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APPLICATION NUMBER: ***61/751,816***
FILING DATE: ***January 11, 2013***
RELATED PCT APPLICATION NUMBER: ***PCT/US14/11361***

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS ***US61/751,816***

UNITED STATES PATENT AND TRADEMARK OFFICE
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET – Page 1 of 2

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. Electronically Filed On January 11, 2013

INVENTOR(S)

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Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max):

METHODS AND COMPOSITIONS FOR CONSUMABLES

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☐ The address corresponding to Customer Number: 021971

OR

☐ Firm or Individual Name

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City	State	Zip
Country	Telephone	Email

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Application Data Sheet. See 37 CFR 1.76

☐ CD(s), Number of CDs _____

☐ Drawing(s) *Number of Sheets* _____

☐ Other (specify) _____

☒ Specification (e.g. description of the invention) *Number of Pages* 123

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☐ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order made payable to the *Director of the United States Patent and Trademark Office* is enclosed to cover the filing fee and application size fee (if applicable).

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PROVISIONAL APPLICATION COVER SHEET
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No

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SIGNATURE Michael Willis Date January 11, 2013

TYPED or PRINTED NAME Michael A. Willis REGISTRATION NO. 53913 *(if appropriate)*

TELEPHONE 650.493.9300 Docket Number: 41751.703.101

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	41751.703.101
	Application Number	
Title of Invention	METHODS AND COMPOSITIONS FOR CONSUMABLES	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
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.)

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	41751.703.101
	Application Number	

Title of Invention	METHODS AND COMPOSITIONS FOR CONSUMABLES

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	41751.703.101
	Application Number	
Title of Invention	METHODS AND COMPOSITIONS FOR CONSUMABLES	

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

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Application Information:

Title of the Invention	METHODS AND COMPOSITIONS FOR CONSUMABLES		
Attorney Docket Number	41751.703.101	**Small Entity Status Claimed**	☐
Application Type	Provisional		
Subject Matter	Utility		
Suggested Class (if any)		**Sub Class (if any)**	
Suggested Technology Center (if any)			
Total Number of Drawing Sheets (if any)		**Suggested Figure for Publication (if any)**	

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	41751.703.101
	Application Number	
Title of Invention	METHODS AND COMPOSITIONS FOR CONSUMABLES	

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☐	Request Early Publication (Fee required at time of Request 37 CFR 1.219)
☐	**Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not**be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

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Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)

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This section allows for the applicant to claim benefit of foreign priority and to identify any prior foreign application for which priority is not claimed. 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(a).

			Remove
Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Priority Claimed
			○ Yes ◉ No

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	41751.703.101
	Application Number	
Title of Invention	METHODS AND COMPOSITIONS FOR CONSUMABLES	

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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 of filing this Authorization.

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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.

◉ Assignee	○ Legal Representative under 35 U.S.C. 117
○ 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 Assignee is an Organization check here. ☒

Organization Name	Sand Hill Foods, Inc.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	41751.703.101
	Application Number	
Title of Invention	METHODS AND COMPOSITIONS FOR CONSUMABLES	

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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	Michael Willis			Date (YYYY-MM-DD)	2013-01-11
First Name	Michael	Last Name	Willis	Registration Number	53913

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WSGR Docket No. 41751.703.101

PROVISIONAL PATENT APPLICATION

METHODS AND COMPOSITIONS FOR CONSUMABLES

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WSGR

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Filed Electronically on: 1/11/13

METHODS AND COMPOSITIONS FOR CONSUMABLES

CROSS-REFERENCE

[0001] This application is related to the following copending patent applications: application Serial No. PCT/US12/46560 ; application Serial No PCT/US12/46552 and application Serial No. 61/611,999 which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

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

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

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

[0005] Plant based meat substitutes have largely failed to cause a shift to a vegetarian diet. The current state of the art for meat substitute compositions involves the extrusion of soy/grain mixture, resulting in products which largely fail to replicate the experience of cooking and eating meat. Common limitations of these products are a texture and mouthfeel that are more homogenous than that of equivalent meat products. Furthermore, as the products must largely be sold 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.

[0006] 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.

[0007] 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 palnts or animals into other food products, mushrooms, bread, yoghurt and the like).

[0008] 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.

[0009] Animals are typically butchered into smaller cuts of meat with specific flavor and handling properties before consumption.

[0010] 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.

[0011] 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 the use of liquid nitrogen, emulsifying agents such as soy lecithin and gelling agents such as calcium alginates to transform food products into unexpected forms.

[0012] 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.

[0013] 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 OF THE INVENTION

[0014] Provided herein are methods and compositions for consumable goods. These can be non-animal based products.

[0015] In some embodiments the invention provides a consumable product comprising: a isolated and purified plant protein in a solution, wherein the isolated plant protein has a solubility in the solution of at least 25 g/L. In one embodiment the plant protein has a solubility of at least 1 mg/ml, wherein the temperature of the solution is 100 C, and the pH of the solution is between pH 8 and at pH 5. In one embodiment the solution comprises an alcohol. In one embodiment the isolated plant protein is at least 10 kDa in size.

[0016] In one embodiment the isolated plant protein does not negatively affect the flavor, texture, or color of a the consumable product as compared to a second consumable product which is identical to the consumable product other than that it does not comprise the isolated plant protein.

[0017] In some embodiments the isolated plant protein is not denatured. In some embodiments the isolated plant protein retains its native conformation. In some embodiments the isolated plant protein is not derived from soy. In one embodiment the isolated plant protein is rubisco. In some embodiments the isolated plant protein comprises a: LEA protein, dehydrin, hydrophilin, intrinsically disordered protein, or protein identified based on its ability to stay soluble after boiling at pH and salt concentrations comparable to foods. In one embodiment the isolated plant protein is Moong 8S globulin.

[0018] In some embodiments the invention further comprising a second isolated and purified protein.

[0019] In some embodiments the invention provides a method for making a consumable product comprising: obtaining an isolated and purified plant protein and dissolving the isolated and purified plant protein in a solution. In some embodiments the method further comprises: obtaining a second isolated and purified plant protein, and dissolving the second isolated and purified plant protein in the solution. In some embodiments the dissolving occurs at a temperature of less than 100 C, and a pH between pH 8 and at pH 5. In some embodiments the isolated and purified plant protein is at least partially hydrolysed when dissolved in the solution.

[0020] In some embodiments the obtaining step comprises: boiling a plant product, filtering the boiled plant product, and concentrating the isolated and purified plant protein. In some embodiments the solution is clear after the dissolving step. In some embodiments the dissolving step comprises dissolving the isolated and purified plant protein is in solution at a greater than 1% concentration. In some embodiments the isolated and purified plant protein is selected from the group consisting of rubisco, moong globulin, soy globulin, pea globulins, pea albumins, prolamins. In some embodiments the isolated and purified plant protein is lyophilized prior to the dissolving step.

[0021] In some embodiments the solution is a liquid to form a high protein drink suitable for human consumption.

[0022] In some embodiments the invention provides a consumable product comprising: an iron-carrying protein with an amino acid sequence with at least 70% homology to a sequence listed in Table 1; wherein the consumable product does not comprise meat. In some embodiments the iron-carrying protein content is visible to the unaided human eye. In some embodiments the iron-carrying protein content account for at least 0.01% of the composition. In some embodiments the product further comprises carbon monoxide or nitrites. In some embodiments the compositions further comprise: ammonium, sodium, potassium, or calcium salts.

[0023] In some embodiments the invention provides a method for extending the shelf-life of a consumable product comprising: obtaining a heme containing protein which is less oxidizable than myoglobin, adding the heme containing protein to a

consumable product. In some embodiments the iron containing protein has an amino acid sequence with at least 70% homology to a sequence listed in Table 1

[0024] In some embodiments the invention provides a consumable product, comprising a gelled emulsion, said gelled emulsion comprising: a protein solution; a first lipid that when not in the consumable product is solid at a first temperature; and; a second lipid that when not in the consumable product is liquid at the first temperature; wherein the first and second lipids are varied in the composition to achieve a melting temperature of the combined first and second lipids in the composition that is similar to the melting temperature of lipids found in meat.

[0025] In some embodiments the melting temperature of the combined first and second lipids in the composition is between 23°C to 27°C. In some embodiments the melting temperature of the combined first and second lipids in the composition is between 26°C to 30°C. In some embodiments the melting temperature of the combined first and second lipids in the composition is between 29°C to 33°C. In some embodiments the melting temperature of the combined first and second lipids in the composition is between 32°C to 36°C. In some embodiments the melting temperature of the combined first and second lipids in the composition is between 35°C to 39°C. In some embodiments the melting temperature of the combined first and second lipids in the composition is between 38°C to 42°C. In some embodiments the melting temperature of the combined first and second lipids in the composition is between 41°C to 45°C. In some embodiments the melting temperature of the combined first and second lipids in the composition is over 45°C and less than 100°C.

[0026] In some embodiments the invention provides a method comprising: obtaining a first lipid, wherein the first lipid is derivedfrom a plant source; obtaining a second lipid, wherein the second lipid is derived from a plant source; combining the first lipid and the second lipid to make a lipid composition with a melting temperature that is not equal to the melting temperature of either the first or second lipid; and combining the lipid composition with a isolated and purified protein to make a consumable product.

[0027] In some embodiments the invention provides a method comprising: obtaining a first plant based fat-analogue and a second plant based fat-analogue, wherein the first and second plant based fat-analogues have different melting temperatures,

constructing a meat analogue composition by distributing the first and second plant based fat-analogues at non-identical locations in the composition. In some embodiments the non-identical locations recapitulates the distribution of fats in a real meat.

[0028] In some embodiments the invention provides a consumable comprising: a plant based fat-analog with a melting temperature above room temperature and below body temperature, and an isolated and purified protein.

[0029] In some embodiments the invention provides a fat tissue replica, comprising a protein composition, the protein composition comprising an isolated and purified non-animal protein; fat droplets, the fat droplets comprising non-animal lipids; and a three-dimensional matrix , the matrix comprising fibers derived from non-animal sources, wherein the fat droplets and protein solution are dispersed in said three-dimensional matrix, and wherein said three-dimensional matrix stabilizes a structure of the fat tissue replica. In some embodiments the fat droplets have a median size of less than 100 microns. In some embodiments a fat content of the fat tissue replica is greater than 50% by weight. In some when cooked at least 30% of the lipids are released from the matrix. In some embodiments the fat tissue replica has a tensile strength equivalent to that of an animal fat.

[0030] In some embodiments the invention provides a connective tissue replica, comprising: a protein content comprising one or more isolated and purified proteins assembled into fibrous structures. In some embodiments the fibrous structures are stabilized by a cross-linking agent.

[0031] In some embodiments the invention provides a connective tissue replica, comprising: a three-dimensional matrix comprising fibers derived from non-animal source, and an isolated and purified plant protein. In some embodiment the fibers have an average diameter of between 10 and 300 microns. In some embodiments the tensile strength of the connective tissue replica is about equivalent to the tensile strength of connective tissue.

[0032] In some embodiments the invention provides a meat replica, comprising: a muscle replica unit, comprising a first isolated and purified protein; a connective tissue replica unit, comprising a second isolated and purified proteins; and a fat tissue replica unit comprising a protein composition with fat droplets suspended therein. In

some embodiments each of said muscle tissue, fat tissue, and connective tissue replica units are purposefully located within the meat replica to approximate the structure of meat. In some embodiments the invention further comprising a heme protein. In some embodiments the invention does not contain meat, looks and smells like meat before the composition is cooked; and looks and smells like meat when the composition is cooked. In some embodiments the invention provides that the replicated meat is ground beef.

[0033] In some embodiments the invention provides a meat replica wherein a muscle tissue unit is $10mm^3$ or less. In some embodiments the connective tissue unit is $10mm^3$ or less. In some embodiments a fat tissue unit is $10mm^3$ or less.

[0034] In some embodiments the invention provides a method of preparing a food or beverage product, comprising adding one or more isolated and purified proteins to said food or beverage product. In some embodiments the one or more isolated and purified proteins does not negatively affect the flavor, texture, or color of said food or beverage product. In some embodiments the one or more isolated and purified proteins improves the flavor, texture, or color of said food or beverage product.

[0035] In some embodiments the invention provides a method for imparting a beef like flavor on a consumable comprising: obtaining a consumable composition adding to the consumable composition an iron-carrying protein with an amino acid sequence with at least 70% homology to a sequence listed in Table 1, wherein after cooking the composition has a more beef like flavor than the composition would have without the addition step.

[0036] In some embodiments the invention provides a method for the production of a consumable comprising: isolating and purifying proteins from plant sources recombining the proteins into an artificial mixture, and producing a consumable comprising the artificial mixture. In some embodiments the invention provides that the artificial mixture is gel. In some embodiments recombining comprises enzymatic crosslinking. In some embodiments recombining comprises thermal denaturation. In some embodiments producing comprises extruding the artificial mixture to form fibers.

[0037] In some embodiments the invention provides a liquid composition comprising: an isolated and purified protein that does not alter the visual appearance

or taste of the liquid as compared to the liquid without the addition of isolated and purified proteins. In some embodiments the beverage has an improved mouthfeel compared to a beverage without the isolated and purified protein. In some embodiments the one or more isolated and purified proteins improves the flavor, texture, or color of said food or beverage product. In some embodiments the one or more isolated and purified proteins does not negatively affect the flavor, texture, or color of said food or beverage product. In some embodiments the beverage contains ethanol. In some embodiments the beverage is a cream liquor that contains no animal products further comprising: a non-dairy lipid emulsion.

[0038] In some embodiments the invention provides a muscle tissue analogue comprising : isolated plant based protein, a heme protein, a crosslinking enzyme and NaCl, wherein the final protein concentration is between 50 and 100 mg/ml.

[0039] In some embodiments the invention provides a fat tissue replica comprising: an emulsion comprising isolated plant protein and isolated plant lipids; plant based fibers wherein the fat replica has a tensile strength greater than 20 kPa.

[0040] In some embodiments the invention provides a meat replica comprising: a plant protein; a fat tissue replica that is solid a room temperature in the meat replica, and a plant based lipid that is a liquid at room temperature in the meat replica.

[0041] In one embodiment the invention provides a method for extending the shelf life of a meat free consumable product, wherein the product comprises a plant-derived heme protein, the method comprising a step of perfusing the product with carbon monoxide gas.

[0042] In one embodiment the invention provides a method for extending the shelf life of a meat free consumable product, wherein the product comprises a plant-derived heme protein, the method comprising a step of reducing heme-$Fe3^{+}$ to heme-$Fe2^{+}$.

[0043] In one embodiment the invention provides a method for making a meat replica comprising: combining at an 80:20 ratio: a plurality of muscle replica pieces and a plurality of fat replica peices, wherein an average individual volume of the pieces is at least $1mm^3$; and grinding the combined composition of step a to make a fine size particle mix.

[0044] In one embodiment the invention provides method for making a poultry composition taste like beef comprising adding a heme protein to the poultry composition.

