶段进行该混合，在 15 至 45 分钟范围内的期间内在具有搅棒和开盖的混合器中进行第一混合阶段，步骤 **20**，其中搅棒在 10 min^{-1} 至 20 min^{-1} 的范围内顺时针旋转并且每在 2 至 10 分钟的范围内各发生一次逆时针旋转；然后，在 10 至 20 分钟范围内的期间内并在-100 kPa 至-50 kPa 的压力下在具有闭盖的混合器中在步骤 **30** 开始第二混合阶段，但其中搅棒在 20 min^{-1} 至 40 min^{-1} 的范围内顺时针旋转并且每在 2 至 10 分钟的范围内各发生一次逆时针旋转。这种两阶段混合和成分的剂量允许从所用的肉类产品中提取大量蛋白质。

在经水合和着色的植物蛋白已与肉类产品混合以后，它与组织改性单位（texturised unit）进行混合，以便获得制备好的糊。以三阶段混合这种混合物：第一混合阶段，步骤 **40**，包括在 5 至 20 分钟范围的期间在混合器中使肉类产品和经水合和着色的植物蛋白的混合物与所述组织改性单位混合，其中混合器具有闭盖并且其搅棒在 10 min^{-1} 至 20 min^{-1} 的范围内顺时针旋转并且每在 2 至 10 分钟的范围内各发生一次逆时针旋转；然后继续进行第二混合阶段，步骤 **50**，其包括在混合器中混合 3 至 10 分钟范围内的时间，其中搅棒在 20 min^{-1} 至 40 min^{-1} 的范围内顺时针旋转并且每在 2 至 10 分钟的范围内各发生一次逆时针旋转；然后进行第三混合步骤，步骤 **60**，

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其包括在-100 kPa 至-50 kPa 范围内的压力下在混合器中在真空下混合 10 至 20 分钟，但其中搅棒在 20 min^{-1} 至 40 min^{-1} 的范围内顺时针旋转并且每在 2 至 10 分钟的范围内各发生一次逆时针旋转。可替换地，在步骤 **40** 期间，以剂量方式加入调味单位，其可以是，例如，具有肉、猪肉、火鸡肉或鸡肉、以及它们的组合的香味的单位。

在步骤 **70** 中，在已制得用于肉类替代食品的糊以后，则按照所期望的肉类替代食品的特性开始选择用于烹调制备好的糊的方法。

图 2 示出了用于烹调制备好的糊的方法的第一种实施方式。在该实施方式中，该方法开始于步骤 **80**，其中制备好的糊被配料成（dose in）在 50 至 150 克范围内变化的多份。通过输送链将制备好的糊的所述份输送并引入到这样类型的烤箱内部，其中烤箱具有安装在相对侧（对边）上并且彼此隔开的上部加热板和下部加热板，使得以基本同时和均匀的方式可调节地碾平和烹调制备好的糊部分，以及它们的上下表面（当与两个加热板接触时），从而所述份获得肉颜色和质地，所有这些均是在步骤 **90** 中。用于该实施方式中的这种类型的烤箱的一个实例是 TwinGrill Model TWG3600/600 Series 614 的电烘箱。

用于生产肉类替代食品的制备好的糊部分在所述烤箱中经受以下操作条件：

i）在烤箱中的停留时间为约 10 秒至约 120 秒；

ii）烤箱的上部和下部加热板的温度应设置为约 100°C 至约 150°C，以便加热板可以在约 100°C 至约 150°C 的温度范围内加热烤箱的上输送带和下输送带；

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iii）烤箱的上输送带与下输送带之间的间距必须调节在 6 mm 至 15 mm 的范围内。

在步骤 **100** 中，在用于肉类替代食品的制备好的糊部分已被碾平和烹调以后，则开始选择一个或多个可替换实施方式，其适合于将最终形式赋予经烹调的糊，以便获得与原先肉类产品具有类似外观的肉类替代食品。第一可替换的步骤 **110** 是，通过这种碾平和烹调的方法并以基本同时和均匀的方式获得的肉类替代食品是自动形成有例如牛排、肉排、侧腹横肌牛排或腊肉的外观的产品，其在一种可替换实施方式，步骤 **120** 中被腌制以便获得具有例如腌制肉（marinated meat）的外观的肉替代品；或在该实施方式的一种可替换的步骤 **130** 中，被洒上面包屑以获得具有例如蘸面横隔膜牛排或蘸面牛肉肉排的外观的肉替代品，其然后可以在步骤 **140** 中被油炸以获得具有例如小牛肉薄片的外观的肉类替代食品。然后，在步骤 **150** 中，将经烹调和形成的糊冷却以达到约 10°C 至约 15°C 的温度。可替换地，加入调味汁以制备所述经烹调和形成的糊。最后，在步骤 **160** 中，包装肉类替代食品。

图 3 示出了直接通过碾平和烹调方法获得的肉类替代食品的表面的一部分。另一方面，图 4 示出了替代肉类食品在电子显微镜下放大 100 倍的照片，其中观察到具有肉形式的长纤维，以及类似于肉的微原纤维，其暗含着与外观、触觉以及口感有关的质地品质，并且包括类似品与口腔的相互作用，以及类似于肉的香味、气味、以及颜色。

回到图 2，在步骤 **170** 中选择其它可替换方式以将最终形式赋予用于肉类替代食品的经碾平和经烹调的糊，其中肉类替代食品具有与原先肉类产品类似的外观，但不具有牛排、肉排、侧腹横肌牛排或腌制牛肉的形式（下面在图 9 中描述）。

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现转向图 5，该图示出了第二种实施方式的方法，其用于根据上面图 1 所描述的方法烹调制备好的糊。在该实施方式中，该方法开始于步骤 **180**，其中在 0°C 至 4°C 范围内的温度下挤压或模塑制备好的糊，使得其纤维以类似于肉纤维的取向的方式加以定向。然后，在步骤 **190** 中，仅可以油炸、或油炸和烹调经挤压或模塑的糊，以获得原始肉的颜色和质地。

在油炸经挤压或模塑的糊的可替换实施方式（步骤 **200**）中，优选在油炸锅中在以下操作条件下进行所述实施方式：

i）在油炸锅中的停留时间为约 10 秒至约 200 秒；

ii）油炸温度为约 150°C 至约 250°C；

iii）在油炸锅中经模塑或挤压的糊达到约 70°C 至 80°C 的内部温度。

在油炸和烹调经挤压或模塑的糊的可替换实施方式（步骤 **210**）中，优选在对流烤箱中在以下操作条件下进行所述实施方式：

i）在对流烤箱中的停留时间为约 10 秒至约 200 秒；

ii）油炸和烹调温度为约 130°C 至约 250°C；

iii）在对流烤箱中经模塑或挤压的糊达到约 70°C 至 80°C 的内部温度。

在步骤 **220** 中，在用于肉类替代食品的制备好的糊已被挤压或模塑和烹调以后，则开始选择下面在图 9 中描述的一个或多个可替换实施方式，其适合于将最终形式赋予经烹调的糊，以便获得与原先肉类产品具有类似外观的肉类替代食品。

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现在在图 6 中示出了用于烹调按照上面在图 1 中所描述的方法的制备好的糊的第三种实施方式的方法。在该实施方式中，该方法开始于步骤 **230**，其中在 0°C 至 4°C 范围内的温度下将制备好的糊装入天然或人造包装（可食用的或不可食用的）中，随后，在步骤 **240** 中，在烤箱或烹调锅中烹调填充的糊（stuffed paste），其中填充的糊达到约 70°C 至约 80°C 的内部温度。

在步骤 **250**，在用于肉类替代食品的制备好的糊已被填充和烹调以后，则开始选择以下在图 10 中所描述的可替换的实施方式，其适合于将最终形式赋予经烹调的糊，以便获得与原先肉类产品具有类似外观的肉类替代食品。

图 7 示出了第四种实施方式的方法，其用于烹调按照上面在图 1 中所描述的方法的制备好的糊。在该实施方式中，上述方法开始于步骤 **260**，其中在对其进行搅拌的情况下，在烹调罐中烹调制备好的糊，其中糊达到在约 70°C 至约 80°C 范围内的内部温度。

在步骤 **270** 中，在用于肉类替代食品的制备好的糊已被烹调以后，则开始选择以下在图 11 中所描述的可替换的实施方式，其适合于将最终形式赋予经烹调的糊，以便获得与原先肉类产品具有类似外观的肉类替代食品。

现在在图 8 中示出了第五种实施方式的方法，用于烹调按照上面在图 1 中所描述的方法的制备好的糊。在该实施方式中，上述方法包括以连续阶段烹调制备好的糊以便获得肉类替代食品，该方法开始于步骤 **280**，其中制备好的糊被配料成多份，其在 50 克至 150 克的范围内变化。将制备好的糊的所述份输送到碾平和烹调的第一阶段，步骤 **290**，其在这样类型的烤箱内进行，其中烤箱具有安装在相对侧并彼此隔开的上部加热板和下部加热板（如上所述）。

随后，在步骤 **300** 中，在油炸锅中油炸、或在对流烤箱中油炸和烹调经碾平和预烹调的部分，以完成烹调。值得注意的是，烹调阶段之间的顺序可以按照性能顺序加以变化并且包括烹调的其它阶段。

在步骤 **400** 中，在用于肉类替代食品的制备好的糊的部分已被碾平和烹调以后，则开始选择可替换的实施方式，其适合于将最终形式赋予经烹调的糊，以便获得与原先肉类产品具有类似外观的肉类替代食品。第一可替换方式，步骤 **410**，是通过这种碾平和烹调的方法并以基本同时和均匀的方式获得的肉类替代食品是自动形成有例如牛排、肉排、侧腹横肌牛排或腊肉的外观的产品，其在一种可替换实施方式、在步骤 **420** 中被腌制以便获得具有例如腌制肉的外观的肉替代品；或在一种可替换实施方式、步骤 **430** 中被洒上面包屑以获得具有例如蘸面横隔膜牛排或蘸面牛肉肉排的外观的肉替代品，然后在步骤 **440** 中其可以被油炸以获得具有例如小牛肉薄片的外观的肉类替代食品。然后，在步骤 **450** 中，经烹调和形成的糊被冷却以达到约 10°C 至约 15°C 的温度。可替换地，可以加入调味汁以制备所述经烹调和形成的糊。最后，在步骤 **460** 中，包装肉类替代食品。

在步骤 **470**（下面在图 9 中描述）中，选择其它可替换方式以将最终形式赋予经碾平和经烹调的糊，从而获得与原先肉类产品具有类似外观的肉类替代食品，但并不具有牛排、肉排、侧腹横肌牛排或腊肉的形式。

图 9 是示出了根据本发明的用于形成经烹调的糊的方法的实施方式的备选方案的框图，其中经烹调的糊用于上面在图 2、图 5、以及图 8 中描述的方法，以获得肉类产品的类似外观。在该实施方式中，上述方法开始于步骤 **480**，其中经烹调的糊被冷却以达到在

约 0°C 至约 15°C 范围内的温度。然后，在步骤 **490** 中，为经烹调的糊选择形式和最终外观的类型以在市场上作为替代肉类食品，例如，在步骤 **500** 中，经烹调的糊在肉切碎机中被切碎以赋予它肉丝（肉丝）的形式；或在步骤 **510** 中，经烹调的糊在食品切块机（food cube cutter）中被切割以赋予它整肉或肉条的形状；或在步骤 **520** 中，经烹调的糊被切碎以赋予它碎肉的形状。可替换地，可以加入调味汁以制备所述经烹调和形成的糊。最后，在步骤 **530** 中，包装肉类替代食品。

图 10 是示出了根据本发明的方法实施方式的备选方案的框图，用来赋予经烹调的糊一定形状，用于上面在图 6 中描述的方法，以及获得肉类产品的类似外观。在该实施方式中，上述方法开始于步骤 **540**，其中经烹调的糊被冷却以达到在约 0°C 至约 15°C 的范围内的温度。然后，在步骤 **550** 中，为经烹调的糊选择形式和最终外观的类型以在市场上作为替代肉类食品进行其商品化，例如，在步骤 **560** 中，经烹调的糊在肉切碎机中被切碎以赋予它肉丝的形式；或在步骤 **570** 中，经烹调的糊在食品切块机中被切割以赋予它整肉或肉条的形状；或在步骤 **580** 中，经烹调的糊被切碎以赋予它碎肉的形状。可替换地，以肉丸的形式粘着经烹调和磨碎的糊。在经烹调的糊获得其形状以后，在油炸锅中在以下操作条件下对其进行油炸：

i）在油炸锅中的停留时间为约 10 秒至约 200 秒；

ii）油炸温度为约 150°C 至约 250°C；

iii）在油炸锅中经模塑或挤压的糊达到约 70°C 至 80°C 的内部温度。

然后，在步骤 **590** 中，经烹调和形成的糊被冷却以达到约 0°C 至约 15°C 的温度。可替换地，可以加入调味汁以制备所述经烹调和形成的糊。最后，在步骤 **600** 中，包装肉类替代食品。

图 11 是示出了根据本发明的方法实施方式的备选方案的框图，用来赋予经烹调的糊一定形式，用于上面在图 7 中描述的方法，以及获得肉类产品的类似外观。在该实施方式中，上述方法开始于步骤 **610**，其中为经烹调的糊选择形式和最终外观的类型以在市场上作为替代肉类食品进行其商品化，例如，在步骤 **620** 中，经烹调的糊在肉切碎机中被切碎以赋予它肉丝的形式；或在步骤 **630** 中，经烹调的糊在绞肉机中被切碎以赋予它碎肉的形状。可替换地，以肉丸的形式粘着经烹调和磨碎的糊。然后，在步骤 **640** 中，经烹调和形成的糊被冷却以达到约 0°C 至约 15°C 的温度。可替换地，可以加入调味汁以制备所述经烹调和形成的糊。最后，在步骤 **650** 中，包装肉类替代食品。

实施例

接着，列出实施方式的多个实施例，其每一个都描述如何获得不同于本发明的肉类替代食品的典型的方式。

实施例 1

将颗粒形式的 500 kg 量的植物蛋白真空水合，并与 1500 升的水和 15 kg 的焦糖色素（caramel color）混合。在约 70 分钟期间内在真空下进行水合和混合，其中混合器的搅棒在旋转的交替循环中以 18 min^{-1} 进行旋转，每个循环持续约 3 分钟，施加的压力为约-90 kPa。

在水合和混合以后，植物蛋白与以一定量加入的以下组分进行混合：

牛脂肪	150 kg
切割的牛肉	100 kg
鸡肉糊	1900 kg
精制盐	45 kg
亚硝酸钠	1 kg
防腐剂	25 kg

首先在具有开盖的混合器中并在 40 分钟内进行混合，其中混合器的搅棒在旋转的交替循环中以 18 min^{-1} 进行旋转，每个循环持续约 7 分钟。接着，关闭混合器以在约 20 分钟内在真空下进行第二混合阶段，其中在旋转的交替循环中混合器的搅棒以 35 min^{-1} 进行旋转，每个循环持续约 5 分钟，同时施加的压力为约-90 kPa。

随后，将 350 kg 的组织改性单位混合于获得的混合物。首先在具有开盖的混合器中并在约 10 分钟内进行该混合，其中混合器的搅棒在旋转的交替循环中以 18 min^{-1} 进行旋转，每个循环持续约 3 分钟。接着，在约 5 分钟期间内进行第二混合阶段，以将在旋转的交替循环中搅棒的旋转变化为 35 min^{-1}，其中每个循环持续约 5 分钟。随后，关闭混合器以在约 12 分钟期间内在真空下进行第三混合阶段，其中混合器的搅棒在旋转的交替循环中以 35 min^{-1} 进行旋转，每个循环持续约 5 分钟，同时施加的压力为约-90 kPa。制备好的糊的温度为 4°C。

在制成以后，糊被配料成 100 克的多份。使用来生产肉类替代食品的制备好的糊的这些份在 TwinGrill Modelo TWG3600/600 Series 614 电烤箱中经受以下操作条件：

i）在烤箱中的停留时间为约 70 秒；

ii）烤箱的上部和下部加热板的温度为约 120°C；

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iii）烤箱的上输送带与下输送带之间的间距为 10 mm。

经烹调的肉类替代食品的份被冷却以达到 8°C 的温度。最后，加入调味汁以获得待包装的肉的食物替代品。由此获得的产品显示在图 3 和图 4 中。

通过这种碾平和一般以基本同时和均匀的方式烹调的方法获得的肉类替代食品是自动形成有例如牛排、肉排、侧腹横肌牛排或腊肉的外观的产品，其可以被腌制以获得具有例如腌制肉的外观的肉类替代食品；或可以被洒上面包屑以便获得具有例如蘸面横隔膜牛排或蘸面牛肉肉排的外观的肉类替代食品，然后其可以被油炸以生产具有例如小牛肉薄片的外观的肉类替代食品。经烹调部分的肉类替代食品被冷却以达到 10°C 的温度。最后，加入调味汁以获得待包装的肉的食物替代品。由此获得的产品显示在图 3 和图 4 中。

实施例 2

利用在实施例 1 中制备的 250 kg 的糊，在烹调的三个阶段在以下操作条件下通过对糊进行搅拌而着手在烹调罐对糊进行烹调：

罐的温度	40°C
烹调时间	10 分钟
罐的旋转	40 min^{-1}

在以下条件下，罐的第一烹调阶段：

罐的温度(蒸汽)	75°C
烹调时间	25 分钟
罐的旋转	10 min^{-1}
糊的温度	60°C

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在以下条件下，罐的第二烹调阶段：

罐的温度(蒸汽)	75°C
烹调时间	15 分钟
罐的旋转	30 min^{-1}
糊的温度	60°C

在以下条件下，罐的第三烹调阶段：

罐的温度(没有蒸汽)	75°C
烹调时间	15 分钟
罐的旋转	10 min^{-1}
糊的温度	72°C

在制备好的糊被烹调为肉类替代食品以后，它在肉切碎机中被切碎以赋予它肉丝的形式；其随后被冷却以达到 8°C 的温度。最后，加入调味汁以获得待包装的肉的食物替代品。

基于上述实施方式，可以认为，对本领域技术人员来说显而易见的是，对于这些实施方式以及可替换实施方式环境的改进将包括在本描述中。因此，可以认为，权利要求涵盖那些在本发明或其等同物的范围内的改进和备选方案。