[0045] In one embodiment the invention provides chocolate flavored spread comprising: sugar, cocoa powder, and a cream fraction from a plant based milk.

[0046] In some embodiments a cross-linking enzyme accounts for 0.5-5% of the composition weight by volume. In some embodiments a cross-linking enzyme accounts for 0.0005% to 0.5% of the composition weight by volume. In some embodiments a cross-linking enzyme accounts for less than 0.001% of the composition weight by volume. In some embodiments a cross-linking enzyme accounts for less not more than 0.001%, 0.01%, 0.1%, or 1% of the composition weight by volume.

[0047] 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. The gelled emulsion can comprise an isolated, purified cross-linking enzyme, wherein the cross-linking enzyme accounts for 0.001%

INCORPORATION BY REFERENCE

[0048] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

DETAILED DESCRIPTION OF THE INVENTION

I. Consumables

[0049] Methods and compositions for the production of consumables are described herein. The consumables can generally be described as replicas of food products 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 food but can 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.

[0050] The advantages of the consumables can include, for example, 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 may also have a higher degree production consistency allowing for improved quality control of the products. Another advantage is that the consumables can designed intentionally to have desirable characteristics for food preparation that are superior to traditional food products.

[0051] The consumables can be for animal consumption, including human consumption. The consumables could be food for domestic animals. For instance, dog food could be produced according to the present inventions. The consumables could be food for wild animals. For instance, the consumables could be provided to non-domesticated predatory animals.

[0052] The consumables can be sold in grocery stores, convenience stores, mass merchandisers, and club stores or prepared in restaurants, fast food, schools, event locations, hospitals, military facilities, prisons, shelters, long-term care facilities, similar to already existing human foods.

[0053] 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.

[0054] 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, seafood such as, e.g, fish, crab, lobster, oyster, muscle, scallop, abalone, squid, octopus, sea urchin, tunicate and others.

[0055] Many meat products are typically derived from skeletal muscle of an animal but it is understood that meat can also come from other muscles or organs of the animal. In some embodiments, the equivalent meat product is a cut of meat derived from skeletal muscle. In other embodiments, the equivalent meat product is an organ such as, e.g., a kidney, heart, liver, gallbladder, intestine, stomach, bone marrow, brain, thymus, lung, tongue. Accordingly, in some embodiments, the compositions of the present invention are consumables similar to skeletal muscle or organs.

[0056] A consumable 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 "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. 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 yet other alternative embodiments, the invention provides meat products substantially derived from animal sources but which are supplemented with one or more of a muscle tissue replica, a fat replica, and/or a connective tissue replica, wherein 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.

[0057] 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, 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 , yoghurt, kefir, sour cream or butter replicas).

[0058] 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. 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.

[0059] The consumables can be made from local products. For instance the consumables can be made from plants grown within a certain radius of the eventual consumer. That radius could be 1, 10, 100, or 1000 miles for example. So, in some embodiments, the invention provides method for producing a consumable which does not contain products which have been shipped over 1, 10, 100, or 1000 miles.

[0060] 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.

[0061] The consumables may require fewer resources to produce than consumables produced from domesticated animals. Accordingly, the present invention provides for meat replicates which require less water or energy to produce than meat. For example a consumable can require less than about 10, 50, 100, 200, 300, 500, or 1000 gallons of water per pound of consumable. For comparison producing beef can require over 2000 gallons of water per pound of meat.

[0062] The consumable may require less land area to produce than a meat product with similar protein content. For example, the consumable may require 30% or less of the land area required to produce a meat product with similar protein content.

[0063] 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 that are indistinguishable to animal products such as ground beef, with reduced or zero cholesterol content will help maintain a low cholesterol diet. In another example the consumable may contain no cholesterol, or higher levels of poly-unsaturated fatty acids compared to the animal product it replaces.

[0064] 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.

[0065] 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, lamb causes the emission of 39 kg equivalents of Carbon dioxide per kilogram of beef that is consumed.

[0066] The consumable may provide alternatives to animal products or combinations of animal products whose consumption is forbidden by religious beliefs. For example, the consumable may be a kosher replica pork chop.

[0067] The consumable can also be shipped in components and produced or assembled at a different location. When available local components can be used for production of the consumable. These can be supplemented with components which are not locally available. This allows for methods of producing consumables, for instance meat replicates, using less energy in shipment than is required for meat. For example, local water can be used in combination with a kit which provides other components of the consumable. Using local water will reduce shipping weight thereby reducing cost and environmental impact.

[0068] The consumables can be produced or assembled wholly or in part in areas where animal farming is not practical or is not allowed. The consumable can be produced or assembled within an urban environment. For example, a kit may be provided to a user to enable the user to produce the consumable. The user could use

local water or use plants from a rooftop garden, for instance in Shanghai. In another example, the consumables could be produced aboard a space craft, space station, or lunar base. Accordingly, the present invention provides methods and systems for the production of meat replicas for use in space travel or for training for the same. For instance the present invention could be used in earth based training for space travel. The consumables could also be produced on an island or upon a manmade platform at sea where the keeping of livestock is difficult or prohibited.

II. Properties of the Consumable

[0069] The consumable is 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 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.

[0070] 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.

[0071] 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. So, 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 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. So, 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.

[0072] 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.

[0073] 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.

[0074] 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. 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.

[0075] Panelists can be served samples in booths, under red lights or under white light, in an open consumer panel. Samples can be assigned random three-digit numbers and rotated in ballot position to prevent bias. Panelists can be asked to evaluate samples for tenderness, juiciness, texture, flavor, and overall acceptability using a hedonic scale from 1=dislike extremely, to 9=like extremely, with a median of 5=neither like nor, dislike. Panelists can be encouraged to rinse their mouths with water between samples, and given opportunity to comment on each sample.

[0076] The results of this experiment can indicate significant differences or similarities between the traditional meats and the compositions of the invention.

[0077] These results will demonstrate that the compositions of the invention are judged as acceptably equivalent to real meat products. Additionally, these results can demonstrate that compositions of the invention are preferred by panelist over other commercially available meat substitutes. So, in some embodiments the present

invention provides for consumables that are significantly similar to traditional meats and are more meat like than previously known meat alternatives.

[0078] 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 cross-sectional area 100 mm2 is measured using, for example, TA.XT Plus Texture Analyzer (Textrue Technologies Corp.) is not significantly different than the force required to tear a sample of animal tissue (muscle, fat or connective tissue) with cross-sectional area 100 mm^2 measured the same way. Accordingly, the invention provides for consumables with similar physical strength characteristics to meat.

[0079] The consumable 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. Similar 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 can also 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.

[0080] In some embodiments the consumable is compared to a food product (e.g. meat) based upon olfactometer readings. In various embodiments the olfactometer can be used to assess odor concentration and odor thresholds, odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of

appreciation, or relative intensity of odors. In some embodiments the olfactometer allows the training and automatic evaluation of expert panels. So in some embodiments the consumable is a product that causes similar or identical olfactometer readings. In some embodiments the differences are sufficiently small to be below the detection threshold of human perception.

[0081] 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.

[0082] 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.

[0083] 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 2C, as the shelf life decreases with exposure to higher temperatures.

[0084] The shelf life of meat is determined through research into 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 High Pressure Pasturization (HPP).

[0085] 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%; pork and veal have 0.1-0.3%; young beef has 0.4-1.0%; and old beef has 1.5-2.0%. 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 colour change of ground meat from red to brown. Meat shelf life extenders have been developed to extend the lifetime of the red colour of meat products including but not limited to carbon monoxide, nitrites, sodium metabisulfite, Bombal, rosemary extract, green tea extract, catechins and other anti-oxidants.

[0086] However an intrinsically more stable heme protein such the hemoblobins isolated from *Aquifex aeolicus* or *Methylacidiphilum infernorum* will degrade more

slowly than a mesophilic heoglobin such as myoglobin. The heme proteins of Table 1 may also have the lifetime of the reduced heme-Fe2+ 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 proteins 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.

[0087] The main determinant of rancidity and production of off flavors 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 to oxygen to create off flavors. In some embodiments the consumable shows increased stability under commercial packaging conditions. In some embodiments the improved shelf life is improved by using components with increased oxidative stability. 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

[0088] The consumable may be substantially or entirely composed of ingredients derived from non-animal sources, e.g. plant based, fungal, or microbial sources. The plant sources can be organically grown sources. The consumables may also be made from a combination of plant based sources and animal based sources. For instance, the consumable may be a ground beef product supplemented with plant based products of the invention.

A. SOURCES OF COMPONENTS OF THE CONSUMABLE

[0089] Isolated and/or purified proteins can be derived from non-animal sources. Non-limiting examples of non-animal sources include plants, funghi, bacteria, yeasts, algae, archaea, genetically modified organisms such as genetically modified bacteria or yeast, chemical or in vitro synthesis. In some embodiments, the one or more isolated, purified proteins are derived from plant sources. Non-limiting examples of plant sources include grains such as, e.g., corn, maize, oats, rice, wheat, barley, rye, triticale, teff, oilseeds including cottonseed, sunflower seed, safflower seed, crambe, camelina, mustard, rapeseed, leafy greens such as, e.g., lettuce, spinach, kale, collard greens, turnip greens, chard, mustard greens, dandelion greens, broccoli, cabbage, green matter not ordinarily consumed by humans, including biomass crops, including switchgrass, miscanthus, arundo donax, energy cane, sorghum, other grasses, alfalfa, corn stover, kelp, other seaweeds, green matter ordinarily discarded from harvested plants, sugar cane leaves, leaves of trees, root crops such as cassava, sweet potato, potato, carrots, beets, turnips, plants from the legume family, such as, e.g., clover, peas such as cowpeas, english peas, yellow peas, green peas, beans such as, e.g., soybeans, fava beans, lima beans, kidney beans, garbanzo beans, mung beans, pinto beans, lentils, lupins, mesquite, carob, soy, and peanuts, coconut, vetch (vicia), stylo (stylosanthes), arachis, indigofera, acacia, leucaena, cyamopsis, and sesbania. Protein can be isolated from the green leafy portion of the plant. 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. One of skill in the art will understand that components (e.g. proteins) that can be isolated from any organism in the plant kingdom may be used in the present invention.

[0090] 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, connective tissue replicas, meat substitute products or others). Accordingly, in some embodiments, the one or more isolated proteins comprises an abundant protein found in high levels in a plant and capable of being isolated and purified in large quantities. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70% of the total

protein content of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5-40%, about 10-50%, about 20-60%, or about 30-70% of the total protein content of the source plant. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50% of the total weight of the dry matter of the source plant. In some embodiments, the abundant protein comprises about 0.5-5%, about 1-10%, about 5-20%, about 10-30%, about 15-40%, about 20-50% of the total weight of the dry matter of the source plant.

[0091] The one or more isolated 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%, 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 ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco). Rubisco is a particularly useful protein for meat replicas because of its high solubility and an amino acid composition with close to the optimum proportions of essential amino acids for human nutrition. In some embodiments, the one or more isolated proteins comprise ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco). In some embodiments, the one or more isolated proteins comprise a vegetative storage protein (VSP).

[0092] 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 are seed storage proteins, e.g., albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin,

hordein, prolamin, phaseolin (protein), proteinoplast, secalin, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins.

[0093] The one or more isolated protein can include highly soluble proteins such as, dehydrins, hydrophilins, natively unfolded proteins and 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. In some cases the highly soluble proteins may be isolated by heating a protein extract to 100°C and, after centrifugation or filtration of insoluble material, concentrating the highly soluble protein fraction by, for example, ultrafiltration. In some cases isoionic pH precipitation, trichloroacetic acid precipitation, ammonium sulfate precipitation steps can be done before or after the heating step to additionally remove non-LEA proteins.

[0094] During an isolation process, the majority of the proteins can be removed from solution, including but not limited to proteases, lipases, amylases albumins, glycinins, conglycinins, globulins, vicilins, convicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins, orother oil body proteins. In some embodiments the nucleic acids are removed from the isolated proteins. In some embodiments the isolated protein has reduced amounts of RNA and DNA compared to the seeds. In some embodiments the lipid membranes are removed from the isolated proteins. In some embodiments the isolated protein has reduced amounts of phospholipids, fats and oils compared to the seeds. In some embodiments, carbohydrates such as for example starch, cellulose and glucans are partially removed from the isolated proteins. In some embodiments, carbohydrates such as for example starch, cellulose and glucans are removed from the isolated proteins. In some embodiments, phenolic and polyphenolic compounds are removed from isolated proteins. In some embodiments, pigments are removed from isolated proteins. In some embodiments, aromatic compounds are removed from proteins.

B. PROTEINS

[0095] 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. The consumable can comprise isolated non-animal based proteins. Proteins can be isolated from plants. The isolated proteins can be purified. In some embodiments, about 0.1%,, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component is comprised of one or more isolated, purified proteins. "Purified protein" can refer to a preparation in which the cumulative abundance by mass of protein components other than the specified protein, which can be a single monomeric or multimeric protein species in aqueous solution, 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.

[0096] The isolated proteins can be isolated from one or more other component 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 may 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 are separated based on their surface charge, for example by isoelectric precipitation, anion exchange chromatography, cation exchange chromatography, Proteins may be separated on the basis of their solubility, for example ammonium sulfate precipitation, isoelectric precipitation, surfactants, detergents or solvent extraction. Proteins may also be separated by their affinity to the resin for example hydrophobic interaction chromatography resins, reactive dye resins, hydroxyapatite resins. Proteins may be purified by affinity chromatography, where the resins may be coated with a antibody to the protein, nickel NTA, lectins, or other molecules which specifically binds the protein. The purified protein may be substantially pure. The purified protein may be 100%, or greater than 99%, 95%, 90%, 85%, 80%, or 70% pure.

[0097] 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.

[0098] The isolated and purified proteins can be produced using modern genetic techniques. For example a recombinant nucleic acid can be introduced to bacteria to produce a particular protein. In some embodiments the isolated and purified proteins are isolated from a genetically modified organism. In some embodiments the genetically modified organism is a genetically modified plant, algae, bacteria, fungal, or yeast organism.

[0099] The isolated and purified proteins are in some instances not isolated from an animal. 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 protein for the consumable.

[00100] 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, texture of the consumable. For example, a meat substitute product can comprises 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.

[00101] Examples of proteins that can be isolated and used in the consumables include: leghemoglobin, non-symbiotic hemoglobin, hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, 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.

[00102] The protein can be an iron-carrying protein. Iron-carrying proteins can be, for example: hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins. Non-symbiotic hemoglobins may be isolated from sprouted soybean, sugar pod, alfalfa, golden flax, black bean, black eyed pea, northern, garbanzo, moong bean, cowpeas, pinto beans, pod peas, quinoa, sesame, sunflower, wheat berries, spelt, barley, wild rice or rice.

[00103] In some embodiments, the iron-carrying protein comprises an amino acid sequence with at least 99%, 95%, 90%, 85%, 80%, 75%, or 70% homology to SEQ ID NO 1, 2, or 3. SEQ ID NO 1: MTTTLERGFTEEQEALVVKSWNVMKKNSGELGLKFFLKIFEIAPSAQKLFSFL RDSTVPLEQNPKLKPHAVSVFVMTCDSAVQLRKAGKVTVRESNLKKLGATH FRTGVANEHFEVTKFALLETIKEAVPEMWSPAMKNAWGEAYDQLVDAIKYE MKPPSS.