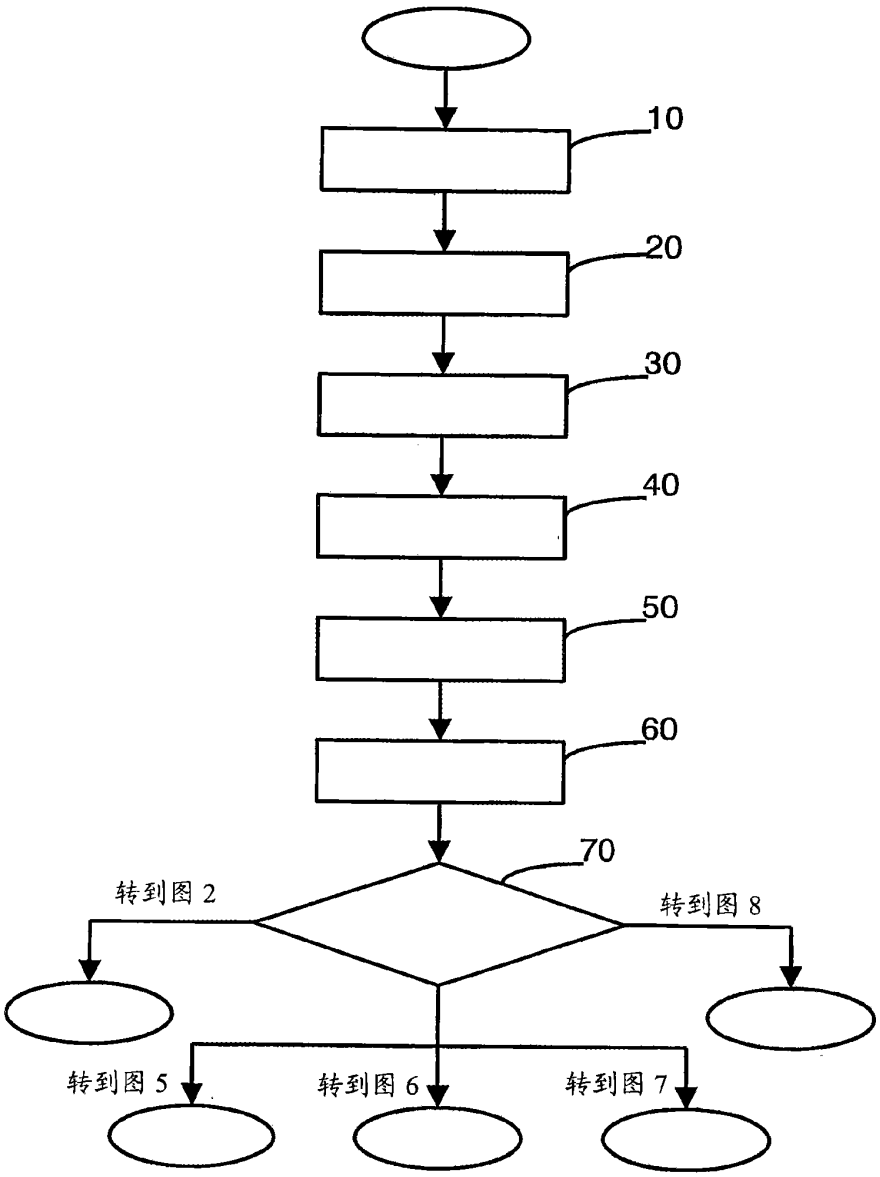

图 1

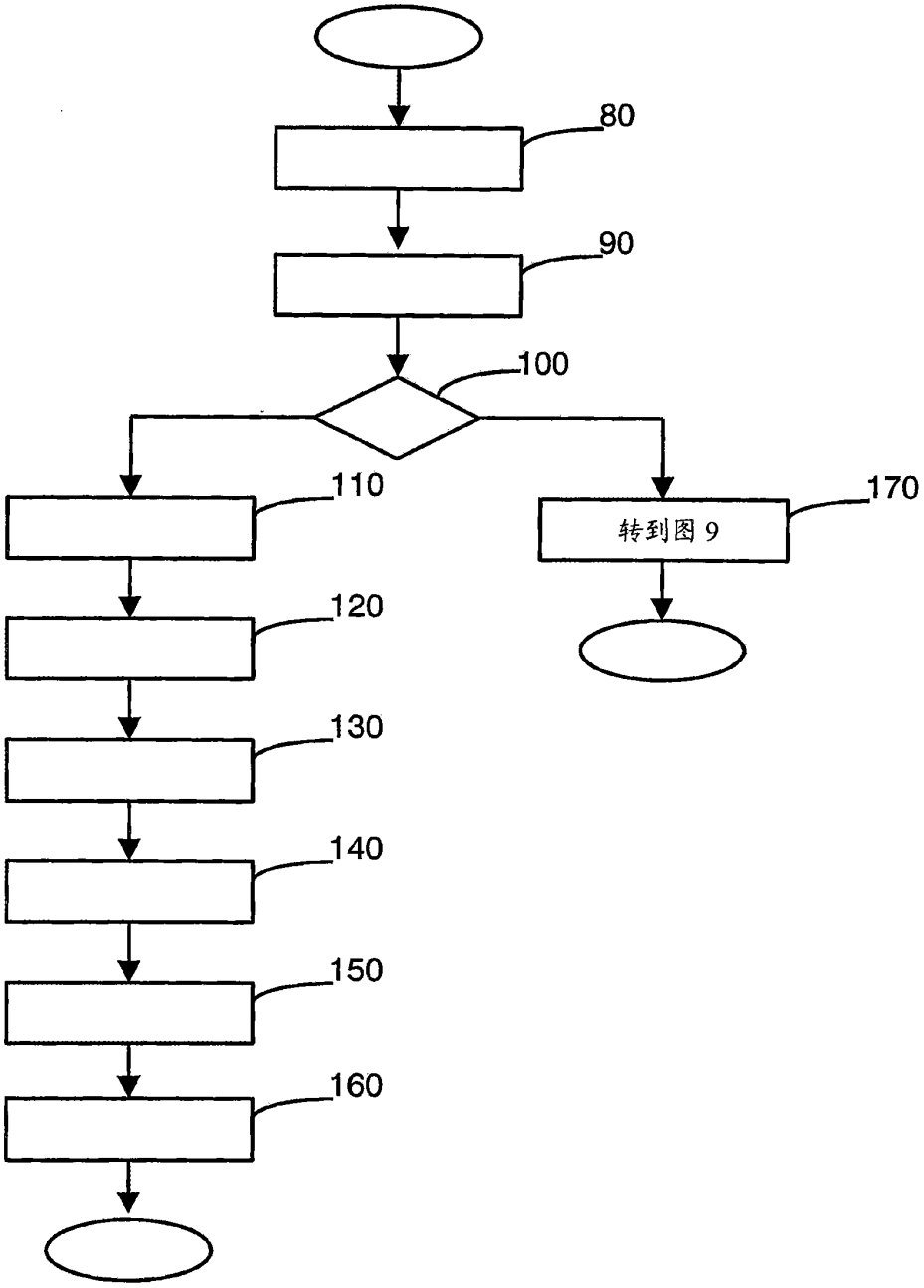

图 2

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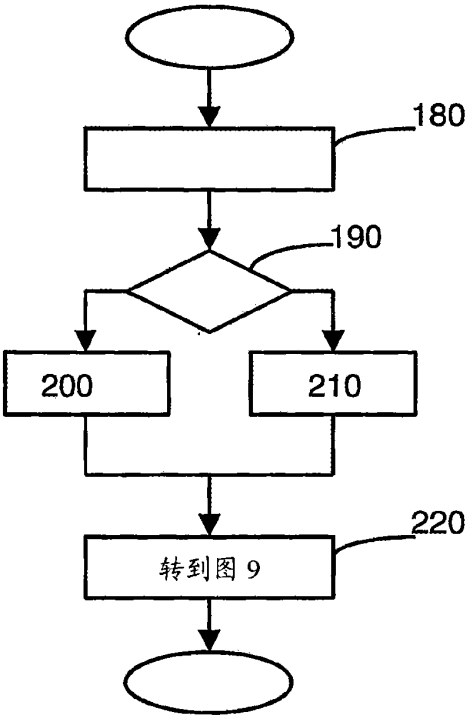

图 5

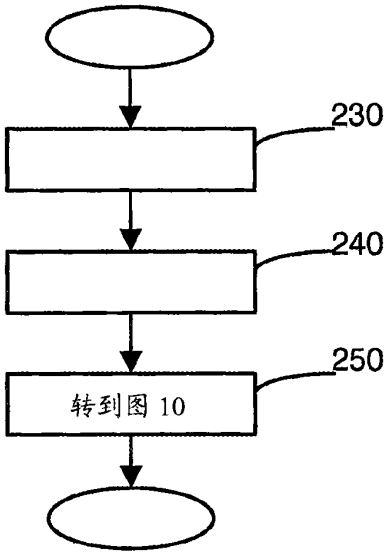

图 6

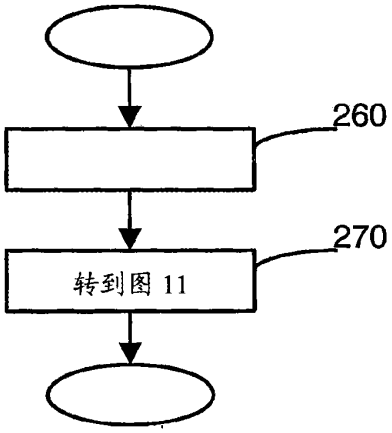

图 7

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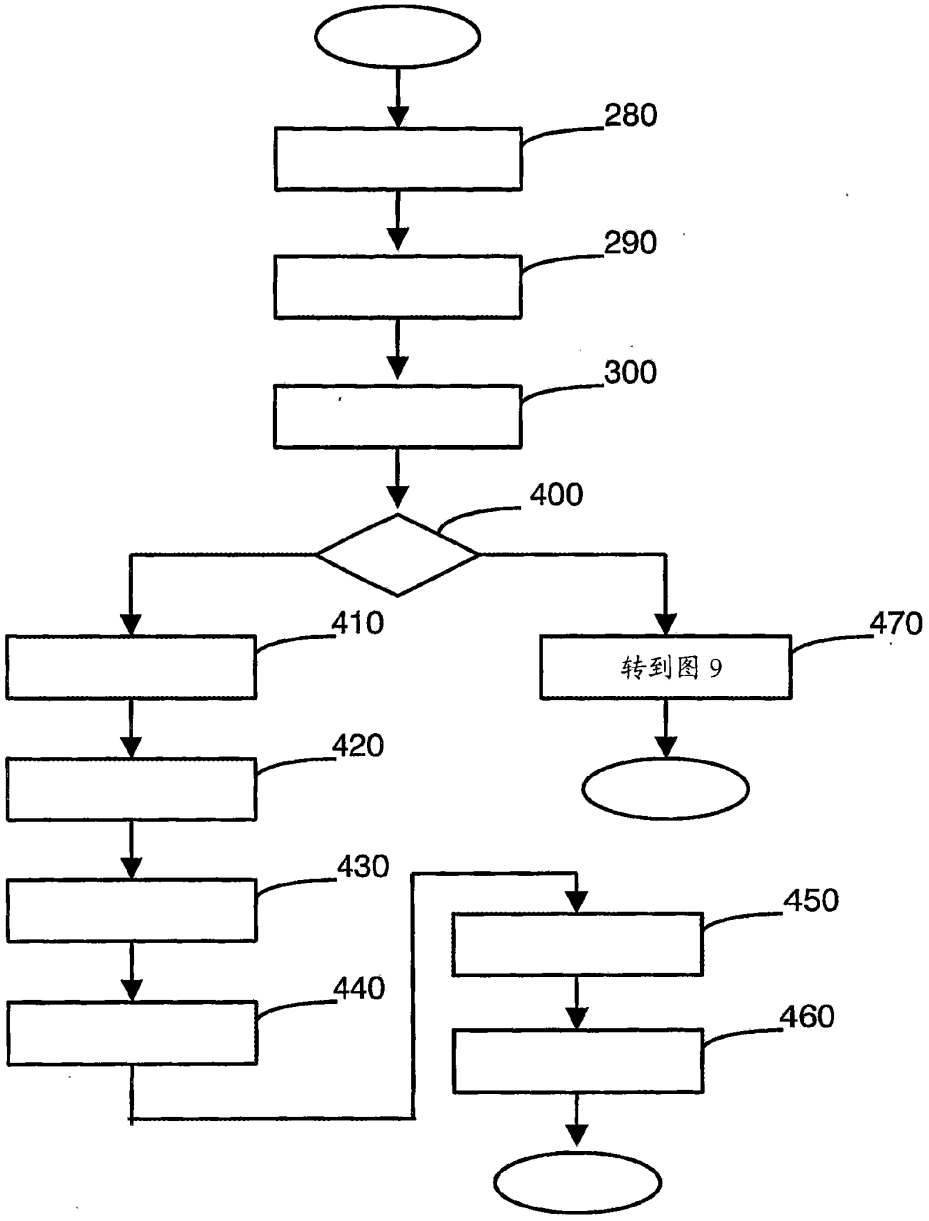

图 8

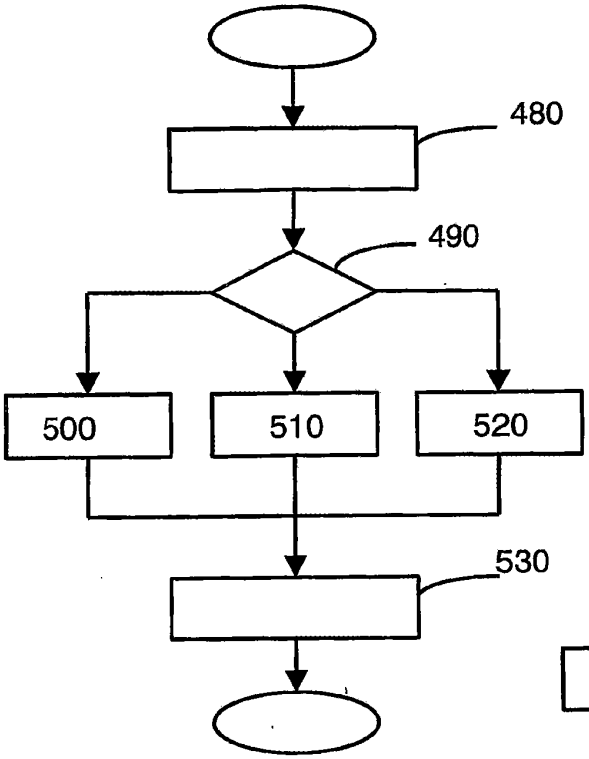

图 9

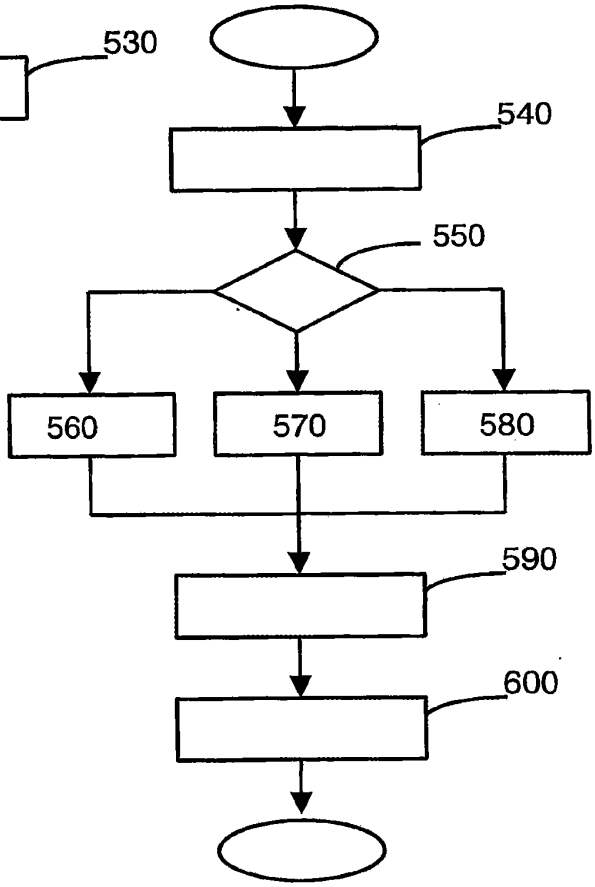

图 10

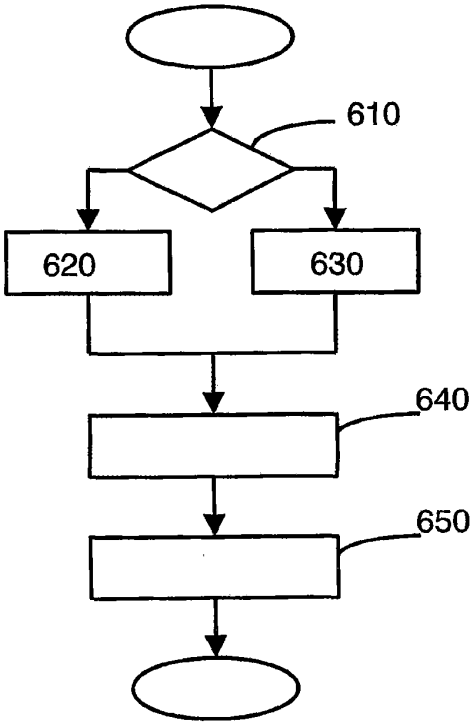

图 11

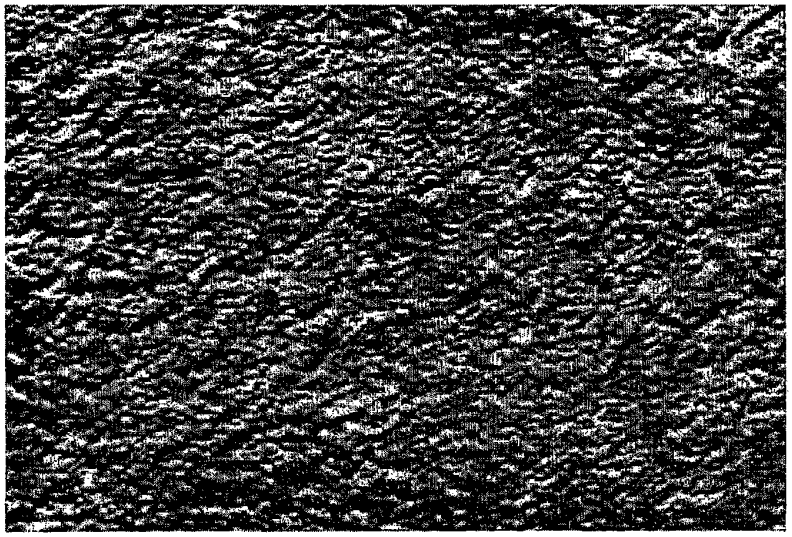

图 3

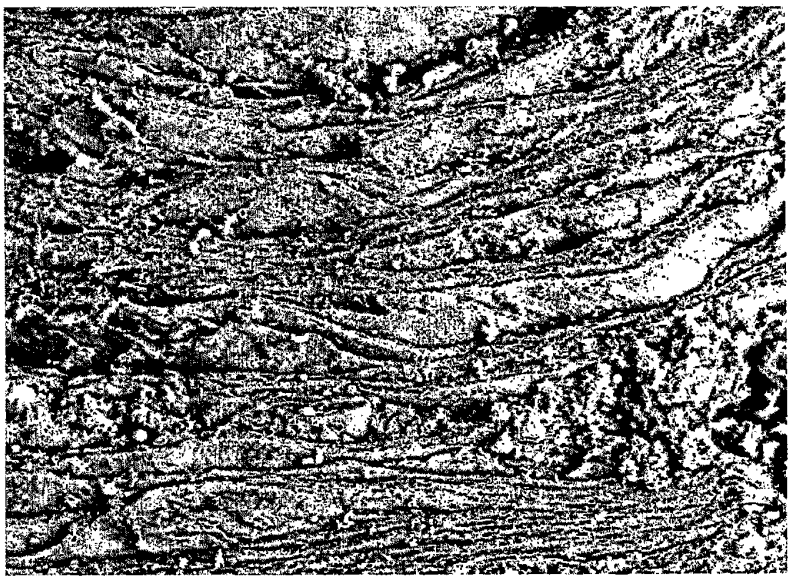

图 4

Espacenet

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Natural high-iron albumen powder and preparation method thereof

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Applicant(s): UNIV CHINA AGRICULTURAL [CN] ± (CHINA AGRICULTURAL UNIVERSITY)

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- cooperative:

Application number: CN2009188248 20090714

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Abstract of CN101606574 (A)

The invention belongs to the field of functional foods, and in particular relates to natural high-iron albumen powder and a preparation method thereof. The method comprises the steps of soybean milk grinding, heated centrifugation, salting precipitation, centrifugal settling and freeze drying. The process comprises the following steps: weighing dry beans, pulping the dry beans by using PBS damping liquid with the quality 1-2 times of that of the dry beans and the concentration of 50mM after soaking the dry beans for 8-12 hours, and filtering and removing bean dregs, wherein polyvinylpyrrolidone in the damping liquid is 0.5-10 grams/liter, heating the pulp for 10-15 minutes in warm bath at 50-60 DEG C, cooling the pulp to room temperature, centrifugalizing at 4500-8000rpm for 10-20 minutes, removing starch and doped protein and taking supernatant liquid; dissolving magnesium chloride, dissolving sodium citrate after 30-120min, evenly mixing and stirring for 8-12 hours at 4 DEG C, wherein the concentration of the magnesium chloride and the concentration of the sodium citrate are respectively 200-600mM and 300-700mM; centrifugalizing at 0-7 DEG C with more than 10000rpm for 20-40 minutes, removing the supernatant liquid and grease and taking deposit parts; and carrying out freeze drying on the deposit parts.