[00104] SEQ ID NO 2. SEQ ID NO 2: MIDQKEKELI KESWKRIEPN KNEIGLLFYA NLFKEEPTVS VLFQNPISSQ SRKLMQVLGI LVQGIDNLEG LIPTLQDLGR RHKQYGVVDS HYPLVGDCLL KSIQEYLGQG FTEEAKAAWT KVYGIAAQVM TAE. SEQ ID NO 3.

[00105] SEQ ID NO 3: MLSEETIRVI KSTVPLLKEH GTEITARMYE LLFSKYPKTK ELFAGASEEQ PKKLANAIIAYATYIDRLEE LDNAISTIAR SHVRRNVKPE HYPLVKECLL QAIEEVLNPG EEVLKAWEEAYDFLAKTLIT LEKKLYSQP.

[00106] In some embodiments, the iron-carrying protein comprises an amino acid sequence with at least 99%, 95%, 90%, 85%, 80%, 75%, or 70% homology to a protein listed in table 1.

Table 1

Class	Name	Organism	Sequence	Peak Absorbance Wavelength (nm)*
Bacterial Hemoglobin	AaTgb	*Aquifex aeolicus*	MLSEETIRVIKSTVPLLKEHGTEITARMYELLFSK YPKTKELFAGASEEQPKKLANAIIAYATYIDRLEE LDNAISTIARSHVRRNVKPEHYPLVKECLLQAIEE VLNPGEEVLKAWEEAYDFLAKTLITLEKKLYSQP	413
	HGbI	*Methylacidi-philum infernorum*	MIDQKEKELIKESWKRIEPNKNEIGLLFYANLFKE EPTVSVLFQNPISSQSRKLMQVLGILVQGIDNLEG LIPTLQDLGRRIIKQYGVVDSIIYPLVGDCLLKSIQ EYLGQGFTEEAKAAWTKVYGIAAQVMTAE	412
Leg-hemoglobin	LGB2	*Glycine max*	MGAFTEKQEALVSSSFEAFKANIPQYSVVFYTSIL EKAPAAKDLFSFLSNGVDPSNPKLTGHAEKLFGL VRDSAGQLKANGTVVADAALGSIHAQKAITDPQ FVVVKEALLKTIKEAVGDKWSDELSSAWEVAYD ELAAAIKKAF	415
Non-symbiotic hemoglobin	HORvu GLB1	*Hordeum vulgare*	MSAAEGAVVFSEEKEALVLKSWAIMKKDSANLG LRFFLKIFEIAPSARQMFPFLRDSDVPLETNPKLKT HAVSVFVMTCEAAAQLRKAGKITVRETTLKRLG GTHLKYGVADGHFEVTRFALLETIKEALPADMW GPEMRNAWGEAYDQLVAAIKQEMKPAE	412
	AFB70892	*Vigna radiata*	MTTTLERGFTEEQEALVVKSWNVMKKNSGELGL KFFLKIFEIAPSAQKLFSFLRDSTVPLEQNPKLKPH AVSVFVMTCDSAVQLRKAGKVTVRESNLKKLG ATHFRTGVANEHFEVTKFALLETIKEAVPEMWSP AMKNAWGEAYDQLVDAIKYEMKPPSS	412

**Bos taurus* myoglobin peak absorbance wavelength is 415 nm.

[00107] The proteins used in the consumable can be altered. The proteins can be hydrolyzed. The proteins can be cleaved. The proteins can be crosslinked. The proteins can be denatured. The proteins can be polymerized. The proteins may be extruded. The proteins may be electrospun. The proteins may be spray dried or lyophilized. The proteins may be derivatised or chemically modified. The proteins can be modified by covalent attachment of sugars, lipids, cofactors, peptides, other

chemical groups including phosphate, acetate, methyl, and other natural or unnatural molecules; their peptide backbone can be broken by exposure to acid or proteases or other means, their 3-dimensional structure can be altered by exposure to heat, cold, changes in pH, exposure to denaturing agents such as detergents, urea, other chaotropic agents, mechanical stress including shear. The alignment of proteins in a solution or colloid or a solid assembly can be controlled to affect the mechanical properties of a material including its tensile strength, elasticity, deformability, hardness, or hydrophobicity.

[00108] A composition of the invention can comprise an isolated and/or purified protein. For clarity, as typically used herein, the isolated and purified protein is described as isolated and purified relative to its starting material (e.g. plants or other non-animal sources). 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.

[00109] The protein used in the consumable may be soluble in a solution. In some embodiments, the isolated and purified proteins are soluble in solution at greater than 25, 50, 100, 150, 200, 250g/L. In some embodiments the proteins are soluble at at least 1 mg/ml at temperatures up to 100°C, at pH 8 and at pH 5.

[00110] 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, 90% in its native fold. In some embodiments, the isolated and purified protein is more than 50, 60, 70, 80, 90% water soluble.

[00111] The proteins can include proteins that interact with lipids and help stabilize lipids in a structure. The proteins can include proteins that bind lipids and help crosslink lipid structures. The proteins can include proteins that bind lipids and help crosslink lipid structures and non-lipid interacting proteins. Without wishing to be bound by a particular theory, such proteins may improve the integration of lipids and/or fat replicas with other components of the meat substitute product, resulting in improved mouthfeel and texture of the final product. A non-limiting example of a lipid-interacting plant protein is the oleosin family of proteins. Oleosins are lipid-interacting proteins that are found in oil bodies of plants. Other non-limiting

examples of plant proteins that can stabilize emulsions include seed storage proteins from Great Northern Beans, albumins from peas, globulins from peas, 8S globulins from Moong bean, 8S globulins from Kidney bean, prolamin and lipid transfer proteins.

[00112] In some embodiments, the one or more isolated and purified proteins accounts for at least 50% of the protein content of the consumable by weight. In some embodiments, the one or more isolated and purified proteins accounts for at least 40% of the protein content by weight. In some embodiments, the one or more isolated and purified proteins accounts for at least 30% of the protein content by weight. In some embodiments, the one or more isolated and purified proteins accounts for at least 20% of the protein content by weight. In some embodiments, the one or more isolated and purified proteins accounts for at least 10% of the protein content by weight. In some embodiments, the one or more isolated and purified proteins accounts for at least 5% of the composition content by weight. In some embodiments, the one or more isolated and purified proteins accounts for at least 1% of the composition content by weight.

C. LIPIDS

[00113] Lipids may also be isolated and/or purified for inclusion in the compositions. 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. The sources can be plant sources. In some embodiments, the sources are plants, algae, yeasts or seaweed. Non-limiting examples of oils include corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, margarine, hydrogenated vegetable oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phosphatidic acids, lysophosphatidic acids, phosphatidyl cholines, phosphatidyl ethanolamines, phosphatidyl serines, sphingomyelins, ceramides, phosphatidyl inositols, stigmasterol, sitosterol., campesterol, brassicasterol, sitostanol,

campestanol, ergosterol, zymosterol, fecosterol, dinosterol, lanosterol, cholesterol and episterololeic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, 20:4 arachidonic acid, 20:5 eicosapentanoic acid, 22:5 docosapentaenoic acid, docosohexanoic acid, 22:1 Erucic acid, 18:2 conjugated linoleic acid, 18:3 linolenic acid, 18:1 elaidic acid, 16:1 trans-vaccenic acid, 16:1 vaccenic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, 20:4arachidonic acid, 20:5eicosapentanoic acid, 22:6 docosohexanoic acid, 22:5 docosapentaenoic acid, 18:2 conjugated linoleic acid, 18:3 linolenic acid, 18:1 elaidic acid, 16:1 trans-vaccenic acid, 16:1 vaccenic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, 20:4 arachidonic acid, 20:5 eicosapentanoic acid, 22:6 docosohexanoic acid, 18:2 conjugated linoleic acid, 18:3 linolenic acid, 18:1 elaidic acid, 16:1 trans-vaccenic acid, 16:1 vaccenic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, 20:4 arachidonic acid, 20:5 eicosapentanoic acid, 22:6 docosohexanoic acid, 18:2 conjugated linoleic acid, 18:3 linolenic acid, 18:1 elaidic acid, 16:1 trans-vaccenic acid, 16:1 vaccenic acid, , 22:5 docosapentaenoic acid or conjugated oleic acid.

[00114] In some embodiments, the fat droplets are the cream fraction created from seeds, nuts, and legumes, including but not limited to sunflowers, safflower, sesame seeds, rape seeds, almonds, macadamia, grapefruit, lemon, orange, watermelon, pumpkin, cocoa, coconut, emulsion comprising fats, proteins mango, butternut squash and pistachios. As used herein, the term "cream fraction" can refer to an isolated emulsion comprising lipids, proteins and water.

[00115] Purification methods for the cream fraction can include some or all of the following steps:. Seeds, nuts or legumes can be blended from 1 minute up to 30 minutes which could include a blending method by increasing the speed gradually to

maximum speed over 4 minute, and 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), NaSO4 (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. Further purification of the cream fraction is possible using urea washing with 0.1M to 2M urea. After blending the slurry, the slurry can be centrifuged. The centrifugation can occur when the slurry is at temperatures of 50°C to 20°C. The slurry can be centrifuged, and the top layer that (also referred to as the "cream"), can be collected, the residual liquid referred to as the "skim" layer that is a solution comprising proteins in water can also be used. The resulting "cream" can then be used as is, or further washed with the solutions described above or heated in solution. Washing and heating removes color and flavor molecules (e.g. unwanted molecules) , or unwanted grainy particles to improve the mouth feel and creaminess. Particularly. washing with a high pH buffer can remove bitter tasting compounds and improves 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, washes with salts can decrease taste compounds. Heating can increase the removal of grainy particles, color and flavor compounds. Heating can be from 0-24 hours, at 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.

[00116] The lipids or fat droplets can comprise phospholipids, sterols or lipids. The phospholipids can comprise a plurality of amphipathic molecules comprising fatty acids, glycerol and polar groups.

[00117] In some embodiments, the fatty acids are , for example, of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic

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

[00118] In some embodiments, the polar groups are, for example, choline, ethanolamine, serine, phosphate, glycerol-3-phosphate, inositol or inositol phosphates.

[00119] In some embodiments the sterols are, for example, stigmasterol, sitosterol, campesterol, brassicasterol, sitostanol, campestanol, ergosterol, zymosterol, fecosterol, dinosterol, lanosterol, cholesterol or episterol.

[00120] In some embodiments, the lipids are, for example, sphingolipids, ceramides, sphingomyelins, cerebrosides, gangliosides, ether lipids, plasmalogens or pegylated lipids.

D. FIBERS

[00121] Fiber can be isolated and/or purified for inclusion in the compositions. Fiber can mean 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 oligosaccharides.

[00122] 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.

[00123] Fiber or fibers can also mean compositions that can form a matrix for a structure for the compositions. A 3-dimensional matrix of fibers can, for example, contains chemicals that promote formation of inter-molecular disulfide cross-links

(mixed glutathione, DTT, BME). In some embodiments, the chemicals are proteins (thioredoxin, glutaredoxin). In some embodiments, the proteins are enzymes (disulfide isomerase).

E. SUGARS

[00124] In some compositions the consumable can also comprise sugars. For example the consumable can comprise: monosaccharides, including but not limited to glucose (dextrose), fructose (levulose), galactose, xylose and ribose, disaccharides including but not limited to sucrose, lactose, and maltose, sugar alcohols, and/or polysaccharides, including plant cell wall degradation products, including, dextran, raffinose, saccharose, trehalose, β -galactosides, β-glucosides, and melibiose and cellobiose.

[00125] The consumable can comprise sugars, sugar alcohols, or polysaccharides. The consumable can comprise plant cell wall degradation products. The consumable can comprise: arabitol, D- and L-xylose, dextran, raffinose, saccharose, mannose, galactose, arabinose, xylose, ribose, trehalose or mannitol, β -galactosides, β-glucosides, galacturonate, glucuronate, gluconate, stachyose, melibiose, salicin, cellobiose, N-acetylglucosamine, dulcitol, and/or sorbitol.

F. GEL FORMATION

[00126] The components of the composition can be formed into a gel. Gels can be formed using a variety of methods. Gels can be stabilized entirely by physical cross-links between the components. For example, gels can be produced by heat/cool cycles, in which case the gel is stabilized by physical interactions (entanglements, hydrophobic interactions) between protein molecules. In some embodiments, the gel is formed by heating a protein solution to a temperature of at least 100°C, 90°C, 80°C, 70°C, 60°C, 50°C, 45°C, 40°C and then cooling to room temperature, or to temperature below 40°C.

[00127] 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 pH of the main protein component by adding hydrochloric acid, or other acids, or sodium hydroxide, or other bases.

[00128] Gels can be also 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 concentrated sodium hydroxide solution. In other examples, protein powder can be soaked in mixed water/ethanol solutions.

[00129] Gels can comprise or be produced (e.g. stabilized by) at least in part by a cross-linking enzyme. A gel can be stabilized by protein cross-links introduced by action of cross-linking enzymes on protein. The cross-linking enzyme can be, for example, transglutaminases, tyrosinases, lipoxygenases, protein disulfide reductases, protein disulfide isomerases, sulfhydryl oxidases, peroxidases, hexose oxidases, lysyl oxidases, and amine oxidases.

[00130] In some cases gels can comprise chemicals that promote formation of inter-molecular disulfide cross-links. In some embodiments, the chemicals are proteins (thioredoxin, glutaredoxin). In some embodiments, the proteins are enzymes (disulfide isomerase).

[00131] 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.

[00132] In some embodiments, more than one of these approaches are used in combination. For example, transglutaminase cross-linked gel can be further stabilized by heat/cool treatment.

[00133] In some embodiments, gels comprise protein, where the protein is derived from non-animal source. In some embodiments, the non-animal source is a plant source. In some embodiments, the non-animal source is a genetically modified yeast or bacteria. In some embodiments, the one or more isolated proteins account for 50% or more of the protein content by weight. In some embodiments, the one isolated and purified protein accounts for 90% or more of the protein content by weight. In some embodiments, each of the one or more isolated proteins is isolated and purified separately.

G. MUSCLE REPLICAS

[00134] 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.

[00135] 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.

[00136] 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.

[00137] 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, transglutaminases, tyrosinases, lipoxygenases, protein disulfide reductases, protein disulfide isomerases,

sulfhydryl oxidases, peroxidases, hexose oxidases, lysyl oxidases, and amine oxidases.

[00138] 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 fat tissue. The list of potential candidates here is essentially open and may include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microorganisms, or synthesized in vitro. The proteins may be artificially designed to emulate physical properties of animal muscle tissue. In some embodiments, one or more isolated, purified proteins accounts for about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component by weight. In some embodiments, one or more isolated, purified proteins accounts for about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein content of a consumable.