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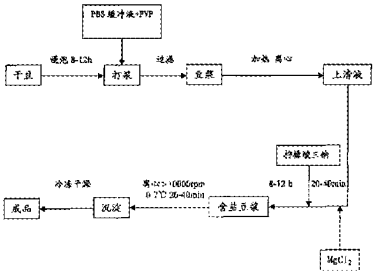
[54] 发明名称

天然高铁蛋白粉及其制备方法

[57] 摘要

本发明属于功能性食品领域，特别涉及一种天然高铁蛋白粉及其制备方法。该方法包括：磨豆浆、加热离心、加盐盐析、离心沉淀和冷冻干燥，过程如下：称取干豆，浸泡 8 ~ 12 小时后，用质量为干豆质量 1 ~ 2 倍的浓度为 50mM 的 PBS 缓冲液打成浆，缓冲液中聚乙烯吡烷酮为 0.5 ~ 10 克/升，过滤去除豆渣；在 50 ~ 60℃温水浴中加热 10 ~ 15 分钟，冷却至室温，4500 ~ 8000rpm 离心 10 ~ 20 分钟，弃淀粉和杂蛋白，取上清液；溶解氯化镁，30 ~ 120 分钟后溶解柠檬酸三钠并混匀，在 4℃搅拌 8 ~ 12 小时，氯化镁和柠檬酸三钠的浓度分别是 200 ~ 600mM 与 300 ~ 700mM；0 ~ 7℃时，以大于 10000rpm 离心 20 ~ 40 分钟，弃上清液和油脂，取沉淀部分；将沉淀部分冷冻干燥。



1. 一种天然高铁蛋白粉的制备方法，其特征在于：

所述制备方法包括以下步骤：

A. 磨豆浆：称取干豆，浸泡 8～12 小时后，添加质量为干豆质量 1～2 倍、浓度为 50mM 的 PBS 缓冲液， PBS 缓冲液中添加的聚乙烯吡烷酮为 0.5～10 克/升，PBS 缓冲液的 pH 值为 7～8，将干豆和 PBS 缓冲液的混合物打成浆，过滤去除豆渣，得到豆浆；

B. 加热离心：将所述豆浆在 50～60℃温水浴中加热 10～15 分钟，冷却至室温后，以 4500～8000rpm 的速度离心 10～20 分钟，弃去淀粉和杂蛋白，取上清液；

C. 加盐盐析：在所述上清液中先溶解氯化镁，30～120 分钟后溶解柠檬酸三钠并混匀，在 1～7℃搅拌 8～12 小时，得到含盐豆浆；其中氯化镁和柠檬酸三钠的浓度分别是 200～600mM 与 300～700mM；

D. 离心沉淀：在 0～7℃时，将所述含盐豆浆以大于 10000rpm 的速度离心 20～40 分钟，弃去上清液和油脂，回收沉淀部分；

E. 冷冻干燥：将所述的沉淀部分冷冻干燥，得到天然高铁蛋白粉。

2. 根据权利要求 1 所述的制备方法，其特征在于：所述干豆为大豆、黑豆或豌豆中的一种。

3. 根据权利要求 2 所述的制备方法，其特征在于：当所述干豆为黑豆时，浸泡 8～12 小时后去皮。

4. 根据权利要求 1 所述的制备方法，其特征在于：在加热离心步骤

中，将加热并冷却至室温的豆浆以 4500～6000 rpm 的速度离心 10 分钟。

5. 根据权利要求 1 所述的制备方法，其特征在于：在加盐盐析步骤中，将所述上清液在 4℃搅拌 10 小时，氯化镁和柠檬酸三钠的浓度分别是 500mM 与 700mM。

6. 根据权利要求 1 所述的制备方法，其特征在于：在离心沉淀步骤中，将所述含盐豆浆在 4 ℃时，以大于 10000rpm 的速度离心 30 分钟。

7. 一种天然高铁蛋白粉，其特征在于：所述天然高铁蛋白粉的制备包括以下步骤：

A. 磨豆浆：称取干豆，浸泡 8～12 小时后，添加质量为干豆质量 1～2 倍、浓度为 50mM 的 PBS 缓冲液， PBS 缓冲液中添加的聚乙烯吡烷酮为 0.5～10 克/升，PBS 缓冲液的 pH 值为 7～8，将干豆和 PBS 缓冲液的混合物打成浆，过滤去除豆渣，得到豆浆；

B. 加热离心：将所述豆浆在 50～60℃温水浴中加热 10～15 分钟，冷却至室温后，以 4500～8000rpm 的速度离心 10～20 分钟，弃去淀粉和杂蛋白，取上清液；

C. 加盐盐析：在所述上清液中先溶解氯化镁，30～120 分钟后溶解柠檬酸三钠并混匀，在 1～7℃搅拌 8～12 小时，得到含盐豆浆，氯化镁和柠檬酸三钠的浓度分别是 200～600mM 与 300～700mM；

D. 离心沉淀：在 0～7℃时，将所述含盐豆浆以大于 10000rpm 的速度离心 20～40 分钟，弃去上清液和油脂，回收沉淀部分；

E. 冷冻干燥：将所述的沉淀部分冷冻干燥，得到天然高铁蛋白粉。

天然高铁蛋白粉及其制备方法

技术领域

本发明属于功能性食品领域，特别涉及一种天然高铁蛋白粉及其制备方法。

背景技术

目前，缺铁导致的疾病是一类严重影响人类健康的疾病。世界卫生组织 2002 年发表的统计数据表明，全球有 20 亿人患有铁缺乏症，缺铁成为第九类健康风险因素。因此，改善铁营养状况，是世界也是我国迫切需要解决的问题。目前使用的补血铁剂主要是二价的非血红素铁，如硫酸亚铁以及有机酸亚铁盐类。由于这些补铁剂具有副作用，易发生铁中毒，铁吸收率不高，且生产或储存都很困难，导致补铁效果不理想，所以开发更好的补铁产品势在必行。

豆类中的铁元素主要存储在铁蛋白中，它的补铁作用主要体现在人体对铁蛋白的吸收。铁蛋白通常为由 24 个亚基组成的中空球状分子，内外直径分别约为 8 以及 12.5 nm。铁蛋白包括 8 个 3 倍轴通道与 6 个 4 倍轴通道，负责铁蛋白与外部环境的物质交换，其中，3 倍轴通道是铁的主要通道和 Fe^{2+}氧化作用位点，铁离子通过此通道进入核中。铁蛋白广泛存在于动物、植物和微生物的细胞中，主要调节铁的代谢平衡。当细胞内 Fe^{2+}浓度高时，铁蛋白催化 Fe^{2+}被氧气分子氧化的反应，并把生成的 Fe^{3+}储藏于其内部空腔中，1 分子铁蛋白可最多储存 4500 个 Fe^{3+}；当细胞需要铁时（Fe^{2+}浓度低时），铁蛋白在还原剂的帮助下将 Fe^{3+}还原为 Fe^{2+}，

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使铁从其内部释放出来供其它蛋白质合成利用，所以铁蛋白在细胞内具有去除 Fe^{2+} 的毒性以及调节铁代谢平衡的双重作用。

近十几年来，铁蛋白（特别是植物铁蛋白）的生物学功能得到了人们极大关注，目前报道人体对大豆中铁（主要以铁蛋白形式存在）的吸收率约为 24.5%; 人体对大豆铁蛋白的吸收与对硫酸亚铁的吸收没有显著性差异，并表明大豆铁蛋白是一种天然的新型补血铁剂。由于植物铁蛋白具有良好的补铁生物活性，它被看作是 21 世纪新型的补铁剂，其应用前景非常光明。

目前，对于动物铁蛋白的研究较多，然而由于其生产成本很高使得很少应用于功能食品的开发中。相对于动物体而言，从植物体中提取铁蛋白一方面成本比较低，另一方面具有高度的安全性。大豆不仅在我国，在全世界都有着非常多的种植面积和非常高的产量，而工业生产主要应用于榨油，对大豆蛋白包括铁蛋白的损失非常大。利用大豆或者豆粕生产一种天然高铁蛋白粉的功能性食品有着非常好的应用前景，这在国内外尚无报道。

传统的补血铁制剂主要是二价的非血红素铁（如：硫酸亚铁、有机酸亚铁盐类等），由于这些补铁剂具有副作用并且铁的吸收效率不高，导致补铁效果不理想；另一方面，如果体内铁等金属离子过多，则会诱发体内产生大量自由基，从而破坏机体组织，损害心脏、大脑等器官，增加罹患癌症的风险。因此，寻找一种安全、高效的补铁制剂势在必行。然而，天然存在于生物体中的铁蛋白的发现给了我们极大的启示，由于它不仅能够贮藏大量的铁（每分子铁蛋白最多可容纳 4500 个 Fe^{3+}，而且可以调节细胞内铁的动态平衡，所以我们预想如果将铁蛋白开发成功能性食品，不仅可以起到补铁作用，而且可以防止由于体内铁素过多而引起的中毒。已经证明铁蛋白具有和硫酸亚铁相同的补铁效果，所以将铁

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蛋白开发成功能食品是可行的。在已报道的植物铁蛋白中，大豆种子中铁蛋白相对比较丰富。

发明内容

本发明的目的之一是提供一种天然高铁蛋白粉的制备方法，采用磨豆浆、加热离心、加盐盐析、离心沉淀和冷冻干燥等步骤，可以大大提高蛋白粉中的铁蛋白和蛋白粉的含量。

本发明的另一目的是得到一种采用上述方法制备的天然高铁蛋白粉。

一种天然高铁蛋白粉的制备方法，包括以下步骤：

A. 磨豆浆：称取干豆，浸泡 8～12 小时后，添加质量为干豆质量 1～2 倍、浓度为 50mM 的 PBS 缓冲液， PBS 缓冲液中添加的聚乙烯吡烷酮为 0.5～10 克/升，PBS 缓冲液的 pH 值为 7～8，将干豆和 PBS 缓冲液的混合物打成浆，过滤去除豆渣，得到豆浆；

B. 加热离心：将所述豆浆在 50～60℃温水浴中加热 10～15 分钟，冷却至室温后，以 4500～8000rpm 的速度离心 10～20 分钟，弃去淀粉和杂蛋白，取上清液；

C. 加盐盐析：在所述上清液中先溶解氯化镁，30～120 分钟后溶解柠檬酸三钠并混匀，在 1～7℃搅拌 8～12 小时，得到含盐豆浆；其中氯化镁和柠檬酸三钠的浓度分别是 200～600mM 与 300～700mM；

D. 离心沉淀：在 0～7℃时，将所述含盐豆浆以大于 10000rpm 的速度离心 20～40 分钟，弃去上清液和油脂，回收沉淀部分；

E. 冷冻干燥：将所述的沉淀部分冷冻干燥，得到天然高铁蛋白粉。

所述干豆为大豆、黑豆或豌豆中的一种。

当所述干豆为黑豆时，浸泡 8～12 小时后去皮。

在加热离心步骤中，将加热并冷却至室温的豆浆以 4500～6000 rpm 的速度离心 10 分钟。

在加盐盐析步骤中，将所述上清液在 4℃搅拌 10 小时，氯化镁和柠檬酸三钠的浓度分别是 500mM 与 700mM。

在离心沉淀步骤中，将所述含盐豆浆在 4 ℃时，以大于 10000rpm 的速度离心 30 分钟。

所述干豆为大豆、黑豆或豌豆中的一种。

当所述干豆为黑豆时，浸泡 8～12 小时后去皮。

在加热离心步骤中，将加热并冷却至室温的豆浆以 4500～6000 rpm 的速度离心 10 分钟。

在加盐盐析步骤中，将所述上清液在 4℃搅拌 10 小时，氯化镁和柠檬酸三钠的浓度分别是 500mM 与 700mM。

在离心沉淀步骤中，将所述含盐豆浆在 4 ℃时，以大于 10000rpm 的速度离心 30 分钟。

一种天然高铁蛋白粉，所述天然高铁蛋白粉的制备包括以下步骤：

A. 磨豆浆：称取干豆，浸泡 8～12 小时后，添加质量为干豆质量 1～2 倍、浓度为 50mM 的 PBS 缓冲液， PBS 缓冲液中添加的聚乙烯吡烷酮为 0.5～10 克/升，PBS 缓冲液的 pH 值为 7～8，将干豆和 PBS 缓冲液的混合物打成浆，过滤去除豆渣，得到豆浆；

B. 加热离心：将所述豆浆在 50～60℃温水浴中加热 10～15 分钟，冷却至室温后，以 4500～8000rpm 的速度离心 10～20 分钟，弃去淀粉和杂蛋白，取上清液；

C. 加盐盐析：在所述上清液中先溶解氯化镁，30～120 分钟后溶解柠檬酸三钠并混匀，在 1～7℃搅拌 8～12 小时，得到含盐豆浆，氯化镁

和柠檬酸三钠的浓度分别是 200～600mM 与 300～700mM;

D. 离心沉淀：在 0～7℃时，将所述含盐豆浆以大于 10000rpm 的速度离心 20～40 分钟，弃去上清液和油脂，回收沉淀部分;

E. 冷冻干燥：将所述的沉淀部分冷冻干燥，得到天然高铁蛋白粉。

本发明的有益效果在于:

通过使用一系列的蛋白分离技术，得到富含铁蛋白的高铁蛋白粉，产品最终铁蛋白含量能达到 9.28%左右。在人类补铁的功能性食品方面开发出了一种新的更天然更有效的产品。本发明所采用的原料简单易得，又是天然食品，加工工艺简单易操作。主要成份为含铁铁蛋白，既是一种天然植物蛋白，又是一种新型有效的补铁剂，通过对豆类种子的简单加工使铁蛋白含量比原来提高了 150 多倍，极大的提升了豆类和豆粕的利用价值，也开拓了补铁性功能食品的市场。

附图说明

图 1——为本发明的一种天然高铁蛋白粉的制备方法流程图。

具体实施方式

下面结合实施例对本发明作进一步说明。

本发明公开了一种天然高铁蛋白粉的制备方法，包括以下步骤:

1. 磨豆浆

本发明所处理的原料选自大豆、黑豆或者豌豆中的一种。根据需要，也可以选用其他富含铁质的豆类原料。

首先，原料需要浸泡处理，所需处理的时间一般为 8～12 小时。随后，进行打浆处理。由于产品补铁功能性成分为铁蛋白，铁蛋白为水溶

性蛋白，在生产过程中过高或者过低的 pH 值溶液会对铁蛋白的稳定性和产品含铁量有比较大的影响。因此，PBS 缓冲液的 pH 值为 7～8。添加质量为干豆质量 1～2 倍、浓度为 50mM 的 PBS 缓冲液（即磷酸缓冲溶液），缓冲液中的 PVP（即聚乙烯吡烷酮）为 0.5～10 克/升，将干豆和 PBS 缓冲液的混合物打成浆，过滤去除豆渣，得到豆浆。

2. 加热离心

由于铁蛋白是一种耐热蛋白，最高可耐 75℃高温。把磨制出的豆浆在 50～60℃温水浴中加热 10～15 分钟，这个步骤是为了下一步离心除去部分淀粉提供方便。

把加热并冷却至室温的豆浆在 4500～8000rpm 速度下离心 10～20 分钟，除去豆浆中部分淀粉和杂蛋白，取上清液。

3. 加盐盐析

在上清液中先溶解氯化镁，30～120 分钟后溶解柠檬酸三钠并混匀，在 1～7℃搅拌 8～12 小时。根据所处理的原料不同，氯化镁和柠檬酸三钠的浓度分别是 200～600mM 与 300～700mM，温度和搅拌时间也可略微调整。

这一步的目的是使铁蛋白沉淀析出，当然会伴随着其他一些杂蛋白的沉淀。加盐种类和浓度对最后产品中铁蛋白的含量至关重要。

4. 离心沉淀

盐析后的豆浆在 0～7℃下以大于 10000rpm 的速度离心 20～40 分钟，弃去上清液和油脂，回收暗红色沉淀部分，此部分即为所需要的高铁蛋白，主要成分为含铁铁蛋白。

5. 冷冻干燥

将上一步骤中离心沉淀得到的高铁蛋白冷冻干燥，即得到天然高铁蛋白粉。

本发明还公开了一种采用上述方法制备的天然高铁蛋白粉。

下面通过描述具体实施例对本发明进行进一步说明。

实施例1

原料为大豆，其铁蛋白含量约为 60 mg/kg，含铁量约为 0.82 mg/ kg。

称取 2 kg 的干大豆，浸泡 10 小时后，用质量为干豆质量 1.5 倍、浓度为 50mM 的 PBS 缓冲液打成浆，PBS 缓冲液中的聚乙烯吡烷酮为 0.5～10 克/升，PBS 缓冲液的 pH 值为 7～8，用纱布过滤除豆渣，得到豆浆。

将上述豆浆在 55℃温水浴中加热 15 分钟，冷却至室温后以 4500rpm 离心 10 分钟，取上清液；

在上清液中先溶解氯化镁，30 分钟后溶解柠檬酸三钠并混匀，4℃下搅拌 10 小时，得到含盐豆浆，氯化镁和柠檬酸三钠的浓度分别为 500 mM 与 700mM ；

将含盐豆浆在 4℃以大于 12000rpm 的速度离心 30 分钟，弃上清液，取沉淀部分；

冷冻干燥：将上一步骤中得到的沉淀部分冷冻干燥，得到天然高铁蛋白粉。

经检测，天然高铁蛋白粉中的最终蛋白粉为 4.83g，铁蛋白含量约为 68.7g/kg，含铁量约为 0.137g/ kg。

实施例 2

原料为黑豆，其铁蛋白含量约为 50mg/kg，含铁量约为 0.7mg/ kg。

Case No.: IPR2023-00208
U.S. Patent No. 10,039,306

称取 2kg 干黑豆，浸泡 10 小时后去皮，用质量为干豆质量 1.5 倍、浓度为 50mM 的 PBS 缓冲液打成浆，PBS 缓冲液中的聚乙烯吡烷酮为 0.5～10 克/升，PBS 缓冲液的 pH 值为 7～8，用纱布过滤除豆渣，得到豆浆。

将上述豆浆在 55℃温水浴中加热 15 分钟，冷却至室温后，以 4500 rpm 离心 10 分钟，取上清液；

在上清液中先溶解氯化镁，30 分钟后溶解柠檬酸三钠并混匀，4℃下搅拌 10 小时，得到含盐豆浆，氯化镁和柠檬酸三钠的浓度分别为 300 mM 与 450mM ；

将含盐豆浆在 4℃以 12000rpm 离心 30 分钟，弃上清液，取沉淀部分；

冷冻干燥：将上一步骤中得到的沉淀部分冷冻干燥，得到天然高铁蛋白粉。

经检测，天然高铁蛋白粉中的最终蛋白粉为 4.21g，铁蛋白含量约为 51.4g/kg，含铁量约为 0.117g/ kg。

实施例 3

原料为豌豆，其铁蛋白含量约为 50mg/kg，含铁量约为 0.49mg/ kg。

称取 2kg 干豌豆，浸泡 10 小时后，用质量为干大豆 1.5 倍、浓度为 50mM 的 PBS 缓冲液打成浆，PBS 缓冲液中的聚乙烯吡烷酮为 0.5～10 克/升，PBS 缓冲液的 pH 值为 7～8，用纱布过滤除豆渣，得到豆浆；

将豆浆在 55℃温水浴中加热 15 分钟，冷却至室温后以 4500rpm 离心 10 分钟，取上清液；

在上清液中先溶解氯化镁，30 分钟后溶解柠檬酸三钠并混匀，4℃下搅拌 10 小时，得到含盐豆浆，氯化镁和柠檬酸三钠的浓度分别为 200 mM 与 300mM ；

Motif Exhibit 1148, Page 322 of 3341

将含盐豆浆在4℃以12000rpm的速度离心30分钟，弃上清液，取沉淀部分；

冷冻干燥：将上一步骤中得到的沉淀部分冷冻干燥，得到天然高铁蛋白粉。

经检测，天然高铁蛋白粉中的最终蛋白粉为3.89g，铁蛋白含量约为96.7g/kg，含铁量约为0.193g/ kg。

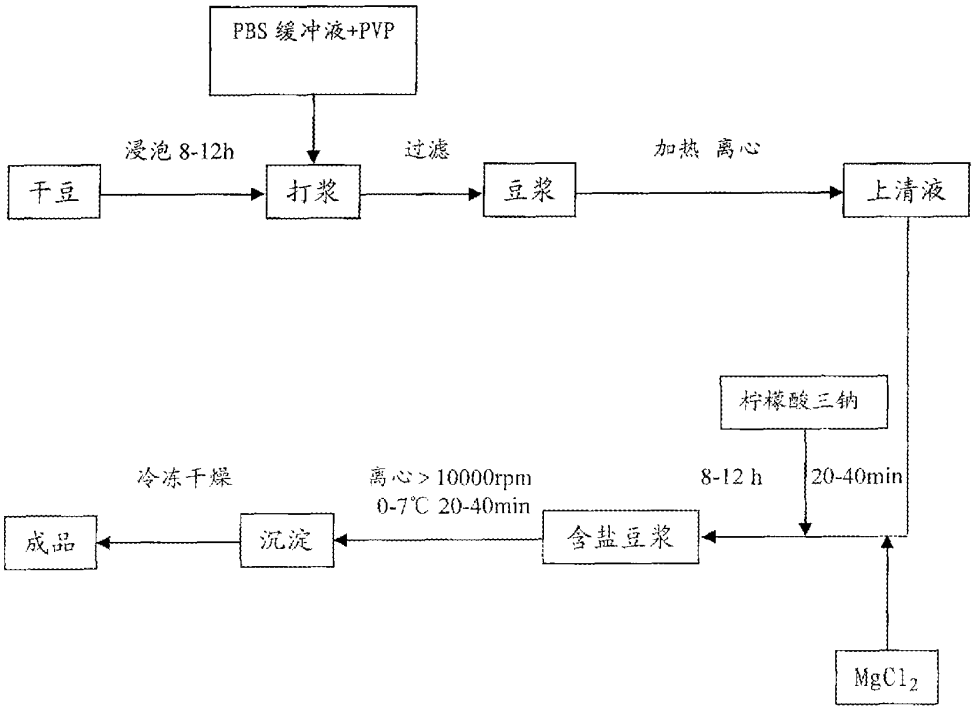
PBS 缓冲液+PVP
浸泡 8-12h
过滤
加热 离心
干豆
打浆
豆浆
上清液
柠檬酸三钠
冷冻干燥
离心 > 10000rpm
0-7℃ 20-40min
8-12 h
20-40min
成品
沉淀
含盐豆浆
$MgCl_2$

图 1

de102007061256

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Producing a substance, useful e.g. as milk protein- or meat substitute product, comprises preparing a liquid mixture of soy milk and finely comminuted kernel and/or nuts and acidifying the mixture by carbohydrate digesting microorganisms

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ABSTRACT

Procedure for producing a substance, comprises: (a) preparing a liquid mixture of soy milk and finely comminuted kernel and/or nuts; and (b) acidifying the mixture by carbohydrate digesting microorganisms until a pH value of 3.8-5, preferably 4.5 is reached, so that the protein is coagulated and a thick milk-like substance is obtained. An independent claim is included for a substance, which is producible by the above method.

DESCRIPTION translated from German

[0001] The invention relates to a method according to claim 1 for preparing a plant substance, and such a substance and the use thereof.

[0002] The offered in the European cultural space protein-rich foods traditionally contain largely animal-derived ingredients, in particular milk ingredients. A small, growing part of the population, however, avoids ethical, especially animal welfare, environmental and climate protection reasons or health reasons, the consumption of products derived from living or dead animals, including animal milk-based products. It is therefore in the interest of vegetarian consumers to get a tasty and varied rich offer pure vegetable foods in health-promoting bringing natural and fresh quality.

[0003] As a vegan protein-rich food base are particularly soybeans. Whole, particularly controlled soybeans organically produced have an ideal nutrient composition. They are rich in high quality protein and contain all eight essential fatty acids. Thus, the soy protein is a complete replacement for animal protein Handsome. In addition, soybeans are rich in vitamins, minerals and fiber. Foods from soybeans are fat and calories and easy to digest. Another advantage is that they contain no lactose or cholesterol. Disadvantages are the typical undesirable odor and flavor of soybean in soy foods.

[0004] There are various methods to eliminate this undesirable off-flavor of soybeans known.

[0005] In the DE-OS 37 30 384 A process for producing a Camembert cheese from soybean is described in which it does not completely managed to eliminate the undesirable flavor of soybeans.

[0006] From GB-A-1383149 is a process for producing a soy cheese is known in which the undesired aftertaste of the soybeans is eliminated by adding a complicated composition of fats.

[0007] In the EP 0923303 B1 a method for the production of cheese, cottage cheese and yoghurt products from soybeans is described in which much of the undesirable tastes and odors of the soy milk are removed in particular by vacuum degassing, and a large similarity of the products with the corresponding one of animal milk is achieved.

[0008] A disadvantage is the addition of animal milk sugar as this is unacceptable for vegans for ethical reasons.

[0009]

CLAIMS (35) translated from German

1. A process for producing a substance comprising the steps of: a) preparation of a liquid mixture of soy milk (soy juice) and finely crushed seeds and / or nuts, b) acidification of the mixture depleting by carbohydrates microorganisms, until a pH in the range of 3 8 to about 5, in particular of 4.5, so that the egg white coagulates and a curd-like substance is obtained.

2. A method according to claim 1, characterized in that the amount of solids of the soy component and the cores and / or nuts to the range less than 50% by weight, in particular 10 to 20% by weight, is adjusted

3. A method according to claim 1 or 2, characterized in that the quantitative ratio of the nuclei and / or nuts for soy proportion desired, particularly in the range of 50 to 150: 100 is set.

4. Method according to one of the preceding claims, characterized in that, the proportion of peeled soy and / or unpeeled soybean derived.

5. Method according to one of the preceding claims, characterized in that the share of soybeans and soy / or soya flour and / or soy concentrate and / or soy isolate is derived

6. Method according to one of the preceding claims, characterized in that soya milk is partially or completely replaced by milk from beans of other papilionaceous plants and / or by a liquid of water with or without constituents obtained from plants, fruits, seeds, or fungi.

7. Method according to one of the preceding claims, characterized in that the cores and / or cashew nuts and / or almonds are.

8. A method according to any one of the preceding claims, characterized in that the seeds and / or nuts are dry or wet fine, almost crushed grain free.

In order to provide the widest possible range of protein-rich vegetarian foods for ethical and / or health-conscious vegans, it is necessary to replace animal protein products by a possible natural vegan substance, which is particularly suitable for further processing into various tasty milk protein substitutes.

The invention is therefore the object of providing a relatively simple method for the preparation of a vegan substance based on soybeans in particular for processing into food, especially to milk protein replacement products such as cottage cheese, cheese and yogurt substitute products, provide, through the typical soybean flavor and odor is removed without great technical, particularly equipment expense, so producing smaller holdings at regional level such products in adequate, even smaller batch manually and as fresh as possible, even probiotic can offer.

This object is achieved by a method of the type mentioned above, which is characterized by the steps of claim 1.

To prepare the vegan substance is initially a liquid mixture of soy milk and finely chopped nuts and / or nuts, especially cashews and / or almonds, cooked. Then this mixture is acidified by microorganisms until attaining a certain pH, a protein coagulation occurs and consequently the invention even slightly firmer, thick milk-like substance is obtained. By a suitable separation method, a curd-like gross weight and a whey-like liquid are separated. The curd-like raw material is processed into food products, especially milk protein substitutes. The whey-like liquid is processed into a whey product replacement or cleaning agent.

With this substance, it is possible, especially tasty vegan cottage cheese, cream cheese, cheese, yogurt, as well as sausage substitute products based on soybeans produce similar with respect to taste, smell, appearance and consistency of the corresponding products of animal sub- stances.

It is particularly advantageous that the undesirable odor and flavor of soybean can be eliminated by the process in a simple manner, particularly when the Men The proportions are the seeds and / or nuts to soy proportion to the range of 50 to 150: 100 is set. Due to the considerably flavor-improving effect of the cores and / or nuts into the mixture with soy milk can be dispensed otherwise in the production of soy foods conventional technically more complicated process steps for the removal of the undesired off-flavor and Sojabohnengeruchs-.

It is also worth the advantage that a new, significant better taste quality by significantly flavor-enhancing effect of the addition of seeds and / or nuts, especially cashews and / or almonds, and acidification than on an exclusive soy base is possible with milk protein substitutes.

A great advantage from an ethical and health point of view is that the provided substance is vegan, so does not contain any animal's constituents. Thus, this substance is best humanely. From a health perspective, it is very valuable because it is an adequate vegetable protein source, but no animal protein Handsome, no animal Royal fat, no cholesterol and no lactose contains, so that it can be used for the manufacture of a dietetic food.

The manufacturing process is simple, because it consists of only a few technical steps and may be practiced without consuming appliances use so that it can be cost-effective and space saving manually applied in-demand smaller batches, eg. In the hotel and catering industry. It can also be dispensed with heat treatment of the final product, so that the products can be deployed very fresh in health-promoting probiotic state and thus also used as a dietary food.

The invention accomplishes the task with high quality standards of taste, smell, appearance and consistency, and with the high ethical and health value, have the vegan sour milk natural foods for vegan consumers.

[0010]

9. Method according to one of the preceding claims, characterized in that vegetable oil or liquid cooked vegetable fat, which has been obtained from nuts or seeds may be added.

10. Method according to one of the preceding claims, characterized in that plant sugars and / or sea salt or sugar-like substances may be added.

11. Method according to one of the preceding claims, characterized in that a kaseinähnlicher substance is added.

12. Method according to one of the preceding claims, characterized in that a substance which serves a firmer consistency, is added.

13. Method according to one of the preceding claims, characterized in that the mixture prior to acidification for the purpose of pasteurisation or sterilization at 70-145 ° C, especially at 85 to 143 ° C, heated and cooled to the necessary temperature for the acidification step.

14. Method according to one of the preceding claims, characterized in that the acidification of the mixture at a temperature between 32 ° and 45 ° C, in particular between 33 to 43 ° C.

15. A method according to claim 15, characterized in that the carbohydrates degrading microorganisms are commercially available, for the acidification of soymilk appropriate mesophilic or thermophilic lactic acid bacteria, which are taken as prescribed, and / or the mixture is commercially available Kombucha tea fungus culture was added.

16. Substance which is obtainable by a process according to any one of the preceding claims.

17. Use of the substance according to claim 16 as a component of a food.

18. Use of the substance according to claim 17 as milk protein or meat substitute product.

19. Use of the substance according to claim 18 as cottage cheese, cream cheese or sausage substitute product, characterized by a pasty consistency.

20. Use of the substance according to claim 19, characterized by separating and removing whey similar liquid as it is customary in the cottage cheese or fresh cheese production, until a pasty consistency is achieved.

21. Use of the substance according to any one of the preceding claims, characterized by the homogenization of the curd-like mass at 10 to 95 ° C, especially at 10 to 33 ° C.

22. Use of the substance according to claims 19 to 21, characterized by the impact with air or inert gases.

23. Use of the substance according to claim 18 as a soft cheese, sausage Schnittkäse- or replacement product, characterized by a physically strong, dimensionally stable consistency.

24. Use of the substance according to claim 23, characterized by separation and removal of whey-like fluid using a conventional in cheese production pressing and molding system, to a molded product of desired physical solid consistency.

25. Use of the substance according to claims 23 and 24, characterized by salts of the product by a physically solid cheese or sausage replacement product will be rolled.

Starting materials for the manufactured substance are soy milk and nearly crushed grains free of seeds and / or nuts, especially cashews and / or almond kernels from which a liquid mixture is prepared.

The soy milk can also be replaced partially or completely by the soymilk from beans other papilionaceous and / or by a liquid of water with or without ingredients derived from plants, fruits, seeds or fungi, thus resulting in a greater taste variety of products.

[0011] The soy milk prepared from soybeans according to a known method, preferably with a soya content 7-13% by weight, or it is a commercially available natural soy milk (soybean juice) is used.

[0012] The seeds and / or nuts are crushed dry or wet grains almost free with the addition of about twice the amount of a liquid.

From the soy milk, finely crushed seeds and / or nuts formed under stirring a mixture. The solids content of soybean and nuts and / or nuts of this liquid mixture is adjusted to the range of less than 50%, preferably to about 10 to 20 weight%. The weight of the solids of the soy milk and the various cores and / or nuts are adjusted to each other as desired, preferably the ratio of the solids from cashew and / or almond kernels is to soy share to 50 to 150: 100 set.

[0013] This mixture is heated provided before the microbiological acidification purposes of pasteurisation at 70-90 ° C and cooled to that prescribed for the addition of microorganisms temperature.

Degrading microorganisms by the addition of carbohydrates occurs at a prescribed temperature, the acidification of the mixture until a pH in the range between about 3.8 and 5, preferably so gained 4.5 that the

[0014] protein coagulation, a thick milk-like substance.

This acidified thick milk-like mass is present even substance can be used in many ways, especially for the more moderate invention processed in foods such as cottage cheese, cream cheese, cheese, sausage or yogurt substitute products.

Cottage cheese, cream cheese or sausage substitute products are manufactured in particular by the claims 19 to 22 and 26, cheese or sausage substitute products by the claims 23 to 26, milk replacement

[0015] drink by the claims 30 or 32, and yogurt substitute products by claim 31, whey replacement drink by claim 29.

Two preferred and advantageous embodiments will be described.

Example 1:

[0016] 1.1. For the production of cottage cheese or cream cheese substitutes 1 liter soy milk (soy juice) with a solids content of 7% by weight (70 g) is used.

1.2. Also 7% by weight (70 g) cashews wet crushed finely with the addition of two parts of water, grain-free.

1.3. From the grains free crushed cashew nuts and soy milk produced with stirring a liquid mixture having a solids content of

[0017] about 14 weight (about 140 g). In this case, the ratio of the cashew is the soy share 100 100 times.

1.4. This mixture is provided for the purpose of pasteurization heated to 85 ° C and cooled to that prescribed for the addition of specific microorganisms temperature.

1.5. By addition of thermophilic carbohydrate degrading microorganisms, particularly Streptococcus thermophilis and / or Lactobacillus bulgaricus is carried out at 33 ° C, the acidification of the mixture until a

[0018] pH of about 4.5, so that by protein coagulation to the invention moderate fat milk-like substance is obtained.

1.6. Then whey-like fluid is usually separated and removed as in cheese production, so that a quark or fresh cheese-like gross mass of desired consistency.

[0019] 1.7. This cottage cheese or cream cheese-like raw material is now taste refined with ingredients that are common for quark or cream cheese, in particular sea salt, pepper, parsley, chives, cumin, other herbs or spices, other herbal ingredients or for a sweet version by plant sugars , fruits or spices.

1.8. A creamier consistency can be achieved, for example with a quark mill.

1.9. In order to increase durability, heat treatment can be carried out before the automated packaging takes place into containers. Transport and storage take place at about 5 ° C.

Example 2:

depending on weight in salt or fingers for some time in a saturated brine, then dried or required under product-dependent temperature and humidity conditions with or without precious mold cultures matures or is smoked.

26. Use of the substance of any one of claims 23 to 25, characterized by the addition of herbs, spices, parts of fruits, vegetables, algae or fungi, seeds, nuts or walnuts, each optionally in processed form, sea salt, vegetable sugar, vegetable oil or liquid vegetable fat, special cheese cultures such as noble mold cultures, as well as a substance that serves a firmer consistency.

27. Use of the substance according to any one of the preceding claims, characterized by pasteurizing and air-tight sealing of the product.

28. Use of the substance according to any one of the preceding claims 1 to 26, characterized in that the cheese or sausage replacement product to increase durability partially or completely enclosed by vegetable oil or vacuum-packed in foil and stored at about 5 ° C. The vegetable oil can herbs, spices or other herbal ingredients, such as olives are added.

29. Use of the substance according to claim 17 as a proportion of whey replacement beverage, characterized by a proportion of fruit ingredients.

30. Use of the substance according to claim 18 as a proportion of a milk substitute beverage, characterized by a proportion of fruit ingredients.

31. Use of the substance according to claim 19 as yogurt replacement product, characterized by a proportion of fruit ingredients.

32. Use of the mixture according to claim 1.a) and 2 to 16. As a proportion of a milk replacement drink, characterized by a proportion of fruit ingredients and pasteurization or sterilization by heating to 70 ° to 145 ° C.

33. Use of the substance according to claim 15 as a proportion of body care products.

34. Use of the substance according to claim 33, characterized by a fraction fragrant imaging or nourishing herbal ingredients.

35. Use of the substance according to claim 15 as a proportion of a cleaning agent.

2.1.) For the preparation of a solid, shaped cheese substitute product, the raw batch as in Example 1) is prepared to 1.6.) According to the steps 1.1..

2.2.) This ingot is taste enhanced by the addition of conventional in cheese production herbs, spices, chopped vegetables or other vegetable ingredients, such as pepper, parsley and chives.

2.3.) From this raw material is separated and removed so that a shaped cream cheese desired consistency is achieved by a similar whey in the cheese industry standard pressing and molding system fluid.

2.4.) This cheese is placed in a saturated sea salt brine. The residence time depends on the weight. For example, remains a cheese with a weight of 200 g for about 30 minutes in the Lake. After half the time the cheese is turned. Then it can be inserted, for example, in herb oil. Transport and storage take place at about 5 ° C.

QUOTES INCLUDED IN THE DESCRIPTION

This list of references cited by the applicant is generated automatically and is included solely to inform the reader. The list is not part of the German patent or utility model application. The DPMA shall not be liable for any errors or omissions.

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PATENT CITATIONS

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EP0923303B1	May 17, 1997	Nov 7, 2001	Wilhelm Hansen	Process for producing cheese, curd and yoghourt products from soya beans
GB1383149A				*Title not available*

CLASSIFICATIONS

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Cooperative Classification	A23L1/366, A23C11/106, C11D3/382, A61Q19/00, C11D7/44, A23J3/28, A61Q19/10, A61K8/97, A23L1/31409, A23C20/025, A23L1/317
European Classification	C11D3/382, C11D7/44, A61Q19/10, A61K8/97, A61Q19/00, A23L1/36D, A23L1/3148, A23L1/317, A23C11/10B2, A23C20/02B, A23J3/28

LEGAL EVENTS

Date	Code	Event	Description
Nov 17, 2011	R119	Application deemed withdrawn, or ip right lapsed, due to non-payment of renewal fee	**Effective date:** 20110701
Sep 10, 2009	8127	New person/name/address of the applicant	**Owner name:** OPPENKOWSKI, KATRIN VON, 81247 MUENCHEN, DE **Owner name:** OPPENKOWSKI, SYLVIA VON, 81247 MUENCHEN, DE
Jul 30, 2009	8122	Nonbinding interest in granting licenses declared	

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[0026]

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A61Q 19/00 (2006.01)

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Die folgenden Angaben sind den vom Anmelder eingereichten Unterlagen entnommen

(54) Bezeichnung: **Verfahren zur Herstellung einer pflanzlichen Substanz sowie derartige Substanz und deren Verwendung**

(57) Zusammenfassung: Verfahren zur Herstellung einer Substanz, die der vielfältigen Verwendung, insbesondere als Nahrungsmittel, dient, gekennzeichnet durch Herstellung eines flüssigen Gemisches von Sojamilch (Sojasaft) und fein zerkleinerten Kernen und/oder Nüssen, insbesondere Cashew- und/oder Mandelkernen, so dass mittels Säuerung durch Kohlenhydrate abbauende Mikroorganismen bis zum Erreichen eines pH-Wertes im Bereich von etwa 3,8 bis 5,0 infolge der Eiweißkoagulation eine dickmilchähnliche Substanz gewonnen wird.

Beschreibung

[0001] Die Erfindung betrifft gemäß Anspruch 1 ein Verfahren zur Herstellung einer pflanzlichen Substanz sowie derartige Substanz und deren Verwendung.