[00139] Skeletal muscle of animals such as beef cattle typically contain substantial quantities of glycogen, which can comprise on the order of 1% of the mass of the muscle tissue at the time of slaughter. After slaughter, a fraction of this glycogen continues to be metabolized yielding products including lactic acid, which contributes to lowering the pH of the muscle tissue, a desirable quality in meat. Glycogen is a branched polymer of glucose linked together by alpha (1->4) glycosidic bonds in linear chains, with branch points comprising alpha (1->6) glycosidic bonds. Starches from plants, particularly amylopectins are also branched polymers of glucose linked together by alpha (1->4) glycosidic bonds in linear chains, with branch points comprising alpha (1->6) glycosidic bonds and can therefore be used as an analog of glycogen in constructing meat replicas. Thus in some embodiments, the muscle or meat replica includes a starch or pectin.

[00140] Additional components of animal muscle tissue include sodium, potassium, calcium, magnesium, other metal ions, lactic acid, other organic acids, free amino acids, peptides, nucleotides and sulfur compounds. Thus, in some embodiments, the

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

[00141] The invention also provides methods for making a muscle replica. In some embodiments, method comprising 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 in US6379738, US3693533, US20120093994, which are herein incorporated by reference. These methods can be applied to making the compositions provided herein.

[00142] 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 is used to limit denaturation of proteins. Protein feed and liquid are added separately: protein is fed 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 50°C, feed rate of 2.3 g/min, and water-flow rate of 11 g/min. Product temperature at the die during extrusion is measured by a thermocouple.

[00143] 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 it 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 other examples, the

bath is filled with a buffer solution at 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.

[00144] 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 cm2, 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

[00145] 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 recapitulates key features of animal fat. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources, which recapitulates animal fat. Such a composition will be labeled herein as a "fat replica". In some embodiments, the fat replica and/or meat substitute product comprising the fat replica are partially derived from animal sources. The consumable can also include fat replica's which recapitulate key features of non-animal fats.

[00146] In some embodiments the consumable has a fat component. In some embodiments the fat content of the consumable is 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, 80%, or 90% fat.

[00147] 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. 1-3 isolated and purified proteins, wherein the solution accounts for 10-70% of the volume of the emulsion. The gelled emulsion can comprise fat droplets, wherein the droplets accounts for 30-90% of the volume of the emulsion. The gelled emulsion can comprise an isolated, purified cross-linking enzyme, wherein the cross-linking

enzyme accounts for 0.5-5% of the emulsion by wt/volume. The fat droplets can be emulsified in the protein solution, wherein the emulsion can be formed into a gel by the cross-linking enzyme. The cross-linking enzyme can be, for example transglutaminase.

[00148] The gelled emulsion can comprise a protein solution comprising multiple proteins (e.g. 1-3 isolated and purified proteins), wherein the 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 0.5-5% of the emulsion weight by volume. 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. The gelled emulsion can comprise an isolated, purified cross-linking enzyme, wherein the cross-linking enzyme accounts for 0.001% or lower of the emulsion weight by volume. The stability of 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. transglutaminase, by gelling proteins via heating and cooling protein solutions, and by combination of heating/cooling and cross-linking enzyme.

[00149] The proteins used in the gel can be plant or microbial proteins. In some embodiments, the proteins used in the fat replica might include Rubisco, oleosins, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microorganisms, or synthesized in vitro. The proteins may be artificially designed to emulate physical properties of animal fat. The proteins may be artificially designed to emulate physical properties of animal fat.

[00150] 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. In some embodiments, the sources are plant sources. Non-limiting examples of oils include corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, margarine, hydrogenated vegetable oil, rice bran oil, oils produced by

bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phophatidic acids, lysophosphatidic acids, oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid. In some embodiments, the fat droplets are derived from animal products (e.g. butter, cream, lard, and suet).

[00151] In some embodiments, fat droplets are derived from pulp or seed oil. In other embodiments, the source may be algae, yeasts, oleaginous yeasts or mold. For instance, in one embodiment the fat droplets comprise triglycerides derived from Mortierella isabellina.

[00152] In some embodiments, the fat droplets are the cream fraction created from seeds, nuts, and legumes, including but not limited to sunflowers, safflower, sesame seeds, rape seeds, almonds, macadamia, grapefruit, lemon, orange, watermelon, pumpkin, cocoa, coconut, mango, butternut squash and pistachios.

[00153] 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.

[00154] In some embodiments, the fat droplets comprise a mixture selected from the group of surfactants, plant oils, microbial oils, animal fats, seed cream fractions and isolated proteins.

[00155] The fat droplets can in some examples range from 100nm, to 150um in diameter. The diameter of these stabilized droplets may be obtained by homogenization, high-pressure homogenization, extrusion or sonication.

[00156] In some embodiments plant oils are modified to resemble animal fats. The plant oils can be modified with flavoring or other agents to recapitulate the taste and smell of meat during and after cooking. Accordingly, some aspects of the invention involve methods for testing the qualitative similarity between the cooking properties of animal fat and the cooking properties of plant oils in the consumable.

[00157] The fat replica can comprise a protein component comprising one or more isolated, purified proteins. The purified proteins contribute to the taste and texture of the meat replica. In some embodiments purified proteins can stabilize emulsified fats. In some embodiments the purified proteins can form gels upon denaturation or enzymatic crosslinking, which replicate the appearance and texture of animal fat. Examples and embodiments of the one or more isolated, purified proteins are described herein. In some embodiments, the one or more isolated proteins comprise a protein isolated from the legume family of plants. Non-limiting examples of legume plants are described herein, although variations with other legumes are possible. In some embodiments, the legume plant is a pea plant. In some embodiments the isolated purified proteins stabilize emulsions. In some embodiments the isolated purified proteins form gels upon crosslinking or enzymatic crosslinking. In some embodiments, the isolated, purified proteins comprise seed storage proteins. In some embodiments, the isolated, purified proteins comprise albumin. In some embodiments, the isolated, purified proteins comprise globulin. In a embodiment, the isolated, purified protein is a purified pea albumin protein. In another embodiment, the isolated, purified protein is a purified pea globulin protein. In another embodiment the isolate purified protein is a Moong bean 8S globulin. In another embodiment, the isolated, purified protein is an oleosin. In another embodiment, the isolated, purified protein is a caloleosin. In another embodiment, the isolated, purified protein is Rubisco. In some embodiments, the protein component

comprises about 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or more of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises about 0.1-5%, about 0.5-10%, about 1-20%, about 5-30%, about 10-50%, about 20-70%, or about 30-90% or more of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises a solution containing one or more isolated, purified proteins.

[00158] In some embodiments, the fat replica comprises cross-linking enzymes that catalyze reactions leading to covalent crosslinks between proteins. Cross-linking enzymes can be used to create or stabilize the desired structure and texture of the adipose tissue replica, to mimic the desired texture of an equivalent desired animal fat. Non-limiting examples of cross-linking enzymes include, e.g., transglutaminase, lysyl oxidases, or other amine oxidases (e.g. Pichia pastoris lysyl oxidase). In some embodiments, the cross-linking enzymes are isolated and purified from a non-animal source, examples and embodiments of which are described herein. In some embodiments, the fat replica comprises at least 0.0001%, or at least 0.001%, or at least 0.01%, or at least 0.1%, or at 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.

[00159] In another aspect, the invention provides methods for making a fat replica. In some embodiments, the fat droplets are suspended in a gel. In some embodiments the present invention provides for methods for producing droplets of fat suspended in the gel. The fat can isolated and homogenized. For example an organic solvent mixture can be used to help mix a lipid. The solvent can then be removed. At this point the lipid can be frozen, lyophilized, or stored. So in some aspects the invention provides for a method for isolating and storing a lipid, which has been selected to have characteristics similar to animal fat. The lipid film or cake can then be hydrated. The hydration can utilize agitation or temperature changes. The hydration can occur in a precursor solution to a gel. After hydration the lipid suspension can be sonicated,

homogenized, high-pressure homogenized or extruded to further alter the properties of the lipid in the solution.

[00160] In some embodiments, the fat replica is assembled to approximate the organization of adipose tissue in meat. In some embodiments some or all of the components of the fat replica are suspended in a gel. In various embodiments the gel can be a proteinaceous gel, a hydrogel, an organogel, or a xerogel. In some embodiments, the gel can be thickened to a desired consistency using an agent based on polysaccharides or proteins. For example fecula, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, alginin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, cellulose, methylcellulose, hydroxymethylcellulose, acadia gum, konjac, starch, pectin, amylopectin or proteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, of fungi can be used alone or in combination to thicken the gel, forming an architecture or structure for the consumable.

[00161] 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 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

[00162] The fat replica can be an emulsion comprising a solution of one or more proteins and one or more fats suspended therein as droplets. In some embodiments, the emulsion is stabilized by one or more cross-linking enzymes into a gel. In some embodiments, the emulsion is stabilized by tissue matrix formed by proteins during heat/cool induced protein denaturation. In some embodiments, the emulsion is stabilized by tissue matrix formed by a combination of one or more protein cross-linking enzymes and heat/cool induced protein denaturation. The one or more proteins in solution can comprise isolated, 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-based oils. The one or more fats can be derived from one or more of: corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phophatidic acids, lysophosphatidic acids, oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid. In some cases the one or more fats is a rice bran oil. In some cases the one or more fats is a canola oil. In some cases the composition comprises, the cross-linking enzyme: transglutaminase, lysyl oxidase, or other amine oxidase. 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 partially-purified moong bean 8S globulin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In some

embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% canola oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In 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 0.0001-1% (wt/vol) transglutaminase into a gel. In some embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% rice bran oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel. In some embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% canola oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel.

[00163] The fat can be suspended in a gel. In some embodiments the present invention provides for methods for producing droplets of fat suspended in the proteinaceous gel. The fat can be isolated from plant tissues and emulsified. The emulsification can utilize high-speed blending, homogenization, high pressure homogenization, 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. In fascia-type connective tissue, the prolamin family of proteins, individually or combinations thereof, demonstrates suitability for the protein component because they are highly abundant, similar in global amino acid composition to collagen (high fraction of proline and alanine), and amenable to processing into films. In addition to zein (found in corn), these include hordein from barley, gliadin from wheat, secalin, extensions from rye, kafirin from sorghum, avenin from oats. Other proteins may be necessary to supplement prolamins in order to achieve targets specifications for physicochemical and nutritional properties. The list of potential candidates here is essentially open and may include any major seed storage proteins, animal-derived or

recombinant collagen, extensins (hydroxyproline-rich glycoproteins abundant in cell walls e.g. Arabidopsis thaliana, monomers of which are "collagen-like" rod-like flexible molecules).

[00164] In one embodiment, the firmness of fat replica formed by stabilizing protein oil emulsion can be modified by varying amount of protein in the fat replica. In one embodiment, fat tissue made from protein Rubisco and 70% sunflower oil is softer at lower concentration of Rubisco than at higher concentration of Rubisco in fat tissue.

[00165] 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, increasing 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.

[00166] Another important property of meat fat is that 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 in phosphatidylcholine and phosphatidylethanolamine in fat provide a more intense beef flavor.

[00167] 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.

[00168] By controlling the melting point of fat tissue replicas in the consumables, it is possible to replicate the cooking experience of different meat types. For example, fat tissue replicas created from fats with a melting point of 23°C to 27°C can have melting points analogous to fat tissue from wagyu beef; fat tissue replicas created from fats with a melting point of 35°C to 40°C can have melting points analogous to fat tissue from regular ground beef; and fat tissue replicas created from fats with a melting point of 36°C to 45°C can have melting points analogous to fat tissue from bacon. Fat tissue replicas can be created and in incorporated into consumables such that a ratio of fat which is released and the ratio of fat which is retained by fat tissue replica during cooking is similar to the fat properties of meat, e.g. from ground beef.

[00169] By controlling melting point of fat in fat tissue replica firmness of fat tissue replica at refrigeration and ambient temperature can be controlled. For example, fat tissue replicas containing triglycerides and phospholipids which are liquid at room temperature will be softer than fat tissue replicas containing triglycerides and phospholipids which are solid at refrigeration and ambient temperatures. In one embodiment, fat tissue replica containing triglycerides and phospholipids with high ratio of saturated fatty acids will be firmer that fat tissue containing higher ratio of monounsaturated and polyunsaturated triglycerides and lipids.

[00170] For example, fat tissue replica containing fat as sunflower oil is softer than fat tissue replica containing fat as cocoa butter.

[00171] In one embodiment, fat tissue replica made as stable emulsion of Rubisco with sunflower oil is softer than fat tissue replica made as stable emulsion of Rubisco and cocoa oil. Fat tissue replicas were formed with 0%, 0.18%, 1.6%, and 2.4% wt/v Rubisco with 70%, 80%, and 90% v/v sunflower or cocoa oils. Each fat tissue replica that contained cocoa oil is firmer than the replicas that were formed with sunflower oil.

[00172] In one embodiment, fat tissue replica made as stable emulsion of Moong Bean 8S protein with sunflower oil is softer than fat tissue replica made as stable emulsion of Moong Bean 8S protein and cocoa oil. Fat 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 oils. Each fat tissue replica that contained cocoa oil is firmer than the replicas that were formed with sunflower oil.

[00173] In one embodiment, fat tissue replica made as stable emulsion of Moong Bean 8S protein of with canola oil is softer than the fat tissue replica made as stable emulsion made of Moong Bean 8S protein with an equal mixture of coconut, cocoa, olive, and palm oils. Fat 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 fat tissue replica that contained mixture of oils is firmer than the replicas that were formed with sunflower oil.

[00174] In one embodiment, fat tissue replica made as stable emulsion of Soy proteins with sunflower oil is softer than the fat tissue replica made as stable emulsion of Soy proteins and cocoa oil. Fat 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 fat tissue replica that contained a mixture of oils is firmer than the replicas that were formed with sunflower oil.

CONTROL OF MELTING POINT BY CONTROL OF FAT COMPOSITION

[00175] Melting temperature of a fat mixture can be controlled by mixing triglycerides and phospholipids whose fatty acids have different chain lengths and chain saturations at different ratios. Melting temperature of fats is governed by chemical composition of fatty acids. In general fats comprising saturated fatty acids (for ex, C10:0, C12:0, C14:0, C16:0, C18:0, C20:0, C22:0) are solid at refrigeration and room temperature. Fats comprising monounsaturated fatty acids (for ex. C16:1, C18:1) are in general solid at refrigeration temperatures and liquid at room temperatures . Fats comprising polyunsaturated fatty acids (for ex. C18:3, C20:5, C22:6) are liquid at refrigeration and room temperatures. For example, virgin coconut oil melts at about 24 °C while hydrogenated coconut oil melts at 36-40 °C.