[0002] Die im europäischen Kulturraum angebotenen eiweißreichen Nahrungsmittel enthalten traditionell noch großenteils von Tieren stammende Bestandteile, insbesondere auch Milchbestandteile. Ein kleiner, zunehmender Teil der Bevölkerung meidet jedoch aus ethischen, insbesondere Tierschutz-, Umweltschutz- und Klimaschutzgründen oder gesundheitlichen Gründen, den Konsum von Produkten, die von lebenden oder toten Tieren stammen, so auch tiermilchhaltige Produkte. Es liegt daher im Interesse veganer VerbraucherInnen, ein schmackhaftes und abwechselungsreiches Angebot rein pflanzlicher Nahrungsmittel in möglichst gesundheitsfördernder natürlicher und frischer Qualität zu erhalten.

[0003] Als eiweißreiche vegane Nahrungsmittelgrundlage eignen sich besonders Sojabohnen. Ganze, insbesondere kontrolliert biologisch hergestellte Sojabohnen haben eine ideale Nährstoffzusammensetzung. Sie sind reich an hochwertigen Eiweißen und enthalten alle acht essentiellen Fettsäuren. Somit ist das Sojaeiweiß ein vollwertiger Ersatz für tierliches Eiweiß. Zudem sind Sojabohnen reich an Vitaminen, Mineral- und Ballaststoffen. Nahrungsmittel aus Sojabohnen sind fett- und kalorienarm sowie leicht verdaulich. Ein weiterer Vorteil ist, dass sie weder Laktose noch Cholesterin enthalten. Nachteilig sind der unerwünschte typische Geruch und Beigeschmack von Sojabohnen in Sojanahrungsmitteln.

[0004] Es sind verschiedene Verfahren zur Beseitigung dieses unerwünschten Beigeschmacks der Sojabohnen bekannt.

[0005] In der DE-OS 37 30 384 wird ein Verfahren zur Herstellung eines Camembertkäses aus Sojabohnen beschrieben, bei dem es aber nicht vollständig gelang, den unerwünschten Beigeschmack der Sojabohnen zu beseitigen.

[0006] Aus GB-A-1 383 149 ist ein Verfahren zur Herstellung eines Sojakäses bekannt, bei dem der unerwünschte Beigeschmack der Sojabohnen durch Zugabe einer komplizierten Zusammensetzung von Fetten beseitigt wird.

[0007] In der EP 0 923 303 B1 wird ein Verfahren zur Herstellung von Käse-, Quark- und Joghurtprodukten aus Sojabohnen beschrieben, bei denen ein Großteil der unerwünschten Geschmacks- und Geruchsstoffe der Sojamilch insbesondere durch Vakuumentgasung entfernt werden und eine große Ähnlichkeit der Produkte mit den entsprechenden aus Tiermilch erreicht wird.

[0008] Nachteilig ist die Zugabe von Tiermilchzucker, da dies für VeganerInnen aus ethischen Gründen inakzeptabel ist.

[0009] Zur Bereitstellung möglichst vielfältiger veganer eiweißreicher Nahrungsmittel für ethische und/oder gesundheitsbewusste VeganerInnen ist es notwendig, die Tiereiweißprodukte durch eine möglichst natürliche vegane Substanz zu ersetzen, die insbesondere zur Weiterverarbeitung zu verschiedenen schmackhaften Milcheiweißersatzprodukten geeignet ist.

[0010] Der Erfindung liegt folglich die Aufgabe zugrunde, ein vergleichsweise einfacheres Verfahren zur Herstellung einer veganen Substanz auf Basis von Sojabohnen insbesondere für die Verarbeitung zu Nahrungsmitteln, vor allem zu Milcheiweißersatzprodukten, wie Quark-, Käse- und Joghurtersatzprodukte, bereitzustellen, durch das der für Sojabohnen typische Beigeschmack und Geruch ohne großen technischen, insbesondere apparativen Aufwand beseitigt wird, so dass auch kleinere Betriebe auf regionaler Ebene derartige Produkte in bedarfsgerechter, auch kleinerer Charge manuell herstellen und möglichst frisch, auch probiotisch anbieten können.

[0011] Diese Aufgabe wird erfindungsgemäß durch ein Verfahren der eingangs genannten Art gelöst, das gekennzeichnet ist durch die Verfahrensschritte gemäß Anspruch 1.

[0012] Zur Herstellung der veganen Substanz wird zunächst ein flüssiges Gemisch von Sojamilch und fein zerkleinerten Kernen und/oder Nüssen, insbesondere Cashew- und/oder Mandelkernen, zubereitet. Dann wird dieses Gemisch mittels Mikroorganismen gesäuert, bis bei Erreichen eines bestimmten pH-Wertes eine Eiweißkoagulation eintritt und infolgedessen die erfindungsmäßige etwas festere, dickmilchähnliche Substanz gewonnen wird. Durch ein geeignetes Trennverfahren werden eine quarkähnliche Rohmasse und eine molkeähnlichen Flüssigkeit getrennt. Die quarkähnliche Rohmasse wird zu Nahrungsmitteln, insbesondere zu Milcheiweißersatzprodukten weiterverarbeitet. Die molkeähnliche Flüssigkeit wird zu einem Molkeersatzprodukt oder Reinigungsmittel weiterverarbeitet.

[0013] Mit dieser Substanz ist es möglich, besonders schmackhafte vegane Quark-, Frischkäse-, Käse-, Joghurt-, aber auch Wurstersatzprodukte auf Basis von Sojabohnen herzustellen, die bezüglich Geschmack, Geruch, Aussehen und Konsistenz den entsprechenden Produkten aus tierlichen Stoffen ähneln.

[0014] Besonders vorteilhaft ist, dass der uner-

wünschte Geruch und Beigeschmack von Sojabohnen durch das Verfahren auf einfache Weise beseitigt werden kann, insbesondere wenn das Mengenverhältnis der Kerne und/oder Nüsse zum Sojaanteil auf den Bereich von 50 bis 150:100 eingestellt wird. Auf Grund der erheblich geschmacksverbessernden Wirkung der Kerne und/oder Nüsse in dem Gemisch mit Sojamilch kann auf sonst bei der Herstellung von Sojanahrungsmitteln übliche technisch aufwändigere Verfahrensschritte zur Beseitigung des unerwünschten Sojabohnengeruchs- und beigeschmacks verzichtet werden.

[0015] Hervorzuheben ist auch der Vorteil, dass durch die erheblich geschmacksverbessernde Wirkung der Zugabe von Kernen und/oder Nüssen, insbesondere von Cashew- und/oder Mandelkernen, und Säuerung eine neue, wesentliche bessere Geschmacksqualität wird, als dies bei Milcheiweißersatzprodukten auf ausschließlicher Sojabasis möglich ist.

[0016] Ein großer Vorteil aus ethischer und gesundheitlicher Sicht ist, dass die bereitgestellte Substanz vegan ist, also keine tierlichen Bestandteile enthält. Somit ist diese Substanz bestmöglich tierschutzgerecht. Aus gesundheitlicher Sicht ist sie sehr wertvoll, da sie ein vollwertiger pflanzlicher Eiweißlieferant ist, jedoch kein tierliches Eiweiß, kein tierliches Fett, kein Cholesterin und keine Laktose enthält, so dass sie auch für die Herstellung eines diätetischen Nahrungsmittels genutzt werden kann.

[0017] Das Herstellungsverfahren ist einfach, denn es besteht aus nur wenigen technischen Verfahrensschritten und ist auch ohne aufwändigen Apparateeinsatz ausführbar, so dass es auch kostengünstig und platzsparend manuell in bedarfsgerechten kleineren Chargen, bspw. im Hotel- und Gaststättengewerbe, angewendet werden kann. Dabei kann auf Wärmebehandlung des Endproduktes auch verzichtet werden, so dass die Produkte sehr frisch im gesundheitsfördernden probiotischen Zustand bereitgestellt und somit ebenfalls als diätetisches Nahrungsmittel genutzt werden können.

[0018] Die Erfindung erfüllt die Aufgabe mit hohem Qualitätsanspruch an Geschmack, Geruch, Aussehen und Konsistenz sowie mit dem hohen ethischen und gesundheitlichen Wert, den vegane milchsaure natürliche Nahrungsmittel für vegane VerbraucherInnen haben.

[0019] Ausgangsstoffe für die herzustellende Substanz sind Sojamilch und annähernd körnerfrei zerkleinerte Kerne und/oder Nüsse, insbesondere Cashew- und/oder Mandelkerne, aus denen ein flüssiges Gemisch zubereitet wird.

[0020] Die Sojamilch kann auch teilweise oder vollständig ersetzt werden durch die Sojamilch aus Bohnen anderer Schmetterlingsblütler und/oder durch eine Flüssigkeit aus Wasser mit oder ohne Bestandteile, die aus Pflanzen, Früchten, Samen oder Pilzen gewonnen wurden, so dass sich eine größere geschmackliche Produktvielfalt ergibt.

[0021] Die Sojamilch wird aus Sojabohnen nach einem bekannten Verfahren hergestellt, bevorzugt mit einem Sojagehalt von 7 bis 13 Gewichts %, oder es wird ein handelsüblicher Sojadrink natur (Sojasaft) verwendet.

[0022] Die Kerne und/oder Nüsse werden trocken oder nass unter Zugabe etwa der doppelten Menge einer Flüssigkeit annähernd körnerfrei zerkleinert.

[0023] Aus der Sojamilch, den fein zerkleinerten Kernen und/oder Nüssen entsteht unter Rühren ein Gemisch. Der Feststoffanteil von Soja und Kernen und/oder Nüssen dieses flüssigen Gemisches wird auf den Bereich kleiner 50%, vorzugsweise auf etwa 10 bis 20 Gewichts %, eingestellt. Dabei werden die Gewichtsanteile der Feststoffe der Sojamilch und der verschiedenen Kerne und/oder Nüsse zueinander beliebig eingestellt, bevorzugt wird das Mengenverhältnis der Feststoffe von Cashew- und/oder Mandelkernen zum Sojaanteil auf 50 bis 150:100 eingestellt.

[0024] Dieses Gemisch wird vor der mikrobiologischen Säuerung zwecks Pasteurisierung auf 70–90°C erhitzt und auf die für die Zugabe der Mikroorganismen vorgeschriebene Temperatur abgekühlt bereitgestellt.

[0025] Durch Zugabe von Kohlenhydrate abbauenden Mikroorganismen erfolgt bei vorgeschriebener Temperatur die Säuerung des Gemisches bis zum Erreichen eines pH-Wertes im Bereich zwischen etwa 3,8 und 5, vorzugsweise 4,5, so dass durch die Eiweißkoagulation eine dickmilchähnliche Substanz gewonnen wird.

[0026] Diese gesäuerte dickmilchähnliche Masse ist die erfindungsmäßige Substanz kann vielfältig verwendet werden, insbesondere für die weitere erfindungsmäßige Verarbeitung zu Nahrungsmitteln, wie Quark-, Frischkäse-, Käse-, Wurst- oder Joghurtersatzprodukten.

[0027] Quark-, Frischkäse- oder Wurstersatzprodukte werden insbesondere durch die Ansprüche 19 bis 22 und 26 hergestellt, Käse- oder Wurstersatzprodukte durch die Ansprüche 23 bis 26, Milchersatzgetränk durch die Ansprüche 30 oder 32 und Joghurtersatzprodukte durch den Anspruch 31, Molkeersatzgetränk durch den Anspruch 29.

[0028] Nachfolgend werden zwei bevorzugte und vorteilhafte Beispiele beschrieben.

Beispiel 1:

1.1. Für die Herstellung von Quark- oder Frischkäseersatzprodukten wird 1 Liter Sojamilch (Sojasaft) mit einem Feststoffgehalt von 7 Gewichts % (70 g) verwendet.
1.2. Ebenfalls 7 Gewichts % (70 g) Cashewkerne werden nass unter Zugabe der doppelten Menge Wasser fein, körnerfrei zerkleinert.
1.3. Aus den körnerfrei zerkleinerten Cashewkernen und der Sojamilch entsteht unter Rühren ein flüssiges Gemisch mit einem Feststoffgehalt von etwa 14 Gewichts (etwa 140 g). Hierbei beträgt das Mengenverhältnis der Cashewkerne zum Sojaanteil 100:100.
1.4. Dieses Gemisch wird zwecks Pasteurisierung auf 85°C erwärmt und auf die für die Zugabe der speziellen Mikroorganismen vorgeschriebene Temperatur abgekühlt bereitgestellt.
1.5. Durch Zugabe von thermophilen Kohlenhydrate abbauenden Mikroorganismen, insbesondere Streptococcus thermophilis und/oder Lactobacillus bulgaricus erfolgt bei 33°C die Säuerung des Gemisches bis zum Erreichen eines pH-Wertes von etwa 4,5, so dass durch die Eiweißkoagulation die erfindungsmäßige dickmilchähnliche Substanz gewonnen wird.
1.6. Dann wird molkeähnliche Flüssigkeit wie bei der Käseherstellung üblich abgetrennt und entfernt, so dass eine quark- bzw. frischkäseähnliche Rohmasse mit gewünschter Konsistenz entsteht.
1.7. Diese quark- oder frischkäseähnliche Rohmasse wird nun geschmacklich veredelt durch Zutaten, die für Quark- bzw. Frischkäse üblich sind, insbesondere Meersalz, Pfeffer, Petersilie, Schnittlauch, Kümmel, sonstige Kräuter oder Gewürze, sonstige pflanzliche Zutaten oder aber für eine süße Variante durch Pflanzenzucker, Früchte oder Gewürze.
1.8. Eine cremigere Konsistenz kann beispielsweise mit einer Quarkmühle erreicht werden.
1.9. Zwecks Haltbarkeitssteigerung kann eine Wärmebehandlung erfolgen, bevor die maschinelle Abpackung in Behälter erfolgt. Transport und Lagerung erfolgen bei etwa 5°C.

Beispiel 2:

2.1.) Zur Herstellung eines festen, geformten Käseersatzproduktes wird die Rohmasse wie im Beispiel 1 gemäß den Schritten 1.1.) bis 1.6.) hergestellt.
2.2.) Diese Rohmasse wird geschmacklich veredelt durch die Zugabe von bei der Käseherstellung üblichen Kräutern, Gewürzen, zerkleinertem Gemüse oder anderen pflanzlichen Zutaten, insbesondere Pfeffer, Petersilie und Schnittlauch.
2.3.) Von dieser Rohmasse wird durch ein in der Käsebranche übliches Press- und Formsystem molkeähnliche Flüssigkeit abgetrennt und entfernt, so dass ein geformter Frischkäse gewünschter Konsistenz entsteht.
2.4.) Dieser Käse wird in eine gesättigte Meersalzlake gelegt. Die Verweildauer hängt vom Gewicht ab. Beispielsweise verweilt ein Käse mit einem Gewicht von 200 g etwa 30 Minuten in der Lake. Nach der Hälfte der Zeit wird der Käse gewendet. Dann kann er beispielsweise in Kräuteröl eingelegt werden. Transport und Lagerung erfolgen bei etwa 5°C.

ZITATE ENTHALTEN IN DER BESCHREIBUNG

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Zitierte Patentliteratur

- DE 3730384 A **[0005]**
- GB 1383149 A **[0006]**
- EP 0923303 B1 **[0007]**

Patentansprüche

1. Verfahren zur Herstellung einer Substanz mit folgenden Verfahrensschritten:
a) Zubereitung eines flüssigen Gemisches von Sojamilch (Sojasaft) und fein zerkleinerten Kernen und/oder Nüssen,
b) Säuerung des Gemisches durch Kohlenhydrate abbauende Mikroorganismen bis zum Erreichen eines pH-Wertes im Bereich von 3,8 bis etwa 5, insbesondere von 4,5, so dass das Eiweiß koaguliert und eine dickmilchähnliche Substanz gewonnen wird.

2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, dass die Feststoffmenge des Sojaanteils und der Kerne und/oder Nüsse auf den Bereich kleiner 50 Gewichts %, insbesondere auf 10 bis 20 Gewichts %, eingestellt wird.

3. Verfahren nach Anspruch 1 oder 2, dadurch gekennzeichnet, dass das Mengenverhältnis der Kerne und/oder Nüsse zum Sojaanteil beliebig, insbesondere auf den Bereich von 50 bis 150:100, eingestellt wird.

4. Verfahren nach einem der vorherigen Ansprüche, dadurch gekennzeichnet dass, der Sojaanteil von geschälten und/oder ungeschälten Sojabohnen stammt.

5. Verfahren nach einem der vorherigen Ansprüche, dadurch gekennzeichnet, dass der Sojaanteil von Sojabohnen und/oder Sojamehl und/oder Sojakonzentrat und/oder Sojaisolaten stammt.

6. Verfahren nach einem der vorherigen Ansprüche, dadurch gekennzeichnet, dass Sojamilch teilweise oder vollständig ersetzt wird durch Milch aus Bohnen anderer Schmetterlingsblütler und/oder durch eine Flüssigkeit aus Wasser mit oder ohne Bestandteile, die aus Pflanzen, Früchten, Samen oder Pilzen gewonnen wurde.

7. Verfahren nach einem der vorherigen Ansprüche, dadurch gekennzeichnet, dass die Kerne und/oder Nüsse Cashew- und/oder Mandelkerne sind.

8. Verfahren nach einem der vorherigen Ansprüche, dadurch gekennzeichnet, dass die Kerne und/oder Nüsse trocken oder nass fein, annähernd körnerfrei zerkleinert werden.

9. Verfahren nach einem der vorherigen Ansprüche, dadurch gekennzeichnet, dass pflanzliches Öl oder flüssig gemachtes pflanzliches Fett, das aus Nüssen oder Kernen gewonnen wurde, zugegeben werden.

10. Verfahren nach einem der vorherigen Ansprüche, dadurch gekennzeichnet, dass Pflanzenzucker und/oder Meersalz bzw. zuckerähnliche Stoffe zugegeben werden.

11. Verfahren nach einem der vorherigen Ansprüche, dadurch gekennzeichnet, dass ein kaseinähnlicher Stoff zugegeben wird.

12. Verfahren nach einem der vorherigen Ansprüche, dadurch gekennzeichnet, dass ein Stoff, der einer festeren Konsistenz dient, zugegeben wird.

13. Verfahren nach einem der vorherigen Ansprüche, dadurch gekennzeichnet, dass das Gemisch vor der Säuerung zwecks Pasteurisierung oder Sterilisierung auf 70 bis 145°C, insbesondere auf 85 bis 143°C, erhitzt und auf die für den Säuerungsschritt nötige Temperatur abgekühlt wird.

14. Verfahren nach einem der vorherigen Ansprüche, dadurch gekennzeichnet, dass die Säuerung des Gemisches bei einer Temperatur zwischen 32° und 45°C, insbesondere zwischen 33 bis 43°C, erfolgt.

15. Verfahren nach Anspruch 15, dadurch gekennzeichnet, dass die Kohlenhydrate abbauenden Mikroorganismen handelsübliche, auch für die Säuerung von Sojamilch geeignete mesophile oder thermophile Milchsäurebakterien sind, die vorschriftsmäßig angewendet werden, und/oder dem Gemisch wird handelsübliche Kombucha-Teepilz-Kultur zugegeben.

16. Substanz, die durch ein Verfahren nach einem der vorherigen Ansprüche herstellbar ist.

17. Verwendung der Substanz nach Anspruch 16 als Bestandteil eines Nahrungsmittels.

18. Verwendung der Substanz nach Anspruch 17 als Milcheiweiß- oder Fleischersatzprodukt.

19. Verwendung der Substanz nach Anspruch 18 als Quark-, Frischkäse- oder Wurstersatzprodukt, gekennzeichnet durch eine pasteuse Konsistenz.

20. Verwendung der Substanz nach Anspruch 19, gekennzeichnet durch Abtrennen und Entfernen molkeähnlicher Flüssigkeit, wie es bei der Quark- bzw. Frischkäseherstellung üblich ist, bis eine pasteuse Konsistenz erreicht ist.

21. Verwendung der Substanz nach einem der vorherigen Ansprüche, gekennzeichnet durch Homogenisierung der quarkähnlichen Masse bei 10 bis 95°C, insbesondere bei 10 bis 33°C.