[00176] Fat tissue replicas can contain oils from a single or multiple sources that are liquid at refrigeration and ambient room temperatures. For example, oil can be canola oil, sunflower oil or hazelnut oil. In one embodiment, fat tissue replica contains oils from single or multiple sources that are solid at refrigeration temperature, but liquid at room temperature. For example, oil can be olive oil, palm oil, rice brain oil. In one embodiment, fat tissue replica contains oils from single or multiple sources that are solid at room temperature but liquid at mouth-temperatures. For example, oil can be palm kernel oil, coconut oil, cocoa oil. In one embodiment, fat tissue replica contains oils from single or multiple sources that are solid at mouth temperature (about 37 °C). For example, fat tissue replica contains oil from mango butter.

[00177] In some embodiments, the fat tissue replicas comprising 0%, 0.18%, 1.6%, and 2.4% wt/v Rubisco with 70%, 80%, and 90% v/v cocoa oil are solid at room temperature but melt at about mouth temperature. In some embodiments, the fat tissue replicas comprising 0.6%, 1.6%, and 2.6% wt/v Soy with 50%, 70%, 80%, and 90% v/v cocoa oil are solid at room temperature but melt at about to mouth temperature. In some embodiments, the fat 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 fat 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 fat tissue replica contains equal amounts of cocoa and mango butters. In some embodiments, the fat tissue replica contains equal amounts of coconut oil, cocoa butter, olive oil and palm oil.

[00178] In one embodiment, the fat tissue replica that comprises triglycerides and phospholipids will contain a ratio of fatty acids similar to one 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%, C20:6 0-2%). For example the fat tissue replica may comprise equal proportions of olive oil, cocoa oil, coconut oil and mango butter. In another example, the fat tissue replica may comprise equal proportions of olive oil and rice brain oil.

[00179] In one embodiment, the melting temperature of fat 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. In some embodiments, the fat tissue replica contains equal amounts of olive oil, rice bran oil, cocoa butter and mango butter.

CONTROL OF MELTING POINT BY CONTROL OF PROTEIN CROSSLINKING.

[00180] The melting temperature of fat replicas may be controlled by manipulation of the structural organization of the fat tissue matrix. In one embodiment, the fat tissue matrix formed by heat/cool denaturation of a preformed protein-oil emulsion, where the protein is Rubisco and the oil is cocoa butter, has a higher melting temperature than a fat replica of similar composition stabilized by cross-linking enzyme.

CONTROL OF FLAVOR BY CONTROL OF PHOSPHOLIPID COMPOSITION

[00181] 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 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 ratio of different phospholipid headgroups.

[00182] In some embodiments, the phospholipids comprise a plurality of amphipathic molecules comprising fatty acids, glycerol and polar groups. In some embodiments, the fatty acids are oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, and/or conjugated oleic acid. In some embodiments, the fatty acids are polyunsaturated fatty acids (ex. 20:4, 20:5, 20:6).

[00183] In some embodiments, the polar groups are choline, ethanolamine, serine, phosphate, glycerol-3-phosphate, inositol and/or inositol phosphates. In some embodiments the sterols associated with the phospholipids are stigmasterol, sitosterol., campesterol, brassicasterol, sitostanol, campestanol, ergosterol, zymosterol, fecosterol, dinosterol, lanosterol, cholesterol and/or episterol. In some embodiments the lipids associated with the phospholipids are sphingolipids, ceramides, sphingomyelins, cerebrosides, gangliosides, ether lipids, plasmalogens or pegylated lipids.

[00184] In some embodiments, the oils are corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower

oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, margarine, hydrogenated vegetable oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, and/or lecithin.

CONTROL OF HOW MUCH FAT IS RELEASED DURING COOKING BY CONTROL OF THE CROSSLINKED PROTEIN MATRIX

[00185] The ratio of fat that is released or retained during the process of cooking contributes to the experience of cooking and eating meat. In one embodiment, the ratio of released and retained fat can be controlled by composition of protein(s), which form the fat tissue matrix. In one embodiment, the tissue matrix is formed from Moong Bean 8S protein. In another embodiment tissue matrix is formed from Moong Bean 8S and sunflower oleosin. In yet another embodiment, tissue matrix is formed by Rubsico.

[00186] In some embodiments, ratio of released and retained fat during cooking can be controlled by arrangement and structural organization of proteins and fat within fat tissue matrix. In some embodiments, fat tissue replica matrix stabilized by crosslinking enzymes releases more fat than fat tissue replica matrix stabilized by heat/cool protein denaturation. some embodiments fat tissue matrix is made from Moong Bean 8S protein. In one embodiment, fat tissue matrix comprised of Moong Bean 8S protein with canola oil retains more mass when formed upon heat/cool denaturation than when formed by cross-liking with an enzyme.In one embodiment, fat tissue replica comprised of Moong Bean 8S protein with an equal mixture of coconut, cocoa, olive, and palm oils retains more mass when formed upon heat/cool denaturation than when formed by cross-liking with an enzyme.

[00187] In one embodiment, fat tissue matrix formed with 2% w/v Rubisco and 50%, 70%, and 90% v/v cocoa oil, with and without Oleosin, retains more mass when formed upon heat/cool denaturation than when formed by cross-liking with an enzyme. In one embodiment, fat tissue matrix formed with Rubisco and sunflower oil, with and without Oleosin, retains more mass when formed upon heat/cool denaturation than when formed by cross-liking with an enzyme. In one embodiment,

ratio of released and retained fat during cooking can be controlled by arrangement and organization of fat tissue matrix, where matrix properties are controlled by amount of protein present in the tissue matrix (wt/wt). In one embodiment, the amount of released fat in fat tissue matrix formed by Rubisco protein decreases with increased Rubisco concentration (wt/wt) in the fat replica. A series of fat tissue replicas formed with varying concentrations of Rubisco including 0%, 1.6%, and 2.4% wt/v Rubisco with 70% v/v sunflower oil, retained more mass in a fat tissue gel after cooking as the concentration of Rubisco increased. In one embodiment, amount of intact tissue replica remaining after cooking can be controlled by arrangement and organization of fat tissue matrix, where matrix properties are controlled by concentration of a particular protein (wt/wt).

[00188] A series of fat tissue replicas constructed from 1.4% wt/v Moong Bean 8S protein with 90% v/v canola oil and 0.45% wt/v soybean lecitin, can be homogenized and increasing concentration of sunflower oleosins added to emulsion. Concentration of oleosins can be varied from 1:10 to $1:10^6$ molar (oleosin:triglyceride, molar ratio). An increase in mass retention after cooking is observed when the concentration of oleosins in fat tissue replica is higher.

[00189] Firmness of fat tissue replica constructed as stabilized protein-fat emulsion increases can be modified by varying concentration of the protein within fat tissue replica matrix. For example, a series of fat tissue replicas formed with varying concentrations of Rubisco with 70-80% v/v sunflower oil, varied in firmness. Fat tissue replicas with 0% and 0.18% (wt/vol) Rubisco were very soft, with 1.6% (wt/vol) Rubisco soft, and with 1.9% (wt/vol) was medium firmness.

[00190] One limitation of meat is that the composition and properties of the tissue are defined by the nature. The art of cooking has been defined by these properties. The ability described above, to provide a meat replica with defined cooking characteristics will allow the production of meat replicas that are optimized for particular cooking techniques. For example, stews require slow cooking to gelatinize tha connective tissue in the meat, whereas meat replicas can be designed wherein the connective tissue replica is more easily gelatinised thus allowing stews to be prepared quickly.

[00191] A fat 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.

I. CONNECTIVE TISSUE REPLICA

[00192] 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.

[00193] Animal connective tissue can generally be divided into fascia-type and cartilage-type tissue. Fascia-type tissue is highly fibrous, resistant against extension (has high elastic modulus), and has a high protein content, a moderate water content (ca. 50%), and low-to-none fat and polysaccharide content. Accordingly, the present invention provides a connective tissue replica that recapitulates key features of fascia type tissue. In some embodiments, the connective tissue replica comprises about 50% protein by total weight, about 50% by liquid weight, and has a low fat and polysaccharide component.

[00194] 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 micron wide. These fibers consist of closely packed thin collagen fibrils 30 to 100 nanometer thick. These fibrils also associate into elastic and reticular fibrous networks with individual fibers that may be 200 nanometer thick.

[00195] In one embodiment, the fascia-type connective tissue replica consists of fibrous or fibrous-like structure which can consist of proteins. In some embodiments, the protein content is derived from non-animal source. In some embodiments, the non-animal source is a plant source, genetically modified yeast or bacteria. In some embodiments, the isolated proteins account for 50%, 60%, 70% 80%, 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.

[00196] The protein used to make the connective tissue replica can be a prolamin family protein. 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, avenin from oats. In some embodiments, the one or more isolated and purified proteins is zein.

[00197] 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. 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.

[00198] 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 will be used to create mixed fibrous tissue 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 compotisions.

[00199] Electrospinning can be used to create fibers in <1-10 micron range. In some embodiments, electrospinning is used to create fibers in <1-10 micron diameter range. For example , concentrated solution of moong bean globulin (140 mg/ml) containing 400 mM sodium chloride is 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 ul/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 a 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.

[00200] 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.

[00201] 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).

[00202] Compositionally, cartilage-type connective tissue will be very similar to fascia-type tissue with the relative ratios of each adjusted to more closely mimic 'meat' connective tissue.

[00203] Methods for forming cartilage-type connective tissue will be similar to those for fascia-type connective tissue, but with a bias towards methods producing isotropic non-fibrous gels.

[00204] 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.

[00205] In fascia-type connective tissue, the prolamin family of proteins, individually or combinations thereof, demonstrates suitability for the protein component because they are highly abundant, similar in global amino acid composition to collagen (high

fraction of proline and alanine), and amenable to processing into films. In addition to zein (found in corn), these include hordein from barley, gliadin from wheat, secalin, extensions from rye, kafirin from sorghum, avenin from oats. Other proteins may be necessary to supplement prolamins in order to achieve targets specifications for physicochemical and nutritional properties. The list of potential candidates here is essentially open and may include any major seed storage proteins, animal-derived or recombinant collagen, extensins (hydroxyproline-rich glycoproteins abundant in cell walls e.g. Arabidopsis thaliana, monomers of which are "collagen-like" rod-like flexible molecules).

[00206] In some embodiments some or all of the components of the consumable are suspended in a gel. In various embodiments the gel can be a hydrogel, an organogel, or a xerogel, The gel can be made thick using an agent based on polysaccharides or proteins. For example fecula, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, alginin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, cellulose, methylcellulose, hydroxymethylcellulose, acadia gum, konjac, starch, pectin, amylopectin or proteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, of fungi can be used alone or in combination to thicken the gel, forming an architecture or structure for the consumable. Enzymes that catalyze reactions leading to covalent crosslinks between proteins can also be used alone or in combination to form an architecture or structure for the consumable. For example 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. In some embodiments multiple gels with different components are combined to form the consumable. For example a gel containing a plant-based protein can be associated with a gel containing a plant-based fat. In some embodiments fibers or stings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

[00207] 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.

[00208] In some embodiments multiple gels with different components are combined to form the consumable. For example a gel containing a plant-based protein can be associated with a gel containing a plant-based fat. In some embodiments fibers or strings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

J. OMISSIONS FROM THE COMPOSITIONS

[00209] 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 do not have certain ingredients that may be not desirable to consumers, for example proteins that some humans are allergic to can be omitted. Additionally, some additives typically added to the consumable can be omitted from the compositions. 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. In some embodiments the consumable contains no 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.

[00210] In some embodiments the consumable contains: no animal products; no methylcellulose; no carrageenan; no Konjac flour; no gum Arabic; no acacia gum; 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, 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.

[00211] In some embodiments, the muscle tissue replica additionally contains less than 1% wheat gluten. In some embodiments, the muscle tissue replica additionally contains less than 5% wheat gluten. In some embodiments, the muscle tissue replica additionally contains less than 10% wheat gluten. In some embodiments, the muscle tissue replica additionally contains 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

[00212] 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, fat tissue replica, and/or connective tissue replica can be assembled in a manner that approximates the physical organization of meat.

[00213] The percentage of different components may also be controlled. For example non-animal-based substitutes for muscle, fat tissue, connective tissue, and blood components can be combined in different ratios and physical organizations to best approximate the look and feel of meat. The various components can be arranged to insure consistency between bites of the consumable. The components can be arranged to insure that no waste is generated from the consumable. For example, while a traditional cut of meat may have portions that are not typically eaten, a meat replicate can improve upon meat by not including these inedible portions. Such an improvement allows for all of the product made or shipped to be consumed, which cuts down on waste and shipping costs. Alternatively, a meat replica may include

inedible portions to mimic the experience of meat consumption. Such portions can include bone, cartilage, connective tissue, or other materials commonly referred to as gristle, or materials included simulating these components. In some embodiments the consumable may contain simulated inedible portions of meat products which are designed to serve secondary functions. For example a simulated bone can be designed to disperse heat during cooking, making the cooking of the consumable faster or more uniform than meat. In other embodiments a simulated bone may also serve to keep the consumable at a constant temperature during shipping. In other embodiments, the simulated inedible portions may be biodegradable.

[00214] In some embodiments the meat substitute compositions contains no animal protein, comprising between 10-30% protein, between 5-80% water, between 5-70% fat, comprising one or more isolated purified proteins. In some embodiments, the meat substitute compositions comprise transglutaminase.

[00215] In some embodiments of the meat substitute product, the muscle replica accounts for 40-90% of the product by weight, the fat 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.

[00216] In some embodiments, the meat substitute product comprises 60-90% water; 5-30% protein content; and 1-20% of a fat or fat replica; wherein the protein content comprises one or more isolated, purified plant proteins.

[00217] 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 can comprise roughly 75% water, 19% protein, 2.5% fat, 1.2% carbohydrates; and 2.3 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 60-90% water, 10-30% protein, 1-20%

fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 60-90% water, 5-10% protein, 1-20% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 0-50% water, 5-30% protein, 20-80%% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments, the replica contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the replica contains between 0.01% and 5% by weight of leghemoglobin. Some meat also contains myoglobin, a heme protein, which accounts for most of the red color and iron content of some meat. It is understood that these percentages can vary in meat and the meat replicas can be produced to approximate the natural variation in meat. Additionally, in some instances, the present invention provides for improved meat replicas, which comprise these components in typically unnatural percentages. The concentration of heme 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.

[00218] 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 oligosaccharidepercentages of components similar to meat cooked over a fire.

[00219] In some embodiments the consumable is a meat replica with a lower that typical water content for meat. In some embodiments the inventions provides for methods for hydrating a meat replica to cause the meat replica to have a content similar to meat. For example a meat replica with a water content that would be low

for meat, for example 1%, 10%, 20%, 30%, 40% or 50% water, is hydrated to roughly 75% water. Once hydrated, in some embodiments, the meat replica is then cooked for human consumption.

[00220] 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.

[00221] 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. 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.

[00222] 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.

[00223] 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 fat 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.

[00224] The physical organization of the meat substitute product can be manipulated by controlling the localization, organization, assembly, or orientation of the muscle, fat, and/or connective tissue replicas described herein. In some embodiments the product is designed in such a way that the replicas described herein are associated with one another as in meat. In some embodiments the consumable is designed so that after cooking the replicas described herein are associated with one another as in cooked meat. In some embodiments, one or more of the muscle, fat, and/or connective tissue replicas are combined in a manner that recapitulate the physical organization of different cuts or preparations of meat. In an example embodiment, the replicas are combined in a manner that approximates the physical organization of natural ground meat. In other embodiments, the replicas are combined in a manner that approximates different cuts of beef, such as, e.g., ribeye, filet mignon, London broil, among others.

[00225] 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.

[00226] In one aspect, the invention provides a meat substitute product (alternatively referred to herein as "consumable") that is substantially or entirely composed of ingredients derived from non-animal sources, yet recapitulates key features associated with the cooking and consumption of an equivalent meat product derived from animals. The equivalent meat product can be a white meat or a dark meat. The equivalent meat product can be derived from any animal. Non-limiting examples of animals used to derive the equivalent meat product include farmed animals such as,

e.g., cattle, sheep, pig, chicken, turkey, goose, duck, horse, dog or game animals (whether wild or farmed) such as, e.g., rabbit, deer, bison, buffalo, boar, snake, pheasant, quail, bear, elk, antelope, pigeon, dove, grouse, fox, wild pig, goat, kangaroo, emu, alligator, crocodile, turtle, groundhog, marmot, possum, partridge, squirrel, raccoon, whale, seal, ostrich, capybara, nutria, guinea pig, rat, mice, vole, any variety of insect or other arthropod, seafood such as, e.g, fish, crab, lobster, oyster, mussel, scallop, abalone, squid, octopus, sea urchin, tunicate and others. Many meat products are typically derived from skeletal muscle of an animal but it is understood that meat can also come from other muscles or organs of the animal. In some embodiments, the equivalent meat product is a cut of meat derived from skeletal muscle. In other embodiments, the equivalent meat product is an organ such as, e.g., a kidney, heart, liver, gallbladder, intestine, stomach, bone marrow, brain, thymus, lung, tongue. Accordingly, in some embodiments the compositions of the present invention are consumables similar to skeletal muscle or organs.

[00227] In some aspects, the present invention provides meat substitute products comprising one or more of a first composition comprising a muscle tissue replica, a second composition comprising an adipose tissue replica, and/or a third composition comprising a connective tissue replica, wherein the one or more compositions are combined in a manner that recapitulates the physical organization of meat. In other aspects, the present invention provides compositions for a muscle tissue replica (herein referred to as "muscle replica"), an adipose tissue replica (herein referred to as "fat replica"), and a connective tissue replica (herein referred to as "connective tissue replica"). In some embodiments, the compositions and meat substitute products are principally or entirely composed of ingredients derived from non-animal sources. In alternative embodiments, the muscle, fat, and/or connective tissue replica, or the meat substitute products comprising one or more of the replicas, are partially derived from animal sources but supplemented with ingredients derived from non-animal sources. In yet other alternative embodiments, the invention provides meat products substantially derived from animal sources but which are supplemented with one or more of a muscle tissue replica, a fat replica, and/or a connective tissue replica, wherein 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 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.

[00228] The physical organization of the meat substitute product can be manipulated by controlling the localization, organization, assembly, or orientation of the muscle, fat, and/or connective tissue replicas described herein. In some embodiments the product is designed in such a way that the replicas described herein are associated with one another as in meat. In some embodiments the consumable is designed so that after cooking the replicas described herein are associated with one another as in cooked meat. In some embodiments, one or more of the muscle, fat, and/or connective tissue replicas are combined in a manner that recapitulate the physical organization of different cuts or preparations of meat. In an example embodiment, the replicas are combined in a manner that approximates the physical organization of natural ground meat. In other embodiments, the replicas are combined in a manner that approximates different cuts of beef, such as, e.g., ribeye, filet mignon, London broil, among others.

[00229] In some embodiments the individual tissue replicas are assembled in layers, sheets, blocks and strings in defined positions and orientations.

[00230] 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.

[00231] In some embodiments, the replicas are combined in the process of passing through the plates of a meat grinder with the holes set at 1/4 inch.

[00232] In some embodiments the individual tissue replicas are formed into small pieces less than 1 cm in diameter before combination with the other tissues.

[00233] In some embodiments the individual tissue replicas are formed into small pieces less than 5 mm in diameter before combination with the other tissues.

[00234] In some embodiments, the connective tissue replica is incorporated into the protein solution before formation of the muscle tissue replica.

[00235] In some embodiments, the connective tissue replica is directly incorporated into the emulsion before formation of the fat tissue replica.

[00236] In some embodiments, the fat tissue replica is added to the muscle tissue replica in strands and sheets to replicate the effect of "marbling".

[00237] In some embodiments, the fat tissue replica is added to the muscle tissue replica in strands and sheets to replicate streaky bacon.

[00238] 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, cocnut/woody/sweet, penetrating/sickening, minty, toasty caramel aromas.

[00239] In some embodiments the mixed meat replicas increase the presence of volatile odorants, such as 2-pentyl-furan; 4-Methylthiazole; Pyrazine, ethyl; Pyrazine, 2,3-dimethyl; 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-H-Pyrrole-2-2carboxaldehyde, 1-methyl; caprolactam; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl. In some embodiments, said 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, said individual tissue replicas increase the presence of volatile odorants including but not limited to Nonane, 2,6-dimethyl, 3-Hexene, 3-methyl; pyridine; Acetoin; Octanal; 2-Propanone, 1-hydroxy; Ethenyl pyrazine. 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).

[00240] In some embodiments, said 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, cocnut/woody/sweet,

penetrating/sickening, minty, toasty caramel aromas. In some embodiments said mixed meat replicas increase the presence of volatile odorants including but not limited to 2-pentyl-furan; 4-Methylthiazole; Pyrazine, ethyl; Pyrazine, 2,3-dimethyl; 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-H-Pyrrole-2-2carboxaldehyde, 1-methyl; caprolactam; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl. In some embodiments, said 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, said individual tissue replicas increase the presence of volatile odorants including but not limited to Nonane, 2,6-dimethyl, 3-Hexene, 3-methyl; pyridine; Acetoin; Octanal; 2-Propanone, 1-hydroxy; Ethenyl pyrazine. 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).

[00241] The production of volatile odorants can be enhanced when the fat, muscle and connective tissues are contacting one another. In some embodiments, said 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 5 mm. In some embodiments, said 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 tissues are intimately mixed with mean size of the individual tissue replicas of 1 mm.

[00242] In some embodiments, the meat substitute is optimized for particular cooking methods.

[00243] In some embodiments, the meat substitute is optimized for cooking in a microwave oven.

[00244] In some embodiments, the meat substitute is optimized for cooking in a stew.

[00245] In some embodiments, the meat substitute is optimized for dehydration.

[00246] In some embodiments, said meat substitute is optimized for fast rehydration upon exposure of the dehydrated meat replica to water.

[00247] In some embodiments, said meat substitute is optimized for use as emergency, camping or astronaut food.

B. INDICATORS OF COOKING MEAT

[00248] The consumable can include compositions which can indicate that the consumable is cooking or has cooked. The release of odorants upon cooking is an important aspect of meat consumption. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking beef. In some embodiments, the consumable when cooked generates an aroma recognizable by humans as typical of cooking pork. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking bacon. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking chicken. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking lamb. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking fish. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking turkey. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with an odorant that is released upon cooking. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with an odorant that is produced by chemical reactions that take place upon cooking. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing mixtures of proteins, peptides, amino acids,

nucleotides, sugars and polysaccharides and fats in combinations and spatial arrangements that enable these compounds to undergo chemical reactions during cooking to produce odorants and flavor-producing compounds. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a volatile or labile odorant that is released upon cooking. In some embodiments the consumable is a method for preparing a meat replica where meat replicas principally or entirely composed of ingredients derived from non-animal sources are heated to release a volatile or labile odorant.

[00249] 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.

[00250] 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.

[00251] 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 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.

[00252] 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%.

[00253] In some embodiments, the one or more isolated, purified proteins are isolated from a genetically modified organism. In one embodiment, the genetically modified organism is a genetically modified plant, algae, bacteria or fungal organism.

[00254] 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.

[00255] 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 taken from the list of Table 1 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.

[00256] Additionally, a hemeprotein taken from the list of Table 1 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, furan 2-ethyl, heptanal, octanal, trans-2-(2-pentenyl)furan, 2-heptenal (Z), 2-octenal (E), pyrrole, 2,4-dodecadienal, 1-octanal, 2-decenal (Z), 2-undecenal.

C. COLOR INDICATORS

[00257] 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.

[00258] The main determinant of the nutritional definition of the color of meat is the concentration of iron carrying proteins in the meat. In the skeletal muscle component of meat products, one of the main iron-carrying proteins is myoglobin. It is estimated that the white meat of chicken has under 0.05%; pork and veal have 0.1-0.3%; young beef has 0.4-1.0%; and old beef has 1.5-2.0%. So, in some embodiments, the consumable is a meat replica which comprises an iron-carrying protein. In some embodiments, the meat replica comprises about 0.05%, about 0.1%, about 0.2%,

about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, or more than about 2% of an iron-carrying protein by dry weight or total weight. In some cases, the iron carrying protein has been isolated and purified from a source. In other cases, the iron carrying protein has not been isolated and purified. In some cases, the source of the iron-carrying protein is an animal source, or a non-animal source such as a plant, fungus, or genetically modified organisms such as, e.g., 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 cases, the iron-carrying protein is leghemoglobin. In some embodiments the consumable is a plant based meat replica that has leghemoglobin added. So, for example a replica of young beef can have about 0.4-1% leghemoglobin. In some cases, the iron-carrying protein is a cytochrome. In some embodiments the consumable is a plant based meat replica that has a cytochrome added. So, for example a replica of young beef can have about 0.4-1% of a cytochrome.

[00259] Another example of iron-carrying proteins is hemoglobin, the iron-containing oxygen-binding protein in the red blood cells of vertebrates. Hemoglobin is similar in color to myoglobin. In some embodiments the invention provides methods of saving and recycling blood from animal farming to supplement the color of a consumable. For example blood is saved from a slaughter house, hemoglobin from the blood is used to enhance the color of a consumable. In some aspects the consumable is a plant-based meat replica containing hemoglobin.

[00260] 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.

[00261] Examples of iron containing proteins include hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate

globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins. Non-symbiotic hemoglobins may be isolated from sprouted soybean, sugar pod, alfalfa, golden flax, black bean, black eyed pea, northern, garbanzo, moong bean, cowpeas, pinto beans, pod peas, quinoa, sesame, sunflower, wheat berries, spelt, barley, wild rice or rice. In various embodiments these iron containing proteins are added to the consumable to alter the visual characteristics or iron content of the consumable.

[00262] In some embodiments the consumable comprises a hemoprotein (e.g. hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins,).

[00263] Leghemoglobin, similar in structure and physical properties to myoglobin, is readily available as an unused by-product of commodity legume crops (eg., soybean, pea). The leghemoglobin in the roots of these crops in the US exceeds the myoglobin content of all the red meat consumed in the US.

[00264] In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a heme protein. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, including a muscle tissue replica, an adipose tissue replica, a connective tissue replica, and a heme protein.

[00265] In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a member of the globin protein family. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a high iron content from a heme protein. In some embodiments the iron content is similar to meat. In some embodiments the consumable has the distinctive red color of meat, such color provided by leghemoglobin.

[00266] A heme protein is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another

embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.

[00267] A heme protein from the group of: Hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin. 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.

[00268] 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%; pork and veal have 0.1-0.3%; young beef has 0.4-1.0%; and old beef has 1.5-2.0%. Normally, myoglobin in meat exists in three states: Oxymyoglobin (Fe2+) (oxygenated = bright red); myoglobin (Fe2+) (non-oxygenated = purplish/magenta); and, metmyoglobin (Fe3+) (oxidized = brown). The transition of oxymyoglobin to metmyoglobin in the presence of oxygen is thought to be the cause of the colour change of ground meat from red to brown. Meat shelf life extenders have been developed to extend the lifetime of the red colour of meat products including but not limited to carbon monoxide, nitrites, sodium metabisulfite, Bombal, rosemary extract, green tea extract, catechins and other anti-oxidants.

[00269] In some embodiments, the increased shelf life is provided by an extension of the lifetime of the desired red color of food products.

[00270] In some embodiments, the increased shelf life is provided by an extension of the lifetime of the desired red color of the non-meat based meat substitute.

[00271] In one embodiment, this invention provides hemoproteins that provide a desired colour 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.

[00272] In some embodiments, the heme proteins are selected from the group of: Hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic

hemoglobin, Hell's gate globin I, bacterial hemoglobins, unicellular eukaryote myoglobins, flavohemoglobins,

[00273] In some embodiments, the non-symbiotic hemoglobins are derived from plants selected from the group consisting of sprouted soybean, sugar pod, alfalfa, golden flax, black bean, black eyed pea, northern, garbanzo, moong bean, cowpeas, pinto beans, pod peas, quinoa, sesame, sunflower, wheat berries, spelt, barley, wild rice or rice.

[00274] In some embodiments the bacterial hemoglobins are derived from thermophilic organisms.

[00275] In some embodiments the bacterial hemoglobins are selected from the group consisting of *A. aeolicus, M. infernorum, Synechocistis SP.*

[00276] In some embodiments, the life time of the oxygenated Fe2+ state of the hemoproteins is similar to that of myoglobin

[00277] In some embodiments, the life time of the oxygenated Fe2+ state of the hemoproteins is 10%, 20%, 30% 50%, 100% or more greater than that of myoglobin.

[00278] In some embodiments the life time of the hemoproteins is extended by treatment with meat shelf life extenders.

[00279] 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.

[00280] In one embodiment, this invention provides hemoproteins that provide a desired flavor profiles to food products.

[00281] In one embodiment, this invention provides hemoproteins that provide a desired flavor profiles to non-meat meat substitutes.

[00282] 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.

[00283] In some embodiments, the heme proteins are selected from the group of: Hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins,

[00284] In some embodiments, the non-symbiotic hemoglobins are derived from sprouted soybean, sugar pod, alfalfa, golden flax, black bean, black eyed pea, northern, garbanzo, moong bean, cowpeas, pinto beans, pod peas, quinoa, sesame, sunflower, wheat berries, spelt, barley, wild rice or rice.

[00285] In some embodiments, the ability of the hemoproteins to generate the desired flavour profile is similar to that of myoglobin

[00286] In some embodiments, the life time of the ability of the hemoproteins to generate the desired flavour profile is 10%, 20%, 30% 50%, 100% or more greater than that of myoglobin.

[00287] Food products comprising isolated, purified heme proteins

[00288] In some embodiments heme proteins of the invention are added to meat to enhance the properties of meat. For example, a heme protein containing solution can be injected into raw or cooked meat. In another example a heme protein solution is dripped over meat or a consumable of the invention to enhance appearance. In one embodiment advertising, photography, or videography of food products such as meat or a meat substitute is enhanced with a heme protein

[00289] In another embodiment, the heme protein is added to the consumable as an iron supplement.