22. Verwendung der Substanz nach den Ansprüchen 19 bis 21, gekennzeichnet durch das Aufschla-

gen mit Luft oder Schutzgasen.

23. Verwendung der Substanz nach Anspruch 18 als Weichkäse-, Schnittkäse- oder Wurstersatzprodukt, gekennzeichnet durch eine körperlich feste, formbeständige Konsistenz.

24. Verwendung der Substanz nach Anspruch 23, gekennzeichnet durch Abtrennen und Entfernen von molkeähnlicher Flüssigkeit mit Hilfe eines bei der Käseherstellung üblichen Press- und Formsystems, bis ein geformtes Produkt gewünschter körperlich fester Konsistenz entsteht.

25. Verwendung der Substanz nach den Ansprüchen 23 und 24, gekennzeichnet durch Salzen des Produktes, indem ein körperlich festes Käse- oder Wurstersatzprodukt je nach Gewicht in Salz gewälzt wird oder eine gewisse Zeit in einer gesättigten Salzlake verweilt, dann trocknet bzw. unter produktabhängig erforderlichen Temperatur- und Luftfeuchtigkeitsbedingungen mit oder ohne Edelschimmelpilzkulturen reift oder geräuchert wird.

26. Verwendung der Substanz nach einem der Ansprüche 23 bis 25, gekennzeichnet durch Zugabe von Kräutern, Gewürzen, Bestandteilen von Früchten, Gemüse, Algen oder Pilzen, Samen, Kernen oder Nüssen, gegebenenfalls jeweils in zubereiteter Form, Meersalz, Pflanzenzucker, pflanzlichem Öl oder flüssigem pflanzlichen Fett, speziellen Käsekulturen wie beispielsweise Edelschimmelpilzkulturen sowie von einem Stoff, der einer festeren Konsistenz dient.

27. Verwendung der Substanz nach einem der vorherigen Ansprüche, gekennzeichnet durch Pasteurisieren und luftdichtes Verschließen des Erzeugnisses.

28. Verwendung der Substanz nach einem der vorhergehenden Ansprüche von 1 bis 26, dadurch gekennzeichnet, dass das Käse- oder Wurstersatzprodukt zur Haltbarkeitssteigerung teilweise oder ganz von Pflanzenöl umschlossen oder in Folie vakuumverpackt und bei etwa 5°C gelagert wird. Dem Pflanzenöl können Kräuter, Gewürze oder andere pflanzliche Zutaten, beispielsweise Oliven zugegeben werden.

29. Verwendung der Substanz nach Anspruch 17 als Anteil eines Molkeersatzgetränkes, gekennzeichnet durch einen Anteil von Fruchtbestandteilen.

30. Verwendung der Substanz nach Anspruch 18 als Anteil eines Milchersatzgetränkes, gekennzeichnet durch einen Anteil von Fruchtbestandteilen.

31. Verwendung der Substanz nach Anspruch 18 als Joghurtersatzprodukt, gekennzeichnet durch einen Anteil von Fruchtbestandteilen.

32. Verwendung des Gemisches nach einem der Ansprüche 1.a) und 2. bis 10. als Anteil eines Milchersatzgetränk, gekennzeichnet durch einen Anteil von Fruchtbestandteilen und Pasteurisierung oder Sterilisierung durch Erhitzen auf 70 bis 145°C.

33. Verwendung der Substanz nach Anspruch 16 als Anteil eines Körperpflegemittel.

34. Verwendung der Substanz nach Anspruch 33, gekennzeichnet durch einen Anteil duftgebender oder pflegender pflanzlicher Zutaten.

35. Verwendung der Substanz nach Anspruch 16 als Anteil eines Reinigungsmittels.

Es folgt kein Blatt Zeichnungen

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fresh vegetable cream translated from German

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ABSTRACT translated from German

Pure vegetable spread containing
a. crushed and soaked nuts
b. Drinking water
b. Mature culture with acidophilus and bulgaricus, among other bacteria
c. Sea salt

DESCRIPTION translated from German

[0001] The invention relates to a novel pure vegetable cream cheese-like spread, which consists of nuts, drinking water, sea salt, matured culture and its pH in the acidic environment is (PH 5).

State of the art

[0002] Vegetarian spreads were formerly produced from plant materials such as soy, grain, vegetable or spice base. These raw materials are mixed, mashed and then bottled and preserved by heating (pasteurization or sterilization). These products are characterized by the fact that they are free of milk protein, lactose and gluten-free and thus represent a pure vegetable foods. However, the PH value of these products Resides in the neutral range.

Task

[0003] The invention has the task to basically produce a pure vegetable cream cheese-like spread by the addition of ripening cultures of a mass of nuts and drinking water whose pH is in the acidic environment. This product is also free of milk protein, lactose and gluten and thus represents a purely herbal and vegan food.

[0004] This object is achieved with a bread spread with the features of claim 1-4.

[0005] The spread of the present invention contains nuts and drinking water, sea salt and ripening cultures with acidophilus and bulgaricus, among other bacteria. To give the desired spread the cream cheese-like consistency, it has been found that the previously crushed, heated and cooled again nut puree must be added to the ripeness culture. As part of a fermentation product after the addition of the aforementioned bacteria culture is changing taste of the maturation and the PH value is reduced in an acidic environment (pH 5), who is known by different cream cheeses from cow's milk her.

[0006] The result is a pure vegetable and in his appearance and taste a cheese very similar spread which has many uses as food.

[0007] Further details of the invention will become apparent from the following embodiment:

Embodiment

[0008] The spread is composed of about 30-45% by weight of nuts, 55-70 parts by weight of water, about 1-2% by weight of sea salt and a maturation culture together. First nuts are soaked for several hours. Basically any type of nuts (walnuts, hazelnuts, Brazil nuts, Makadamias and sunflower seeds) may provide a basis for the spread of the invention. In the present embodiment, however, cashew nuts are used. The nuts are finely ground after soaking. The puree is then mixed with water and heated and then cooled to about 40 ° C again, at this stage a mature culture with acidophilus and bulgaricus bacteria is stirred into the mixture. Thereafter, an about 10-hour fermentation is carried out. Then the dry mass is adjusted to about 33% with water and then treated with 1% sea salt. Finally the product is packaged and pasteurized.

CLAIMS (4) translated from German

1. Pure vegetable spread containing a. Crushed and soaked nuts b. Drinking water b. Mature culture with acidophilus and bulgaricus, among other bacteria c. Sea salt

2. Pure vegetable spread according to claim 1, characterized in that cashews, walnuts, hazelnuts, Brazil nuts, macadamia nuts, as well as sunflower seeds can be used as nuts.

3. Pure vegetable spread according to claim 1 or 2, characterized in that it comprises the addition of a maturing culture with, among other things acidophilus and bulgaricus bacteria and fermentation, a cream cheese-like consistency and a pH in the acidic environment (pH 5).

4. Pure vegetable spread, in accordance with claims 1-3, characterized in that it comprises the following composition: Nuts: about 30-45% parts by weight Drinking water: about 55-70% parts by weight Sea salt: about 1-2% parts by weight such as Mature Culture

CLASSIFICATIONS

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Abstract of EP0680751 (A1)

New lipid vesicles have an average particle size of 0.1-20 microns and comprise a shell consisting of practically all the proteins and phospholipids present in the oil bodies of oil seeds surrounding a core comprising exogenous lipids and/or exogenous lipophilic substances. Also claimed is an aq. emulsion contg. lipid vesicles as above dispersed in an aq. phase. Also claimed is a process for preparing an aq. emulsion as above. Also claimed are cosmetic, food and therapeutic compsns. contg. lipid vesicles as above.

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(54) Vésicules lipidiques.

(57) La présente invention a pour objet des vésicules lipidiques ayant une taille moyenne de 0,1 à 20 micromètres et comprenant une enveloppe constituée pratiquement de l'ensemble des protéines et des phospholipides présents dans les corps huileux des graines d'oléagineux entourant un noyau comprenant des lipides exogènes et/ou des substances lipophiles exogènes.

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La présente invention concerne des vésicules lipidiques et des émulsions aqueuses les contenant et un procédé pour leur fabrication.

La présente invention concerne plus spécifiquement des vésicules lipidiques qui comprennent comme enveloppe les protéines et les phospholipides présents dans les graines d'oléagineux.

On a considéré jusqu'à présent que dans les graines d'oléagineux, l'huile (triglycérides essentiellement) était présente sous la forme de corps huileux d'un diamètre moyen de 1 à 10 micromètres comprenant un noyau central d'huile entouré d'une enveloppe constituée de protéines (oléosines) et de phospholipides (J. Tzen et al., J. Cell Bio, 117, 327, 1992).

On a maintenant découvert que les protéines constituant l'enveloppe des corps huileux sont de deux types : certaines protéines ne sont pas glycosylées et d'autres protéines sont glycosylées.

La présente invention a pour but de fournir des vésicules lipidiques qui comprennent l'ensemble des protéines et des phospholipides présents dans l'enveloppe des corps huileux et donc qui comprennent à la fois des protéines non glycosylées et des protéines glycosylées identiques à celles présentes dans l'enveloppe des corps huileux.

Par ailleurs, on sait que lors de l'extraction des huiles végétales des oléagineux, on récupère des tourteaux qui contiennent encore une fraction importante des protéines et des phospholipides présents dans l'enveloppe des corps huileux. Ces tourteaux contiennent en outre un taux d'huile non extraite qui peut représenter de 10 à 30 % en poids.

La présente invention vise plus spécifiquement à fournir de nouvelles vésicules lipidiques et des émulsions les contenant, en valorisant les tourteaux d'oléagineux.

A cet effet, la présente invention a pour objet des vésicules lipidiques ayant une taille moyenne de 0,1 à 20 micromètres, notamment 1 à 8 micromètres, et comprenant une enveloppe constituée pratiquement de l'ensemble des protéines et des phospholipides présents dans les corps huileux des graines d'oléagineux, entourant un noyau comprenant au moins des lipides exogènes et/ou des substances lipophiles exogènes.

L'invention a également pour objet une émulsion aqueuse comprenant des vésicules lipidiques selon l'invention dispersées dans une phase aqueuse.

Dans la présente invention l'expression "pratiquement l'ensemble des protéines et des phospholipides présent dans les corps huileux des graines d'oléagineux" signifie que l'on retrouve dans l'enveloppe des vésicules pratiquement les mêmes constituants que dans l'enveloppe des corps huileux et que les protéines comprennent à la fois des protéines non glycosylées et des protéines glycosylées. En pratique, les protéines et les phospholipides présents dans les graines d'oléagineux sont ceux présents dans les tourteaux d'oléagineux.

L'invention a également pour objet un procédé de fabrication d'une telle émulsion aqueuse, qui comprend :

- le broyage de tourteaux d'oléagineux,
- l'addition aux tourteaux broyés d'une phase lipidique de façon à avoir un pourcentage global de lipides de 50 à 95 % en poids,
- le malaxage des tourteaux broyés et de la phase lipidique jusqu'à obtenir une pâte homogène, notamment à une température de 0 à 90° C,
- l'addition d'une phase aqueuse à la pâte dans un rapport pondéral pâte/phase aqueuse d'environ 40/60 à 5/95, notamment à une température de 0 à 90° C,
- l'agitation de la pâte et de la phase aqueuse pour former une émulsion,
- et éventuellement la décantation et/ou la filtration de l'émulsion pour éliminer les particules solides,
- et éventuellement la centrifugation de l'émulsion pour obtenir une émulsion concentrée.

Comme exemples de tourteaux d'oléagineux, on peut citer les tourteaux de soja, pistache, macadamia, tournesol, colza, arachide, amande, noisette, sésame, bourrache, germe de blé, jojoba. On utilise de préférence des tourteaux obtenus à partir de graines à forte teneur en huile (pistache, macadamia, arachide, jojoba, noisette, amande).

Le broyage des tourteaux peut être effectué par des broyeurs classiques, tels que des broyeurs à couteaux, avantageusement jusqu'à obtenir une taille de particules inférieure à 0,1 mm.

Les tourteaux broyés peuvent être éventuellement soumis à une irradiation pour une débactérisation jusqu'à une intensité de 10 kgray et conservés sous atmosphère inerte en emballage hermétiquement clos.

Par lipides exogènes on désigne les lipides d'apport, c'est-à-dire que l'on ajoute et qui ne sont pas présents dans les tourteaux broyés. Ces lipides que l'on ajoute aux tourteaux broyés peuvent être des lipides du même type que ceux présents dans l'oléagineux utilisé (lipides endogènes) ou d'autres lipides. En général, il reste dans les tourteaux une proportion relativement importante de triglycérides et l'on ajoute des lipides de façon à avoir un pourcentage global de lipides de 50 à 95 % en poids.

On règle la quantité de lipides en fonction de la taille des particules de l'émulsion finale que l'on veut obtenir. Ainsi, si l'on veut avoir des tailles de particules plus faibles, on diminue la quantité de lipides ajoutés.

Outre ces lipides, on peut ajouter des substances lipophiles telles que des vitamines (vitamines D, A, E, K), des filtres solaires (tels que Parsol MCX et Parsol 1789 de Givaudan ou de la benzophénone-3), des

mélanges lipophiles complexes (Titan M262CD de Kemira Oy) additionnés éventuellement d'huile minérale, de carotène, de silicone (par exemple DC 200 de Dow Corning), d'esters gras.

Le malaxage des tourteaux et de la phase lipidique ajoutée est alors effectué jusqu'à obtenir une pâte homogène. On opère avantageusement sous atmosphère non oxydante (sous vide ou sous azote).

Les vésicules lipidiques peuvent contenir de 0,01% à 100% en poids de lipides exogènes ou de substances lipophiles exogènes.

La phase aqueuse est ensuite ajoutée, avantageusement à température ambiante et sous atmosphère inerte (sous vide ou sous azote). Cette phase aqueuse peut comprendre, outre de l'eau, différents constituants hydrophiles.

L'addition de la phase aqueuse est généralement réalisée de façon à avoir un rapport pondéral pâte/phase aqueuse d'environ 40/60 à 5/95. On préfère un rapport 30/70 à 5/95.

Pour obtenir une émulsion, on soumet l'ensemble à une agitation qui peut être importante, à l'aide par exemple d'un broyeur colloïdal.

L'émulsion obtenue est alors soumise éventuellement à une décantation et/ou filtration, par exemple sur un tamis de 200 micromètres.

L'émulsion obtenue est relativement diluée et en général il est nécessaire de concentrer cette émulsion par centrifugation. A cet effet, on peut utiliser une centrifugeuse à assiettes adaptée au taux de la phase lipidique. Le produit obtenu est un concentrat de particules lipidiques dont le taux de composants lipophiles peut varier d'environ 20 à 70 % en poids suivant les conditions de fabrication.

Avant ou après cette centrifugation, la suspension lipidique peut subir un traitement thermique dont les conditions peuvent varier de 2 secondes à 10 minutes pour des températures de 80° C à 140° C. On peut alors procéder, si désiré, à une opération d'homogénéisation à l'aide d'un appareil de type Gaulin par exemple, laquelle opération peut avoir des conditions de pression variant de 5 à 400.10^5 Pa.

Ce concentrat de particules lipidiques peut être repris et dilué avec une phase aqueuse du type décrit plus loin. Cette phase aqueuse peut être épaissie à l'aide de gélifiants tels que gomme xanthane, gomme sclerane, bentone et dérivés, cellulose et dérivés, carbopol et dérivés, caroube, carraghénanes et dérivés, présents à des concentrations de 0 à 2 % en poids.

Le concentrat de particules lipidiques peut en outre être baratté suivant les techniques de laiteries pour obtenir un beurre que l'on comprime pour en exprimer l'eau.

Les émulsions ainsi obtenues ont en général des tailles moyennes de particules de 0,1 à 20 micromètres.

Les émulsions ainsi obtenues trouvent notamment des applications dans le domaine des produits cosmétiques et la présente invention a également pour objet des compositions cosmétiques comprenant des vésicules lipidiques selon l'invention (par exemple des compositions hydratantes, des compositions de protection solaire, des compositions nourrissantes).

L'utilisation dans ces compositions d'un système émulsionnant naturel (oléosines, phospholipides) entraîne une meilleure tolérance cutanée du produit final.

Dans le cas de préparation d'une émulsion à usage cosmétique, on peut par exemple utiliser une phase aqueuse comprenant:

- Glycérine 5 à 10 %
- Propylène glycol 5 à 10 %
- Butane diol 5 à 10 %
- Urée 1 à 5%
- Sodium PCA 0,5 à 5 %
- EDTA 0,05 à 0,1 %
- Méthyl paraben 0,05 à 0,2 %
- Propyl paraben 0,05 à 0,1 %
- Butyl paraben 0,05 à 0,1 %
- Ethanol 0,05 à 0,5 %
- B H T 0,05 à 1 %
- Alginate de sodium 1 à 5 %
- Vitamine C et dérivés 0,05 à 1 %
- Vitamine B et dérivés 0 à 1 %
- Acide sorbique 0 à 1 %
- Préparations aqueuses diverses d'oligo-éléments
- Sulfite de sodium 0 à 0,4 %.

Les émulsions selon l'invention trouvent également des applications dans le domaine des produits alimentaires. La présente invention a donc également pour objet des compositions alimentaires comprenant des vésicules lipidiques selon l'invention. Comme exemple on peut citer des suppléments lactés vitaminés sans cholestérol, des desserts lactés fermentés, des yaourts et des crèmes fouettées. Les desserts lactés fermentés et les yaourts peuvent être fabriqués par addition de souches de lactobacilles à une émulsion selon l'invention puis incubation 5 heures à 35° C environ. Les crèmes fouettées peuvent être obtenues par addition de 90 parties d'un propulseur (CO_2 ou N_2O) à 10 parties d'une émulsion concentrée selon l'invention, additionnée des additifs alimentaires habituels (arômes et sucres).

Les émulsions selon l'invention trouvent également des applications dans le domaine des produits pharmaceutiques à usage humain ou vétérinaire, en liaison avec les qualités intrinsèques des oléosines (petite taille et lipophilie).

La présente invention a ainsi également pour objet des compositions pharmaceutiques. Comme exemple on peut citer des compositions pour l'apport de vitamine E, de vitamines D ou d'hormones via des patchs transdermiques.

En outre, il est à noter que les émulsions obte-

nues comprennent des protéines qui présentent une grande similitude avec les apolipoprotéines des mammifères; autrement dit, on pense que les lipases des mammifères reconnaissent les protéines des émulsions selon l'invention, ce qui permet la fixation des lipases sur les membranes des particules et par suite la dégradation des lipides substrats des lipases concernées.

Les exemples suivants illustrent la présente invention.

EXEMPLE 1

On broie un tourteau d'arachide à l'aide d'un broyeur à couteaux jusqu'à obtenir une taille inférieure à 0,1 mm environ.

Le tourteau contient 20 % en poids environ de triglycérides.

On ajoute à 50 parties de tourteau broyé 50 parties d'huile végétale (triglycérides d'arachide ou autre oléagineux). L'ensemble est malaxé sous vide à température ambiante jusqu'à obtenir une pâte homogène.

On ajoute à cette pâte 900 parties d'eau. On soumet l'ensemble à température ambiante et sous vide à une vive agitation à l'aide d'un broyeur colloïdal réglé sur l'ouverture maximale.

On filtre sous tamis de 200 micromètres. On centrifuge éventuellement le filtrat qui est un lait très fluide dans une centrifugeuse de laiterie.

On obtient un concentrat de particules lipidiques ayant une taille moyenne de 3 micromètres, contenant environ 60 % de triglycérides.

EXEMPLE 2

On opère comme à l'exemple 1 avec un tourteau de macadamia (contenant 25 % en poids de triglycérides), en ajoutant à 30 parties en poids de tourteaux, 70 parties en poids de triglycérides (huile de macadamia ou d'autres oléagineux).

L'eau est ajoutée à la pâte à raison de 900 parties en poids.

On obtient finalement un concentrat de particules lipidiques ayant une taille moyenne de 3 micromètres, ce concentrat contenant environ 60 % de triglycérides.