[00290] In some embodiments heme proteins of the invention are added to meat to enhance the properties of meat. For example, a heme protein containing solution can be injected into raw white meat. The heme proteins of the invention would then improve the organoleptic properties of the meat during cooking adding a "beefy" flavor to white meats such as chicken.

[00291] In one application of the invention, hemeproteins may be used as food dyes.

[00292] 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.

[00293] In some embodiments, the present invention provides methods for obtaining hemeproteins from plant. In some embodiments, the hemoprotein is a leghemoglobin.

[00294] In some embodiments the present invention provides methods for obtaining leghemoglobin from plants. Leghemoglobin can be obtained from a variety of plants. Various legumes species and their varieties, for example, Soybean, Fava bean, Lima bean, Cowpeas, English peas, Yellow peas, Lupine, Kidney bean, Garbanzo beans, Peanut, Alfalfa, Vetch hay, Clover, Lespedeza and Pinto bean, contain nitrogen-fixing root nodules in which leghemoglobin has a key role in controlling oxygen concentrations (for example root nodules from a pea plant). Ion-exchange chromatography purification of leghemoglobin from root-nodules of legume plants (soybeans, favabeans and peas) and recombinant (E.coli expressed) non-symbiotic hemoglobin from moong bean plant.

[00295] In one embodiment leghemoglobin protein is purified from root nodules of legume plants using ion-exchange chromatography. In an embodiment, leghemoglobin is purified from soybean, favabean and sweet pea root nodules.

[00296] 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 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 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%.

[00297] Proteins in legume root-nodule lysate can be transferred into 10mM 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%.

[00298] 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 lysate, thus decreasing color quality of leghemoglobin solution. In one embodiment, anion-exchange resin is FFQ. In one embodiment anion-exchange resin is DEAE. In one embodiment anion-exchange resin is Amberlite IRA900. In one embodiment, anion-exchange resin is Dowex 22. In one embodiment, anion-exchange resin is 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.

[00299] In other embodiments, hemoproteins useful for the invention can be sourced from non-animal sources, such as from genetically modified organisms such as plants, algae, bacteria or fungus which express high levels of hemoproteins.

[00300] In some embodiments, the hemoprotein is a leghemoglobin.

[00301] Leghemoglobin can also be produced by genetically engineering a bacterium or fungus to produce it.

[00302] In one embodiment, non-symbiotic hemoglobin from Moong Bean is recombinately expressed in E.coli and purified using anion-exchange chromatography.

[00303] 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 10ug/ml 5-aminolevulinic acid. Expression was induced by 0.2mM IPTG and grown at 30°C for 20hr. E.coli cells expressing Moong Bean non-symbiotic hemoglobin were collected and resuspended in 20mM Na-carbonate pH 9.5, 50mM NaCl, 1mM $MgCl_2$, 1mM $CaCl_2$, 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-Q resin on fast protein liquid chromatography instrument (GE Healthcare). Moong Bean non-symbiotic hemoglobin eluted in 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.

[00304] In one embodiment, non-symbiotic hemoglobin from Moong Bean is recombinately expressed in E.coli and purified using cation-exchange chromatography.

[00305] Non-symbiotic hemoglobin from Moong Bean can be cloned into pJexpress401 vector (DNA2.0) and transformed into E.coli BL21. Non-symbiotic hemoglobin from Moong Bean can be cloned into pJexpress401 vector (DNA2.0) and transformed into E.coli BL21. Cells can be grown in LB media containing soytone instead of tryptone, kanamycin, 0.1mM ferric chloride and 10ug/ml 5-aminolevulinic acid. Expression can be induced by 0.2mM IPTG and cells grown at 30°C for 20hr. E.coli cells expressing Moong Bean non-symbiotic hemoglobin can be collected and resuspended in 20mM MES buffer pH 6.5, 50mM NaCl, 1mmM $MgCl_2$, 1mM $CaCl_2$. DNAaseI, and protease inhibitors can be added. Cells can be lysed by sonication. Lysate can be cleared from cell debris by centrifugation at 16 000g for 20 min, followed by filtration over 200nm filter. Cell lysate can then be loaded over FF-S

resin on 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.

[00306] Heme proteins of the invention can also be produced by genetically engineering a bacterium or fungus to produce it. Recombinant heme proteins carrying His-tag and TEV protease His-tag removal site can be expressed in E.coli BL21 strain and purified using His-tag affinity chromatography (Talon resin, CloneTech).

[00307] In some embodiments, the hemoprotein is selected from the group consisting of bacterial hemoglobins algal hemoglobins, protozoan heme proteins and non-symbiotic plant hemoglobins.

[00308] In some embodiments, the non-symbiotic plant hemoglobin is selected from the group consisting of non-symbiotic plant hemoglobins isolated from sprouted soybean, sugar pod, alfalfa, golden flax, black bean, black eyed pea, northern, garbanzo, moong bean, cowpeas, mustards, pinto beans, pod peas, quinoa, sesame, sunflower, wheat berries, spelt, barley, wild rice or rice.

[00309] In some embodiments, the bacterial hemoglobin is derived from thermophilic bacteria.

[00310] In some embodiments,the thermophilic bacteria grow at a temperature of greater than 45°C.

[00311] In some embodiments the bacterial hemoglobins are selected from the group consisting of *Aquifex Aolifex*, *Thermobifida fusca*, and *Mycobacterium Infernorum* (Hells Gate). *Synechocystis SP, Bacillus subtilis.*

[00312] In some embodiments, the algal hemoglobin is derived from *Chlamydomonas eugametos*

[00313] In some embodiments the protozoan hemoglobins are derived *from Paramecium caudatum and Tetrahymena pyriformis*

[00314] Hemoproteins of the invention were tasted by a panel of volunteers and in each case described as tasting like blood.

[00315] In some embodiments, the heme proteins are utilized as ingredients in food products where the flavour of blood is desired.

[00316] Heme proteins, for example leghemoglobin, can be combined with other plant based meat replica components. In some embodiments the heme proteins are captured in a gel which contains other components, for example lipids and or proteins. In some aspects a multiple gels are combined with non-gel based heme proteins. In some embodiments the combination of the heme proteins and the other compounds of the consumable are done to insure that the heme proteins are able to diffuse through the consumable. In some embodiments the consumable is soaked in a heme-protein containing solution, for instance a leghemoglobin solution,. In some embodiments the consumable is soaked in a heme protein containing solution, for instance a leghemoglobin solution for 1, 5, 10, 15, 20 or 30 hours. In some embodiments the consumable is soaked in a heme containing solution, for instance a leghemoglobin solution for 1, 5, 10, 15, 30, or 45 minutes.

[00317] 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 or proximity-ligation assay or luminex assay or western blot analysis can be performed to determine whether leghemoglobin is present in a food product such as meat or a meat replica. In one embodiment the detection methods are performed to determine whether meat has been altered with leghemoglobin.

B. MAYONNAISE SPREAD REPLICA.

[00318] 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.

[00319] 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 or multiple sources as described herein. Non-traditional mayonnaise product can be used for all the culinary applications that traditional mayonnaise is used for. In one embodiment, vinegar and/or lemon, lime juice are added as flavor additives. In one embodiment, purified plant proteins are not soy proteins. In one embodiment, flavor can be modified by addition of mustard, spices, herbs pickles.

[00320] The mayonnaise replica can comprise a mixture comprising non-animal proteins. In one embodiment, mayonnaise replica is mixed from 50% (wt/v) rice bran oil, 7% (wt/v) Moong Bean 8S protein. In one embodiment, mayonnaise replica is mixed from 70% (wt/v) sunflower oil, 2.4% (wt/v) rubisco, 0.29% (wt/wt) soybean lecithin. In one embodiment, mayonnaise replica is mixed from 70% (wt/v) sunflower oil, 2.4% (wt/v) rubisco, 0.29% (wt/wt) soybean lecithin, and 8 uM oleosin In one embodiment, mayonnaise replica is mixed from 70% (wt/v) cocoa oil, 2.4% (wt/v) rubisco, 0.29% (wt/wt) soybean lecithin.

[00321] The mixture can be emulsified, and stability of emulsion controlled by modifying size of the oil-water-protein particles by high pressure homogenization. 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 oil and protein solution. A resulting oil, protein and lecithin solution can then homogenized, for example first at 5000psi and then 8000psi. Properties (e.g. thickness, texture and creaminess) of a resulting product can be similar to one of traditional mayonnaise.

[00322] In one embodiment, resulting mixture is emulsified and stability of emulsion controlled by modifying size of the oil-water-protein particles by sonication. Resulting oil, protein and lecithin solution is 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 a some instances (e.g. Moong Bean 8S protein and Rice Bran Oil) a product is light off-white in color.

C. CREAM LIQUOR REPLICA

[00323] Traditionally cream liquors contain dairy cream and liquor as their base. Examples of liquor include Irish whiskey, Scotch whiskey, rum, vodka, fermented fruits. 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 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 can be from single or multiple sources as described herein. In one embodiment, plant fats can be from single or multiple sources as described herein. In one embodiment, liquor can be, for example: whiskey, rum, vodka, grappa, fermented fruits (for ex. cherry liquor), plum brandy, tequila, herbal bitters.

D. PROTEIN ENRICHED ALCOHOLIC BEVERAGE

[00324] 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, 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 (4-7% alcohol), beer (~ 3-10% alcohol), wine (8-14% v/v alcohol), dessert wines (17-20%), whiskey (~ 40% alcohol), and vodka (35-50% alcohol). In addition, some traditional alcoholic beverages include sugars (for example, Bacardi Razz at 10% wt/v).

[00325] Accordingly beverages containing alcohol are supplemented by addition of purified plant proteins at for example 0.1-5% wt/v. In one embodiment, beverages containing alcohol are supplemented by addition of purified plant proteins at 0.1-5% wt/v and sugar (1-15% wt/v). The sugar can be cane sugar, brown sugar, sucrose, glucose. For example, purified Rubisco at 180mg/ml in 20mM K-phosphate pH 7.0, 150mM NaCl was added whiskey. Jameson supplemented with 5% wt/v Rubsico formed a soft gel, its consistency similar to traditional Jello shots.

[00326] For example, Rubsico, Moong Bean 8S and Pea Globulin enriched alcoholic beverage was made by adding purified Rubsico, Moong Bean 8S and Pea Globulin proteins at final protein concentrations of 0.5%, 1% and 5% wt/v to Corona beer, Pino Grigio wine and Jameson whiskey. Zein was added at 0.5%, 1% and 5% wt/v to 60% ethanol, 5% sucrose solution in water.

[00327] Pea proteins were extracted from pea flour by resuspending flour in 5%, 20% and 40% 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.

[00328] 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 soften the aroma and mouthfeel of Jameson. Addition of 0.5% wt/v of Moong Bean added slightly creamy flavor to Jameson, with aroma similar to traditional White Russian coctail. Addition of 5% wt/v Zein to Jameson generated aromas and flavor characterized as moldy beans and raw potato.

[00329] In another example, where Corona beer was enriched with 0.5% wt/v Pea Globulin aroma changed to hoppy and resembled one of Indian Pale Ale. Flavor changed to carrying pea notes. Addition of 0.5% wt/v and 5% wt/v of Moong Bean 8S protein changed Corona aroma towards sweet peony flower with an intensified hop aroma. Flavor was neutral in case of 0.5% wt/v Moong Bean 8S and carried planty-nutty notes in case of 5% wt/v Moong Bean 8S.

[00330] In another example, where Pino Grigio wine was enriched with 1% wt/v Moong Bean 8S protein, additional aroma notes of sweet and citrus were detected. Flavor changed to that of carrying notes of peanut-butter. Addition of 1% wt/v of Pea Globulins modified 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.

[00331] A zein enriched 60% ethanol, 5% sucrose solution carrried burnt tortilla chip aroma notes compared to solution without zein. There was no difference in flavor.

[00332] 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 alchohol content.

E. CHOCOLATE SPREAD

[00333] 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. Product can be used as a spread on bread, crepes, pancakes, icing for cakes and cookies, filling for chocolate confectionary, non-dairy chocolate cake filling.

[00334] In one embodiment, dairy milk and milk products are substituted by non-dairy cream fraction made as described herein. In one embodiment, non-dairy cream fraction can come from a single 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.

D. OTHER APPLICATIONS:

[00335] 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.

[00336] 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.

[00337] In one embodiment, non-dairy plant cream fraction can be used as substitute for dairy milk and dairy milk products to make non-dairy milk chocolate confectionary.

[00338] 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 confectionary.

[00339] 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 would be substituted by non-dairy plant cream fraction. In one embodiment,

dairy butter and eggs would be substituted by non-dairy plant cream fraction and air-water foam stabilizing plant seed storage proteins.

[00340] Vegan consumables: pate analog. Vegan pate analog is 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 is chopped into ½-inch cubes and browned over in the fat and shallots mix for another 3-5 min. The mixture is forced through a sieve until homogeneous. The pan, while still warm, is 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 a 15 min chilling in a refrigerator, pate is ready to be served.

[00341] 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% fat tissue replica.

[00342] In some embodiments, muscle tissue replica with higher iron content can be used for pate to make it 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 heme protein.

[00343] 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 heme protein.

[00344] Vegan consumables: blood sausage analog Vegan blood sausage is made from blood analog created by mixing solutions of heme protein and purified plant protein. For example, 35 ml of mixed solution of leghemoglobin (120 mg/ml) and pea albumin (100 mg/ml), which approximates composition of blood, is carefully mixed with a slurry of corn flour in salt water (6:5 w/v flour to water ratio). A table spoon of chopped onion is fried with10 g of chopped fat tissue replica, mixed with a few raisins and cooled to room temperature before mixing with blood/flour mix. The mixture is seasoned to taste (for example, using salt, pepper, parsley and cinnamon), loaded into vegetarian sausage casings, and poached in near-boiling water for about 45 min. After cooling, sausage can be consumed as is or further cooked, for example smoked, crisped in an oven or roasted.

[00345] In some embodiments, muscle replica can be included in the recipe to imitate meat/blood sausages.

[00346] In some embodiments, barley, buckwheat, oat, rice, rye, sorghum, wheat or other grains can be used in the blood sausage.

[00347] 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.

[00348] All steps were carried out at 4°C whenever possible. Centrifugation steps were at 8000 g for 20mins, 4°C. 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 50mM KPhosphate buffer pH 7.4 + 0.5M NaCl. 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. Protein composition at individual fractionation steps was monitored by SDS-PAGE and protein concentrations were measured by standard UV-Vis methods.

[00349] (i) Pea-albumins: Dry green pea flour was used as a source of pea albumins. The flour was suspended in 10 volumes of 50mM sodium acetate buffer pH 5 and stirred for 1hr. Soluble protein was separated from un-extracted protein and pea seed debris by either centrifugation (8000 g, 20 minutes) or filtration over 5 micron filter. Supernatant and filtrate respectively were collected. To this crude protein extract, solid ammonium sulfate was added to 50% wt/v saturation. The solution was stirred for 1hour 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 0-500mM sodium chloride.