On donnera ci-après des exemples de compositions obtenues selon le procédé de l'invention.

Exemple A - Lait pour le visage

. Eau qs. 100 %
. Gomme xanthane 1 à 2 %
. Concentrat A 20 à 40 %
. Parfum 0,1 à 0,5 %
. Conservateurs qs.

Le concentrat A est fabriqué comme décrit à l'exemple 1, à partir d'une huile végétale.

Le pH varie de 5,5 à 6,5 (qs = acide citrique). Viscosité de 2000 à 3000 cP.

Exemple B - Lait solaire écran total

. Eau qs 100 %
. Gomme xanthane 1 à 2 %
. Concentrat B 20 à 40 %
. Parfum 0,1 à 0,5 %
. Conservateurs qs

Le concentrat B est fabriqué comme décrit à l'exemple 1, à partir d'une huile composée par exemple de :

. Triglycérides qs 100 %
. PARSOL 1789 1 à 5 %
. PARSOL MCX 1 à 15 %
. TITAN M262CD 1 à 10 %

Le pH varie de 5,5 à 6,5 (qs = acide citrique). Viscosité 2000 à 3000 cP.

Exemple C - Lait hydratant pour le corps

. Eau qs 100 %
. Glycérine 5 à 10 %
. Gomme xanthane 1 à 2 %
. Urée 1 à 5%
. Concentrat C 20 à 40 %
. Parfum 0,1 à 0,5 %
. Conservateurs qs

Le concentrat C est fabriqué comme décrit à l'exemple 1, à partir d'huile végétale additionnée d'esters gras du jojoba (5 à 10 %).

Le pH varie de 6,0 à 6,5 (qs = acide citrique), la viscosité de 2000 à 3000 cP.

Exemple D - Supplément lacté vitaminé sans cholestérol

. Eau qs 100 %
. Vitamine C 1 %
. Gomme xanthane 0,5 à 1 %
. Concentrat D 20 à 40 %
. Extrait de vanille qs
. Conservateurs qs

Le concentrat D est fabriqué comme décrit à l'exemple 1, à l'aide d'huile à forte teneur en triglycérides gamma linoléniques additionnée de vitamine D (500 UI/g max) ou A (1500 UI/g max).

Les conservateurs pourront être éliminés si les conditions suivantes sont adoptées :

- débactérisation initiale du tourteau,
- traitement UHT (120° C x 3 secondes) de la suspension, avant ou après les ajouts décrits,
- conservation en Tétrapak ou équivalent, sinon, les conservateurs alimentaires seront choisis, et de préférence :
- acide sorbique

- parabens divers
- sodium sulfite
- BHT

le pH sera réglé à 6,5 ± 0,2 à l'aide d'acide citrique.

On donnera ci-après des résultats d'essais mettant en évidence les natures différentes des protéines présentes dans l'enveloppe des corps huileux.

EXEMPLE 3

On a procédé comme à l'exemple 2 en partant d'un tourteau de macadamia.

A partir d'un concentrat de vésicules lipidiques, on a séparé les protéines par la méthode sur gradients d'urée après extraction des lipides à l'éther diéthylique (Millichip, Colloque Int. Lipides Végétaux).

Les protéines ont été remises en solution dans un tampon Tris/HCl 10 mM, SDS 0,07 M, pH 8.

La purification des protéines a été réalisée par chromatographie de tamisage moléculaire sur colonne de Superose 6 équilibrée et éluée en tampon Tris/HCl 10 mM SDS 0,07 M, pH 8,2.

On a séparé 50 fractions et testé les fractions 7, 36 et 43 correspondant à des pics d'élution. A cet effet on a utilisé une technique de type "dot-blot" avec un sérum dirigé contre la β-glucosidase du soja qui réagit avec les motifs glucidiques complexes communs à la plupart des plantes, qui contiennent des résidus α-1-3 glucose et β-1-2-xylose.

Les fractions 7 et 36 sont reconnues par le sérum dirigé contre la β-glucosidase, ce qui n'est pas le cas de la fraction 43. La reconnaissance est donc imputable à la présence de chaînes glycosidiques dans les fractions 7 et 36 qui n'apparaissent pas pour la fraction 43.

Revendications

1. Vésicules lipidiques ayant une taille moyenne de 0,1 à 20 micromètres et comprenant une enveloppe constituée pratiquement de l'ensemble des protéines et des phospholipides présents dans les corps huileux des graines d'oléagineux, entourant un noyau comprenant des lipides exogènes et/ou des substances lipophiles exogènes.

2. Vésicules lipidiques selon la revendication 1, dans lesquels les protéines et les phospholipides sont ceux présents dans les tourteaux d'oléagineux.

3. Emulsion aqueuse comprenant des vésicules lipidiques selon la revendication 1 ou la revendication 2 dispersées dans une phase aqueuse.

4. Procédé de fabrication d'une émulsion aqueuse selon la revendication 3, qui comprend :
   - le broyage des tourteaux d'oléagineux,
   - l'addition aux tourteaux broyés d'une phase lipidique de façon à avoir un pourcentage global de lipides de 50 à 95 % en poids,
   - le malaxage des tourteaux broyés et de la phase lipidique jusqu'à obtenir une pâte homogène,
   - l'addition d'une phase aqueuse à la pâte dans un rapport pondéral pâte/phase aqueuse d'environ 40/60 à 5/95,
   - l'agitation de la pâte et de la phase aqueuse pour former une émulsion,
   - et éventuellement le décantation et/ou la filtration de l'émulsion,
   - et éventuellement la centrifugation de l'émulsion pour obtenir une émulsion concentrée.

5. Procédé selon la revendication 4, dans lequel on effectue l'addition de la phase aqueuse et son mélange avec la pâte à température ambiante.

6. Procédé selon la revendication 4 ou la revendication 5, dans lequel on effectue l'addition de la phase aqueuse et son mélange avec la pâte sous atmosphère non oxydante.

7. Procédé selon l'une des revendications 4 à 6, dans lequel on effectue en outre un barattage de l'émulsion concentrée.

8. Composition cosmétique comprenant des vésicules lipidiques selon la revendication 1.

9. Composition alimentaire comprenant des vésicules lipidiques selon la revendication 1.

10. Composition thérapeutique comprenant des vésicules lipidiques selon la revendication 1.

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DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie	Citation du document avec indication, en cas de besoin, des parties pertinentes	Revendication concernée	CLASSEMENT DE LA DEMANDE (Int.Cl.6)
D,A	J. OF CELL BIOLOGY, vol.117, no.2, Avril 1992, US pages 327 - 335 TZEN ET AL. 'SURFACE STRUCTURE AND PROPERTIES OF PLANT SEED OIL BODIES' * le document en entier * ---	1-10	A61K7/48 A61K7/00 A23D9/00 A23L1/22
A	WO-A-91 11169 (BONNE AT AL.) * le document en entier * ---	1-10	
A	PARFUMS, COSMETIQUES, AROMES, vol.56, Mai 1984, FR page 57 RAYMOND ET AL. 'LES PROTEINES D'OLEAGINEUX: PROPRIETES FONCTIONNELLES UTILISABLES EN COSMETOLOGIE' * le document en entier * -----	1-10	
			DOMAINES TECHNIQUES RECHERCHES (Int.Cl.6) A61K A23D A23L

Le présent rapport a été établi pour toutes les revendications

Lieu de la recherche	Date d'achèvement de la recherche	Examinateur
LA HAYE	10 Août 1995	Fischer, J.P.

CATEGORIE DES DOCUMENTS CITES

X : particulièrement pertinent à lui seul
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(54) Iron-containing protein composition

(57) Addition of a specific sugar compound to an iron-containing protein composition solution can provide an excellent freezing and thawing stability and re-strained precipitate productivity in a thawed solution of its frozen product and increase the efficiency in the use of the iron-containing protein useful for enrichment of iron in a food or drink, production of iron preparations.

EP 1 166 653 A2

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Description

1.Field of the Invention

[0001] The present invention relates to an iron-containing protein composition solution which is superior in freezing-thawing stability and low in precipitating tendency when frozen and thawed; a frozen product of the solution; a liquid composition obtained by thawing the frozen product; and a powder obtained by drying the solution or the liquid composition. The iron-containing protein composition solution of the present invention has an excellent adaptability for freezing to storage or transfer it in the frozen state. This composition solution has restrained precipitate productivity in the solution obtained by thawing a frozen product of the composition solution. The composition solution has no astringent taste uniquely possessed by iron. Therefore, the composition solution can be suitably used in production of an iron-enriched food or drink or an iron preparation. This composition solution is also useful for prevention or treatment of anemia.

2.Description of the Related Art

[0002] Since 1975, the iron intake by Japanese has been flat at a sufficiency ratio of about 100% relative to the iron requirement. Iron is important for maintenance of the homeostasis of human body and is one of the nutrients to be taken with care in meals. The iron sufficiency ratio particularly in young to middle aged women is between 80 to 90%, and the iron shortage in these women is pointed out in "Status of National Nutrition, 1998 Edition", published by Daiichi Shuppan K.K. Thus, many of the young to middle aged women suffering from iron shortage or anemia are supplementing iron in the form of iron-enriched food or drink or of medicine such as iron preparation or the like.

[0003] However, inorganic irons used in iron preparations, such as iron citrate, iron pyrophosphate, iron sulfate and the like generally have an astringent taste or a metallic taste, both uniquely possessed by iron, or have harmful side effects such as damages to stomach, intestines and mucosa in some cases. Heme iron, which is an organoiron, has a problem of possessing a metallic taste, a raw fish-like smell, etc. although it is highly absorbable. Therefore, when the above iron preparations are added to a food or a drink or are used as such, there are cases that the addition amount must be restricted in some cases, often making it difficult to intake the required amount.

[0004] Meanwhile, as iron-containing proteins, an iron-lactoferrin complex (Japanese Patent Laid-Open (Kokai) No. 304798/'95), an iron-casein complex (Japanese Patent Laid-Open (Kokai) No. 77793/'97), etc. are known in the form of a composition. These iron-containing protein compositions are free from astringent taste or metallic taste uniquely possessed by iron, have no harmful side effects, and moreover are superior in solubility and thermal stability; therefore, they are suited for use in foods, drinks and iron preparations.

[0005] Incidentally, one of the present inventors filed as a co-inventor an Japanese Application No. 89741/99 concerning an iron-whey protein complex, which is published as Japanes Patent Laid-Open (Kokai) No. 279143/2000.

SUMMARY OF THE INVENTION

[0006] As describe above, iron-containing protein compositions are being used in foods, drinks and iron preparations. However, they may have a problem in generating precipitate when a solution of these compositions is frozen for storage, transfer or the like and then thawed.

[0007] One of the objects of present invention is to provide a solution containing an iron-containing protein composition useful for iron enrichment in foods and drinks or as an effective ingredient of iron preparation, which solution has restrained precipitate productivity after freezing and thawing. The other object of the present invention is to provide a frozen product of the above solution and a solution obtained by thawing the frozen product, both useful for iron enrichment in foods and drinks as well as for production of iron preparation.

[0008] The present inventors proceeded with a study in view of the above objects. As a result, the present inventors found out that precipitation in a resulting solution by thawing a frozen product of the original solution of the iron-containing protein composition, can be suppressed by adding at least one compound selected from monosaccharides, disaccharides, sugaralcohols, non-reducing saccharides and dextrin to the original solution. The present invention has been completed based on this above findings.

[0009] In the present invention, "superior in freezing-thawing stability" refers to that, when a sample of an iron-containing protein composition solution is placed in an appropriate container and stored in a frozen state at -20°C for 2 weeks, then the frozen sample is thawed, for example, by contacting the container with a flowing water or by allowing the container to stand at room temperature, and 50 ml of the thawed sample is taken into a 50-ml graduated test tube and subjected to centrifugation at 3,000 rpm for 15 minutes using a centrifuge (Kubota Ks-5000P), the volume of the precipitate generated in the centrifuged sample is 0.5 ml or less. When the volume of the precipitate generated is as small as above, the loss of iron-containing protein by precipitation is suppressed effectively; the thawed solution has

a good quality for use in a food, a drink or an iron preparation; and its storability and transferability in a frozen state, etc. can give an improved economic efficiency.

[0010] The present invention can provide an iron-containing protein composition solution which has restrained precipitate productivity after freezing and thawing, and superior freezing-thawing stability. The iron-containing protein composition solution according to the present invention has no astringent taste uniquely possessed by iron and, therefore, can be suitably used in production of an iron-enriched food or drink or an iron preparation and is useful for prevention or treatment of anemia.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Fig. 1 is a graph showing the change of turbidity of samples when they were subjected to repeated freezing-thawing operations in Example 2. The numbers 0 to 10 means the repeat times of a unit operation including freezing of a solution and thawing of the resultant frozen product.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

[0012] A iron-containing protein composition solution, i. e., a solution containing an iron-containing composition, according to the present invention is a solution of an iron-containing protein composition in an aqueous medium and has excellent freezing-thawing stability. The iron-containing protein composition is a soluble and dispersible agglomerate, or aggregate, obtained by allowing iron to act on a protein.

[0013] As an example of the iron-containing protein composition, there can be mentioned a composition containing mainly a reaction product (soluble and dispersible agglomerates or aggregates) obtained by mixing iron, protein and at least one of a carbonate and a bicarbonate.

[0014] When the iron-containing composition is added to an aqueous medium, the components comprising the protein are dispersed as particles in the aqueous medium and the water-soluble mineral components are dissolved in the aqueous medium. Therefore, the composition forms fine particles comprising the protein(s) in an aqueous medium which are dispersible therein.

[0015] Incidentally, as the aqueous medium, a medium composed mainly of water can be mentioned. Ordinarily, water is used as the aqueous medium, per se, and an aqueous solution of the iron-containing protein composition is formed.

[0016] The aqueous solution of an iron-containing protein composition can be obtained when water is used as the aqueous medium, by, for example, preparing a solution containing at least either of a carbonate and a bicarbonate (as a solution A), a solution containing iron (as a solution B) and a solution containing a protein (as a solution C) and mixing these solutions A to C to allow the individual components to act on each other. In this case, the molar concentration of iron ion in the solution B is 1/3 or less, preferably 1/10 or less, more preferably 1/30 or less, further preferably 1/60 or less, most preferably 1/100 or less of the molar concentration of carbonate ion and/or bicarbonate ion dissolved in the solution A (the total molar concentration of both the carbonate ion and the bicarbonate ion is used when both the carbonate ion and the bicarbonate ion are present). The preferable lower limit of the ratio of the molar concentration of iron (A) to that of the molar concentration (B) of carbonate ion and/or bicarbonate ion, i. e., A/B is 1/10,000.

[0017] The molar concentration of protein in the solution C is preferably 1/2 to 1/1,000 of the molar concentration of iron ion in the solution B. Incidentally, when there is used, as the protein in the solution C, a protein decomposition product obtained by proteolysis, its molar concentration can be the molar concentration of the protein before proteolysis and is preferably 1/2 to 1/1,000 of the molar concentration of iron ion in the solution B.

[0018] The solution B and the solution C may be prepared separately and mixed with the solution A. Alternatively, the solutions A and C may be first mixed and the resultant mixture may be mixed with the solution A. In these cases, the solution containing a protein is preferably added before the solution containing iron is added, in order for the mixed solution to have higher stability. The reaction between carbonate and/or bicarbonate, iron and protein takes place in the mixed solution. Either of the iron and the protein to be added may be used in a solid form.

[0019] Further, in order to keep a high concentration of carbonate ion and bicarbonate ion in the mixed solution, the solution A may be allowed to contain a carbonate and/or a bicarbonate in an amount exceeding the solubility. For the same purpose, when a mixture of the solutions B and C is used, a carbonate and/or a bicarbonate, or a carbonate solution and/or a bicarbonate solution may be added in the middle point of the mixing process by adding the mixture of the solutions B and C to the solution A, i. e., at a timing when part of the mixture of the solutions B and C has been added to the solution A.

[0020] The solution of an iron-containing protein composition can also be obtained by mixing a solution containing a protein and at least one of a carbonate and a bicarbonate (as a solution D) and a solution containing iron (as a solution E) to give rise to a reaction between them. In this case, the molar concentration of iron ion in the solution E is 1/3 or less, preferably 1/10 or less, more preferably 1/30 or less, further preferably 1/60 or less, most preferably 1/100

or less of the molar concentration of at least one of carbonate ion and bicarbonate ion dissolved in the solution D (the total molar concentration of carbonate ion and bicarbonate ion is used, when both the carbonate ion and the bicarbonate ion are present). The preferable lower limit of the ratio of the molar concentration of iron (A) to that of the molar concentration (B) of carbonate ion and/or bicarbonate ion, i. e., A/B is 1/10,000.

[0021] The molar concentration of protein in the solution D is preferably 1/2 to 1/1,000 of the molar concentration of iron ion in the solution E. Incidentally, when there is used, as the protein in the solution D, a protein decomposition product obtained by proteolysis, its molar concentration can be the molar concentration of the protein before proteolysis and is preferably 1/2 to 1/1,000 of the molar concentration of iron ion in the solution E.

[0022] Then, the solution of an iron-containing protein composition is sterilized or pasteurized. The solution may be concentrated, if required. The resultant solution is then subjected to particle diameter adjustment to preferably 50 nm or less. To the resulting solution is added a saccharide for imparting freezing-thawing stability to the solution; then, pH adjustment is made; thereby, the desired iron-containing protein composition solution superior in freezing-thawing stability can be obtained. This solution, when filled in an appropriate container, can be stored or transferred in a frozen state, and, in the solution after thawing, almost no precipitate is generated or the amount of precipitate generated is effectively very small.

[0023] The pasteurization and sterilization can be conducted according to an conventional method, for example, by heat pasteurization, sterilization, or bacteria removal by microfiltration (MF). Heat pasteurization can be conducted, for example, by low-temperature pasteurization of about 65°C for 30 minutes or high-temperature short-time pasteurization at about 120°C for 2 to 3 seconds.

[0024] The concentrating treatment is useful for enhancement of iron concentration in the final product and can be conducted, for example, by a method using a membrane such as ultrafiltration (UF) membrane, nanofiltration (NF) membrane, reverse osmosis (RO) membrane or the like, or by vacuum concentration. Besides the concentration, adjustment of iron concentration to a desired level is also possible by subjecting the iron-containing protein composition solution to spray-freezing, freeze-drying or the like according to an conventional method to powderize the solution and diluting the powder to obtain an appropriate concentration in a solution using water or the like.

[0025] The iron-containing protein composition is dissolved in an aqueous medium in the form of particles. The diameters of the particles are adjusted to preferably 200 nm or less, i. e., at most 200 nm, in terms of average particle diameter, by subjecting the above solution to a homogenization treatment at 100 kg/cm^2 or more, preferably at 300 kg/cm^2 or more, or by using a MF membrane of 400 nm. The average particle diameter is preferably 50 nm or less and, when the average particle diameter of the iron-containing protein composition is at such a level, the composition can have even higher stability.

[0026] When the concentration, the drying and the adjustment of average particle diameter are conducted, these treatments can be conducted at any timing after preparation of an iron-containing protein composition solution (this may contain or may not contain a saccharide). When heat pasteurization is conducted, however, the above treatments are conducted preferably after the heat pasteurization.

[0027] As the protein used in preparation of an iron-containing protein composition, there can be mentioned lactoferrins, casein proteins, whey proteins, etc. At least one selected from these proteins can be used.

[0028] As the lactoferrins, there can be mentioned, for example, lactoferrins separated from liquid secretions (e.g. milks) of mammals such as human, cattle and the like; transferrins separated form blood, internal organs, etc.; lactoferrins (e.g. ovotransferrins) separated form egg, etc.; and decomposition products obtained by enzymatic decomposition of these lactoferrins. As the casein proteins, there can be mentioned, for example, casein proteins separated from liquid secretions (e.g. milks) of mammals (e.g. human and cattle), such as casein, acid casein, casein sodium, lactic acid casein, α-casein, β-casein, κ-casein and the like; and decomposition products obtained by enzymatic decomposition of these caseins. As the whey proteins, there can be mentioned, for example, decomposition products obtained by enzymatic decomposition of whey proteins separated from liquid secretions (e.g. milks) of mammals such as human, cattle and the like. There can be mentioned decomposition products obtained by subjecting a whey protein to component separation to isolate individual components such as β-lactoglobulin, α-lactoalbumin and the like and then subjecting each component to enzymatic decomposition. A number of methods are known for separation of these proteins in a large quantity. A protein separated according to any method is usable in the present invention. A protein produced by a microorganism, animal cells or a transgenic animal by genetic engineering is also usable.

[0029] Incidentally, when there is used, as the casein protein, a casein mixture (e.g. crude casein) of α-casein, β-casein and κ-casein, the average molecular weight of the caseins in the mixture is determined from the proportions of individual protein components, from which the molar concentration of the casein dissolved can be calculated.

[0030] When there is used, as the whey protein, a mixture of various protein components, the average molecular weight of the protein components in the mixture is determined from the proportions of individual protein components, from which the molar concentration of the whey protein dissolved can be calculated.

[0031] When a preparation containing various protein components or a hydrolysis product thereof is used, each of which is a mixture of protein components of different molecular weights, the protein molar concentration can be cal-

culated by the step of measuring the proportions and molecular weights of the individual protein components in the same manner as described above, according to, for example, electrophoresis using a gel medium; calculating the average molecular weight of the protein components based on the measured proportions and molecular weights; and calculating the protein molar concentration of the mixture based on the average molecular weight and the total amount of each protein component.

[0032] Meanwhile, the enzymatic decomposition product of a lactoferrin, a casein protein or a whey protein can be obtained by using a protease. As the protease, there can be used, for example, enzymes obtained from animals, such as trypsin, chymotrypsin, pepsin and the like; enzymes obtained from plants, such as papain, bromelain, ficin and the like; and enzymes obtained from microorganisms (e.g. mold, bacteria and yeast). As the form of enzyme when the enzyme is allowed to act on a protein, there can be mentioned an isolated and purified enzyme; a crude enzyme; a cultured product of a microorganism containing an enzyme; an enzyme-containing material obtained by removing the microorganism from the above cultured product; microorganism cells or a crushed product thereof; a recombinant protease; and so forth. As such an enzyme, there can be used those prepared from various materials, or commercial enzyme preparations. As the enzymatic decomposition product, there is preferred, for example, one contains decomposition products having molecular weights in a range not exceeding 10,000.

[0033] As the iron used in preparation of an iron-containing protein composition, there can be mentioned, for example, iron salts mostly of trivalency which show a pH of 4 or less when dissolved in deionized water, such as ferric chloride, ferric nitrate, ferric sulfate and the like.

[0034] As the solution containing at least one of a carbonate and a bicarbonate, there can be mentioned, for example, a carbonic acid water, an ammonium bicarbonate solution, a sodium bicarbonate solution, a potassium bicarbonate solution, a sodium carbonate solution, a calcium carbonate solution and a mixture of two or more of these solutions. To these solutions is preferably added, as a pH-adjusting agent, sodium hydroxide, ammonia, potassium hydroxide, hydrochloric acid, citric acid, lactic acid or the like.

[0035] As the saccharide added for securing freezing-thawing stability, there can be mentioned, for example, monosaccharides such as grape sugar, fruit sugar and the like; disaccharides such as cane sugar, milk sugar and the like; sugaralcohols such as sorbitol, maltitol, erythritol and the like; non-reducing saccharides such as trehalose, palatinit, reduced thick malt syrup and the like; and dextrin. Of these, particularly preferred are sugaralcohols, non-reducing saccharides and dextrin. At least one kind selected from the above monosaccharides, disaccharides, sugaralcohols, non-reducing saccharides and dextrin is added to the iron-containing protein composition solution in an amount of preferably 3 to 50% by weight in order to more effectively suppress not only precipitation in the resulting solution obtained by thawing a frozen product of the solution of the iron-containing protein composition, but also increase of viscosity of the resulting solution which may reduce its workability.

[0036] When pH adjustment is carried out after a saccharide has been added to the iron-containing protein composition solution, the pH of the resulting solution is adjusted to preferably 5 to 10, more preferably 5.8 to 8.5, in order to more effectively suppress not only separation of iron ion during the storage, resulting in deteriorated taste, but also generation of lysinoalanine. For this pH adjustment, there can be used, for example, sodium hydroxide, ammonia, potassium hydroxide, hydrochloric acid, citric acid, or lactic acid.

[0037] The iron-containing protein composition solution of the present invention can be filled in an appropriate container to use as a product. The filled product can be frozen according to an conventional method and the frozen product can be stored and transferred. The frozen product can be thawed into a solution and can be favorably used for production of an iron-containing drink, iron enrichment of a food or a drink, or production of an iron preparation.

[0038] The iron-containing protein composition solution of the present invention is superior in freezing-thawing stability; therefore, when thawed from a frozen state, the solution has no precipitate or a very small amount of a precipitate. As a result, the loss of the iron-containing protein in the solution can be prevented and the efficiency in the use of iron-containing protein can be strikingly increased.

[0039] The iron-containing protein composition solution and the liquid composition obtained by thawing the frozen product of the iron-containing protein composition solution, according to the present invention, can be made into a powdery product via an conventional drying process. The period and efficiency in the storage and transfer of the iron-containing composition can be more greatly improved by combining the storage or transfer in the frozen state and the storage or transfer in the powder state.

Examples

[0040] The present invention is described in more detail below by way of Examples.

Example 1

Solution F:

[0041] A solution obtained by dissolving 0.92 mM of lactoferrin (a product of TATUA) in 4.02 kg of an aqueous solution containing 3.1 M of sodium bicarbonate.

Solution G:

[0042] 43.6 kg of an aqueous solution containing 0.46% by weight (17 mM) of ferric chloride.

[0043] The solution F and the solution G were mixed. The mixed solution was heat-pasteurized at 70°C for 18 minutes and then concentrated to 1/20 using an UF membrane to obtain 2.2 kg of a concentrate of an iron-containing protein composition solution. The concentrate was subjected to a homogenization treatment of 500 kg/cm^2. The resulting concentrate was divided into five portions each of 400 g. To the four portions was added 80 g of sorbitol, erythritol, trehalose and dextrin, respectively, and nothing was added to the remaining one portion.

[0044] Each of the thus-prepared five samples was evaluated for freezing-thawing stability and measured for average particle diameter, as follows.

Evaluation of freezing-thawing stability

[0045] Each sample was stored in a frozen state at -20°C for 2 weeks. The resulting frozen samples were thawed on a flowing water. Each 50 ml of the thawed sample was placed in a 50-ml graduated test tube and subjected to centrifugation at 3,000 rpm for 15 minutes using a centrifuge (Kubota ks-5000P) to measure the volume of the precipitate generated. In the present invention, when the volume of the precipitate generated was 0.5 ml or less, the solution (the sample) was regarded as "superior in freezing-thawing stability".

Measurement of average particle diameter

[0046] This measurement was conducted at a sample temperature of 25°C using a light-scattering photometer (Autosizer 4700, Malvern Instrument Ltd. UK) and an argon laser of 488 nm (Air-Cooled Argon Ion Laser System, Model 2031, Uniphase, California).
The results are shown in Table 1.

Table 1

	Sorbitol	Erythritol	Trehalose	Dextrin	No addition
Precipitate (ml)	0.1	0.1	0.1	0.1	2.0
Average particle diameter (nm)	38.3	34.5	36.2	35.8	330.6

[0047] As is clear from Table 1, it was confirmed that freezing-thawing stability can be imparted to an iron-containing protein composition solution by adding thereto a sugaralcohol, a non-reducing saccharide or dextrin.

Example 2

Solution H:

[0048] 450 kg of an aqueous solution containing 5% by weight (0.6 M) of sodium bicarbonate.

Solution I:

[0049] 50 kg of an aqueous solution containing 10% by weight (1.25 mM) of lactoferrin (a product of DMV)

Solution J:

[0050] 740 kg of an aqueous solution containing 0.5% by weight (18.5 mM) of ferric chloride.

[0051] The solution H and the solution I were mixed. To the mixed solution was added the solution J. The resulting mixed solution was heat-pasteurized by heating up to 80°C. The pasteurized solution was concentrated to 1/25 using

a rotary evaporator (RE-10ED-120, a product of Shibata Kagaku), to obtain 48 kg of a concentrate of an iron-containing protein composition solution. The concentrate was subjected to a homogenization treatment of 300 kg/cm^2. The resulting concentrate was divided into five portions each of 9 kg and each portion was placed in a container. To the four portions were added 1.9 kg of sorbitol, erythritol, trehalose and dextrin, respectively, and nothing was added to the remaining one portion.

[0052] Each of the five samples was stored in a frozen state at -20°C for 2 days and then thawed by contacting the outer wall of the container with a flowing water. The thawed sample was measured for turbidity. Thereafter, the sample was again stored in a frozen state at -20°C for 2 days and thawed using a flowing water. This operation of freezing and thawing was repeated 10 times, and turbidity measurement was conducted after each operation.

[0053] The turbidity measurement was conducted as follows: The sample after 10 times of the freezing and thawing operations was evaluated for freezing-thawing stability and measured for average particle diameter, according to the same methods as in Example 1.

Measurement of turbidity

[0054] A sample was diluted ten-fold with deionized water and measured for absorbance at 660 nm using a spectrophotometer (U-2000, a product of Hitachi Ltd.). The absorbance was taken as the turbidity of the sample.

[0055] The results are shown in Table 2 and Fig. 1.

Table 2

	Sorbitol	Erythritol	Trehalose	Dextrin	No addition
Precipitate (ml)	0.1	0.1	0.1	0.1	2.3
Average particle diameter (nm)	39.2	35.7	35.9	37.0	320.8

[0056] As is clear from Table 21, it was confirmed that freezing-thawing stability can be imparted to an iron-containing protein composition solution by adding thereto a sugaralcohol, a non-reducing saccharide or dextrin.

[0057] Regarding the results in Fig. 1, it was confirmed that an iron-containing protein composition solution to which a sugaralcohol, a non-reducing saccharide or dextrin has been added, as compared with the same solution to which the above material is not added, is low in turbidity and very small in precipitate amount.

Example 3

Solution K:

[0058] Two liters of an aqueous solution containing 2 M of sodium bicarbonate and 16 μM of α-casein (a product of Sigma).

Solution L:

[0059] Two liters of an aqueous solution containing 2.4 mM (as iron ion) of ferric sulfate.

[0060] The solution K and the solution L were mixed to obtain a mixed solution. The mixed solution was subjected to desalting and concentration using an UF membrane (molecular weight cutoff: 5,000) to obtain 0.4 kg of a concentrate of an iron-containing protein composition solution.

[0061] The concentrate was diluted with a buffer solution of pH 7.5 containing 0.05 M/1 of imidazole and 0.15 M/1 of sodium chloride so that the iron ion concentration after dilution became 27 mg/100 ml. The diluted solution was placed in a test tube with a screw head. The test tube was sealed, heated at 90°C for 10 minutes, allowed to cool to room temperature, and subjected to a homogenization treatment at 300 kg/cm^2. There were prepared two kinds of samples, that is, a sample obtained by adding 2 g of dextrin to 50 ml of the solution after the above homogenization treatment and a sample which was the solution after the homogenization treatment per se.

[0062] The thus-prepared two samples were evaluated for freezing-thawing stability and measured for average particle diameter, according to the same methods as in Example 1. The results are shown in Table 3.

Table 3

	Dextrin	No addition
Precipitate (ml)	0.1	3.0

Table 3 (continued)

	Dextrin	No addition
Average particle diameter (nm)	33.6	360.4

[0063] From the results of Table 3, it was confirmed that freezing-thawing stability can be imparted to an iron-containing protein composition solution by adding dextrin thereto.

Example 4

Solution M:

[0064] Fifteen liters of an aqueous solution containing 10 M of sodium bicarbonate.

Solution N:

[0065] Three liters of an aqueous solution containing 180 mM (as iron) of ferric chloride.

Solution O:

[0066] Twelve liters of an aqueous solution containing 1 mM (as a whey protein before enzyme hydrolysis) of a whey protein hydrolysis product.

[0067] In the solution O, adjustment of molar concentration was conducted using average molar weight. This average molecular weight was determined by examining the proportions of the individual protein components in the whey protein by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and making calculation using the proportions and the molecular weights of the individual protein components.

[0068] The whey protein hydrolysis product used in the solution O was produced as follows. That is, 1 kg of a whey protein (purity: 80%) concentrate (a product of New Zealand Daily Board) was dissolved in 19 kg of deionized water. The resulting solution was adjusted to pH 8. Thereto were added 2.4 l (400,000 units) of alcalase (a product of Novo Nordisk) and 4,000,000 units of actinase AS (a product of Kaken Pharmaceutical Co., Ltd.). The mixture was kept at 50°C for 6 hours with the pH being maintained at 8, to give rise to hydrolysis. The reaction mixture was adjusted to pH 7, then heated at 90°C for 10 minutes to deactivate the enzymes, and cooled. The resulting solution was passed through a filtration apparatus using an UF membrane (molecular weight cutoff: 3,000), to obtain a whey protein hydrolysis product.

[0069] The solution N (3 liters) and the solution O (12 liters) were mixed to prepare a mixed solution (N+O)(15 liters). This mixed solution (N+O) (15 liters) was added to the solution M (15 liters) to prepare a mixed solution (M+N+O). This mixed solution was heat-pasteurized at 90°C for 10 minutes and then concentrated to 1/20 using a rotary evaporator (RE-10ED-120, a product of Shibata Kagaku) to obtain 600 g of a concentrate of an iron-containing protein composition solution. The concentrate was subjected to a homogenization treatment at 300 kg/cm^2. To 300 g out of 600 g was added 15 g of erythritol, and nothing was added to the remaining 300 g.

[0070] The thus-prepared two samples were stored in a frozen state at -20°C for 2 weeks, then thawed at room temperature, and evaluated for freezing-thawing stability and measured for average particle diameter according to the same methods as in Example 1. The results are shown in Table 4.

Table 4

	Erythritol	No addition
Precipitate (ml)	0.1	2.9
Average particle diameter (nm)	37.1	370.8

[0071] As is clear from the results of Table 4, it was confirmed that freezing-thawing stability can be imparted to an iron-containing protein composition solution by adding erythritol thereto.

Example 5

Solution P:

[0072] One liter of an aqueous solution containing 1,300 mM of sodium bicarbonate.

Solution Q:

[0073] 0.2 liter of an aqueous solution containing 12 mM (as iron) of ferric sulfate.

Solution R:

[0074] 0.8 liter of an aqueous solution containing 0.1 mM (as lactic acid casein before partial decomposition) of a lactic acid casein decomposition product.

[0075] In the solution R, adjustment of molar concentration was conducted using average molar weight. This average molecular weight was determined by examining the proportions of α-casein, β-casein and κ-casein by urea-sodium dodecyl sulfate (SDS) electrophoresis and making calculation using the proportions and the theoretical molecular weights of the individual caseins.

[0076] The lactic acid casein decomposition product used in preparation of the solution R was produced as follows. That is, there was prepared a solution containing 5% by weight of lactic acid casein (a product of New Zealand Daily Board). Thereto was added sodium bicarbonate for pH adjustment to 8.0. To the resulting solution was added 1,000 units (per g of casein) of trypsin (a proteolytic enzyme, a product of Novo Nordisk). While pH was being maintained at 8.0 by addition of sodium hydroxide, partial decomposition was conducted at 50°C for 6 hours. The reaction mixture was kept at 85°C for 20 minutes to deactivate the enzyme, whereby the partial decomposition was completed. The resulting solution was passed through a filtration apparatus using an UF membrane (molecular weight cutoff: 10,000), to obtain a lactic acid casein hydrolysis product.

[0077] The solution Q (0.2 liter) and the solution R (0.8 liter) were mixed to prepare a mixed solution (Q+R) (1 liter). This mixed solution (Q+R) (1 liter) was added to the solution P (1 liter) to prepare a mixed solution (P+Q+R). This mixed solution was heat-pasteurized at 65°C for 30 minutes and then subjected to a homogenization treatment at 300 kg/cm^2 to obtain an iron-containing protein composition solution (1 liter). To 500 ml of the 1-1 solution was added 50 g of trehalose, and nothing was added to the remaining 500 ml.

[0078] The thus-prepared two samples were each stored in a container in a frozen state at -20°C for 2 weeks, then thawed by contacting the outer wall of the container with a flowing water, and evaluated for freezing-thawing stability and measured for average particle diameter according to the same methods as in Example 1. The results are shown in Table 5.

Table 5

	Trehalose	No addition
Precipitate (ml)	0.1	1.8
Average particle diameter (nm)	34.9	360.2

[0079] As is clear from the results of Table 5, it was confirmed that freezing-thawing stability can be imparted to an iron-containing protein composition solution by adding trehalose thereto.

Example 6

[0080] Solutions having the following compositions were prepared.

Solution A:

[0081] 16.5 kg of an aqueous solution containing 4% by weight (0.48 M) of sodium bicarbonate.

Solution B:

[0082] 1.76 kg of an aqueous solution containing 10% by weight (1.25 mM) of lactoferrin

Solution C:

[0083] 24.31 kg of an aqueous solution (a product of Junsei Kagaku) containing 0.45% by weight (16.6 mM) of ferric chloride.

[0084] The solution A, the solution B and the solution C were mixed. The mixed solution was pasteurized until its temperature reached 90°C. The solution after sterilization was concentrated to 1/20 using a rotary evaporator (RE-10ED-120, a product of Shibata Kagaku). The resulting concentrate was subjected to a homogenization treatment at 300 kg/cm^2 using a homogenizer (GM-1, a product of K.K. SMT). To the homogenized concentrate was added 20% by weight of dextrin. One liter of the resulting mixture and 1 liter of the homogenized concentrate per se were each powderized using a spray drier (Pulvis Mini-Spray, GB-21). Using each of the resulting powders, 100 g of an aqueous solution containing 25% by weight of the powder was prepared.

[0085] The thus-prepared two samples (two solutions) were evaluated for freezing-thawing stability and measured for average particle diameter according to the same methods as in Example 1. The results are shown in Table 6.

Table 6

	Dextrin	No addition
Precipitate (ml)	0.1	1.9
Average particle diameter (nm)	35.6	400.2

[0086] As is clear from the results of Table 6, it was confirmed that freezing-thawing stability can be imparted to an iron-containing protein composition solution by adding dextrin thereto.

Claims

1. An iron-containing protein composition solution comprising an aqueous medium and an iron-containing protein composition dispersed in the aqueous medium,
   said iron-containing protein composition solution further containing at least one sugar compound selected from the group consisting of monosaccharides, disaccharides, sugaralcohols, non-reducing saccharides and dextrin to provide said iron-containing protein composition solution an excellent freezing-thawing stability.

2. An iron-containing protein composition solution according to Claim 1, wherein the iron-containing protein composition comprises at least one of a carbonate and a bicarbonate; iron; and at least one protein selected from the group consisting of lactoferrins, casein proteins and whey proteins.

3. A frozen product of an iron-containing protein composition solution set forth in Claim 1 or 2.

4. A liquid composition obtained by thawing a frozen product set forth in Claim 3.

5. A powder obtained by drying of an iron-containing protein composition solution set forth in Claim 1.

6. A powder obtained by drying of an iron-containing protein composition solution set forth in Claim 2.

7. A powder obtained by drying a liquid composition set forth in Claim 4.

8. A method of preparing an iron-containing protein composition solution including a sugar compound, which comprises the steps of preparing an iron-containing composition solution comprising an aqueous medium and an iron-containing protein composition dispersed in the aqueous medium; and adding at least one sugar compound selected from the group consisting of monosaccharides, disaccharides, sugaralcohols, non-reducing saccharides and dextrin to the an iron-containing composition solution to provide said iron-containing protein composition solution an excellent freezing-thawing stability.

9. An method of preparing an iron-containing protein composition solution including a sugar compound according to Claim 8, wherein the iron-containing protein composition comprises at least one of a carbonate and a bicarbonate; iron; and at least one protein selected from the group consisting of lactoferrins, casein proteins and whey proteins.