[00350] (ii) Pea-globulins: Dry green pea flour was used to extract pea globulin proteins. The flour was suspended in 10 volumes of 50mM 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.

[00351] 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 20mins or clarified by 5 micron filtration and the supernatant was collected. This mixture contains both the 7S and 11S globulins (crude protein extract). The solution was then 0.2 micron filtered and concentrated by 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.

[00352] Soybean proteins can also be extracted by suspending the defatted soy flour in 4-15 volumes of 20mM sodium carbonate, pH9 (or water, pH adjusted to 9 after addition of the flour) to decrease off-flavors in the purified protein. The slurry is centrifuged at 8000 xg for 20minutes or clarified by filtration. The extracted proteins are ultrafiltered and then processed as above.

[00353] (iv) Moong bean 8S globulins: Moong bean flour was used to extract 8S globulins by first suspending the flour in 4 volumes of 50mM 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 in section 0002.

[00354] Moong bean globulins can also be extracted by suspending the flour in 4 volumes of 20mM sodium carbonate buffer, pH9 (or water adjusted to pH9 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.

[00355] (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, suspension 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. Supernatant was then heated to 95°C for 15 minutes, and then centrifuged. 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.

[00356] (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 supernatant collected. Ethanol in supernatant was evaporated by heating solution to 85°C. Resulting solution was cooled to room temperature. and ice-cold acetone added (1: 4 v/v) to precipitate proteins. Solution is then centrifuged (4000g, 20min), and protein recovered as the light-beige colored pellet.

[00357] (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, then solution is then centrifuged, and protein recovered as the pellet.

[00358] (viii) RuBisCO was fractionated from alfalfa greens by first grinding leaves with 4 volumes of cold 50mM 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.

[00359] RuBisCO can also be purified by applying the crude lysate adjusted to 0.1M NaCl to an anion exchange resin. The weakly bound protein contaminants are washed

with 50mM KPhosphate buffer pH 7.4 buffer + 0.1M NaCl. RuBisCO was then eluted with high ionic strength buffer (0.5M NaCl).

[00360] 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.

[00361] 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.

[00362] (ix) Leghemoglobin. Soy root nodules were suspended and lysed 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 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 flowthrough 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.

[00363] (x) Non-symbiotic hemoglobin from Moong Bean was cloned into pJexpress401 vector (DNA2.0) and transformed into E.coli BL21. 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 10ug/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.

[00364] (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, 0.1mM ferric chloride and 10ug/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.

[00365] (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 50mM 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.

EXAMPLE 2: CONSTRUCTING A MUSCLE TISSUE ANALOG

[00366] To prepare 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). Mixed solution was concentrated using Amicon spin concentrators (10 kDa cut-off) to final concentration of moong bean 8S globulin 61 mg/ml, and of leghemoglobin 6.5 mg/ml. 400 mg of transglutaminase powder were added, solutions thoroughly mixed, 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 FAT TISSUE REPLICA

[00367] A 40ml aliquot of rice bran oil and a 40ml aliquot of moong bean protein (114mg/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.

[00368] In an 18cm x 18cm x 2.5cm synthetic rubber Ikea Plastis ice cube tray, 48mg of electrospun fibers (from connective tissue example) were laid longitudinally and as homogenously as possible across the bottom of one triangular 13.97cm x 1.27cm x 1.5875cm mold. 20ml of the resulting emulsions from above were then poured on top

of the fibers. An additional 20ml of the emulsions were then poured into a similarly sized blank mold on the same tray to be used as a control.

[00369] The ice cube tray was then floated in boiling water for 15 minutes, removed, and cooled to room temperature.

[00370] 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. The fiber containing fat replica had tensile strength 23 kPa, whereas the fat replica with no fibers had tensile strength 20 kPa.

EXAMPLE 4 HIGH PERCENTAGE FAT FAT-REPLICA

[00371] A fat tissue matrix of a protein-oil emulsification, formed with 3.3% wt/v Pea Globulin with 70% v/v oil that consisted of an equal mixture of coconut, cocoa, olive, and palm oils, and with 0.5% wt/v lecitin, was cross-linked with an transglutaminase. After draining and dehydrating, resulting gel was medium soft and the fat content was confirmed to be 75% (wt/wt).

[00372] A fat tissue matrix of a protein-oil emulsification was formed with 1.6 % wt/v Rubisco and 80% v/v cocoa oil. Resulting gel was soft.

EXAMPLE 5: METHOD OF PREPARING FAT TISSUE REPLICAS

[00373] 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. Lecitin 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 transglutaminase enzyme or (c) heating/cooling followed by addition of 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 cool to room temperature. All emulsifications are incubated for 8-12 hours at 37°C.

EXAMPLE 6: METHOD TO ANALYZE FAT TISSUE REPLICAS

[00374] 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 fat 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: FAT REPLICA OF BEEF FAT

[00375] A fat tissue replica was made by gelling emulsion of purified Moong Bean 8S protein and equal amounts of cocoa butter, coconut butter, olive oil and palm oil. Moong Bean 8S protein was purified. Concentration of Moong Bean 8S protein was 140 mg/ml, in 20mM K-phosphate pH 7.4, 400mM NaCl. Fat mixture was prepared by melting individual fats from solid to liquid state at 45°C for 30min. Individual fats (cocoa butter, coconut butter, olive oil and palm oil) in liquid state where then mixed at 1:1:1:1 (v/v) ratio. Protein-fat emulsion was formed by mixing 70% v/v liquid fat mixture with 4.2% wt/v Moong Bean 8S protein, 0.4% wt/v soybean lechitin and emulsified by vortexing for 30sec followed by sonication for 1 min. After homogenization fat-protein emulsion was in single liquid phase as judged by visual observation. One fat 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. Resulting fat tissue replicas were in a single

phase. Fat tissue replica matrix formed by action of transglutaminase was softer solid than fat tissue replica matrix formed by heat/cool induced gelling.

EXAMPLE 8: A FAT REPLICA OF WAGYU BEEF FAT

[00376] Fat tissue replica was made by gelling emulsion of purified pea globulin proteins and equal amounts of cocoa butter, coconut butter, olive oil and palm oil. Pea globulin proteins were purified. Concentration of pea globulin solution was 100mg/ml, in 20mM K-phosphate pH 8, 400mM NaCl. 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) in liquid state where then mixed at 2:1:1 = (olive oil: cocoa butter: mango butter) v/v ratio. Protein-fat emulsion was formed by mixing 50% v/v liquid fat mixture with 5% wt/v Pea globulins, and emulsifying using hand-held homogenizer at maxium setting for 30sec. After homogenization fat-protein emulsion was in 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. Resulting fat tissue replica was in a single phase, soft solid and salty in flavor.

EXAMPLE 9: FAT TISSUE REPLICA WITH FATTY ACID DISTRIBUTION OF BEEF:

[00377] Fat tissue replica was made by gelling 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. Concentration of pea protein solution was 100mg/ml, in 20mM K-phosphate pH 8, 400mM NaCl. 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 liquid state where then mixed at 1:1:1:1 v/v ratio. Protein-fat emulsion was formed by mixing 50% v/v liquid fat mixture with 5% wt/v Pea globulins, and emulsifying using hand-held homogenizer at maxium setting for 30sec. After homogenization fat-protein emulsion was in 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. Resulting fat tissue replica was in a single phase, soft solid and salty in flavor.

EXAMPLE 10: FAT TISSUE REPLICA WHERE FIRMNESS OF FAT TISSUE AT REFRIGERATION AND AMBIENT TEMPERATURES IS CONTROLLED BY MELTING TEMPERATURE OF FAT IN FAT TISSUE REPLICA.

[00378] Fat tissue replica made as stable emulsion of Rubisco with sunflower oil is softer than fat tissue replica made as stable emulsion of Rubisco and cocoa oil. Fat 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 oils. Each fat tissue replica that contained cocoa oil is firmer than the replicas that were formed with sunflower oil.

[00379] Fat tissue replica made as stable emulsion of Moong Bean 8S protein with sunflower oil is softer than fat tissue replica made as stable emulsion of Moong Bean 8S protein and cocoa oil. Fat 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 oils. Each fat tissue replica that contained cocoa oil is firmer than the replicas that were formed with sunflower oil.

[00380] Fat tissue replica made as stable emulsion of Moong Bean 8S protein of with canola oil is softer than the fat tissue replica made as stable emulsion made of Moong Bean 8S protein with an equal mixture of coconut, cocoa, olive, and palm oils. Fat 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 fat tissue replica that contained mixture of oils is firmer than the replicas that were formed with sunflower oil.

[00381] Fat tissue replica made as stable emulsion of Soy proteins with sunflower oil is softer than the fat tissue replica made as stable emulsion of Soy proteins and cocoa oil. Fat 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 fat tissue replica that contained mixture of oils is firmer than the replicas that were formed with sunflower oil.

EXAMPLE 11: FAT TISSUE REPLICA: CONTROL OF MELTING POINT BY CONTROL OF FAT COMPOSITION

[00382] Fat tissue replicas were constructed as described above comprised of 0.18%, 1.6%, and 2.4% wt/v Rubisco with 70%, 80%, and 90% v/v cocoa oil are solid at room temperature but melt close to mouth temperature.

[00383] Fat tissue replicas comprised of 0.6%, 1.6%, and 2.6% wt/v Soy proteins with 50%, 70%, 80%, and 90% v/v cocoa oil are solid at room temperature but melt close to mouth temperature.

[00384] A fat tissue replica comprised of 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 close to mouth temperature.

EXAMPLE 12: FAT TISSUE REPLICA COOKING: STRUCTURE OF FAT TISSUE MATRIX CONTROLS MELTING POINT DURING COOKING.

[00385] A fat tissue matrix of 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 oil, melts at a higher temperature when formed upon heat/cool denaturation and at a lower temperature when formed by cross-liking with transglutaminase.

EXAMPLE 13: COOKING FAT TISSUE: ARRANGEMENT AND STRUCTURAL ORGANIZATION OF PROTEINS AND FAT WITHIN A FAT TISSUE MATRIX CONTROLS AMOUNT OF FAT RELEASED AND FAT RETAINED BY FAT TISSUE REPLICA DURING COOKING.

[00386] During cooking of fat tissue matrix of a protein-oil emulsification formed with 2% w/v Rubisco and 50%, 70%, and 90% v/v cocoa oil, more fat tissue replica mass is retained after cooking when fat tissue replica is formed upon heat/cool denaturation than when formed by cross-liking with transglutaminase. Released mass is liquid and appears oily.

[00387] During cooking of fat tissue matrix of a protein-oil emulsification formed with 2.6 and 0.6 % w/v Soybean protein and 50%, 70%, and 90% v/v cocoa oil, more fat tissue replica mass is retained when formed upon heat/cool denaturation than when formed by cross-liking with transglutaminase. Released mass is liquid and appears oily.

EXAMPLE 14: COOKING A FAT TISSUE REPLICA: CONCENTRATION OF PARTICULAR PROTEINS WITHIN FAT TISSUE MATRIX CONTROLS MASS OF FAT TISSUE REPLICA THAT REMAINS AFTER COOKING

[00388] A series of fat tissue replicas constructed from 1.4% wt/v Moong Bean 8S protein with 90% v/v canola oil and 0.45% wt/v soybean lecitin, were homogenized and increasing concentration of sunflower oleosins were added to emulsion. 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 concentration of oleosins in fat tissue replica was higher.

[00389] A series of fat 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. Fat tissue replicas containing rubisco at 0% wt/v completely melted, while 1.9% wt/v Rubisco retained 10% mass, and fat tissue replica containing 2.4% wt/v Rubsico retained 20% mass upon cooking.

EXAMPLE 15: FAT TISSUE REPLICA: FIRMNESS OF FAT TISSUE REPLICA CAN BE CONTROLLED BY AMOUNT OF PROTEIN WITHIN FAT TISSUE MATRIX

[00390] A series of fat tissue replicas were formed with varying concentrations of Rubisco (0.18, 1.6 1.9 % wt/v) with 70-80% v/v sunflower oil were firmer as amount of protein in fat tissue matrix increased. Fat tissue replicas with 0.18% wt/v Rubisco were very soft, with 1.6% wt/v Rubisco soft, and with 1.9% wt/v was medium firmness.

EXAMPLE 16: CONNECTIVE TISSUE ANALOG

[00391] Conective 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 ul/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 a 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 17: EXTENDING THE LIFETIME OF REDUCED (HEME-FE2+) LEGHEMOGLOBIN

[00392] Equine myoglobin was purchased from Sigma. Myoglobin was resuspended at 10 mg/ml in 20mM potassium phosphate, pH 8.0, 100mM NaCl. SDS-PAGE analysis suggested that protein purity is ~ 90%. Soy leghemoglobin 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 20mM potassium phosphate, pH 8.0, 100mM NaCl. The leghemoglobin eluted in flowthrough fractions. SDS-PAGE analysis suggested that protein purity is ~ 70%. Leghemoglobin was buffer exchanged into 20mM potassium phosphate, pH 7.4, 100mM NaCl and concentrated to 10mg/ml on 3.5kDa membrane concentrators.

[00393] Carbon monoxide treatment: Myoglobin at 10mg/ml in 20mM potassium phosphate, pH 8.0, 100mM NaCl and leghemoglobin at 10mg/ml in 20mM potassium phosphate, pH 7.4, 100mM 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-$Fe3^+$ to heme-$Fe2^+$ state by adding 10mM sodium dithionite 0.1mM sodium hydroxide for 2 minutes. Sodium dithionite and sodium hydroxide were removed from protein solution by using size-exclusion chromatography (PD-10

desalting column) in 20mM potassium phosphate, pH 8.0, 100mM NaCl and 20mM potassium phosphate, pH 7.4, 100mM NaCl respectively. Globins fractions were collected as a peak red colored fractions as evaluated by visual estimation. UV-VIS spectra confirmed presence of heme-$Fe2^+$ state for both proteins. After desalting, I again perfused the solution with gas for another 2 minutes. I evaluated the color of the solutions by taking UV-Vis spectra (250nm- 700nm) every 20 minutes using the nanodrop spectrophotometer. Control samples were not treated with carbon monoxide.

[00394] Sodium nitrite treatment: Myoglobin at 10mg/ml in 20mM potassium phosphate, pH 8.0, 100mM NaCl and leghemoglobin at 10mg/ml in 20mM potassium phosphate, pH 7.4, 100mM NaCl were reduced from heme-$Fe3^+$ to heme –$Fe2^+$ by adding 10mM sodium dithionite 0.1mM sodium hydroxide for 2 minutes. Sodium dithionite and sodium hydroxide were removed from protein solution by using size-exclusion chromatography (PD-10 desalting column) in 20mM potassium phosphate, pH 8.0, 100mM NaCl and 20mM potassium phosphate, pH 7.4, 100mM NaCl respectively. Globins fractions were collected as a peak red colored fractions as evaluated by visual estimation. UV-VIS spectra confirmed presence of heme-$Fe2^+$ state for both proteins. Sodium nitrite was then added to a final concentration of 1mM from 100mM nitrite in phosphate buffer pH 7.4. Lifetime of heme-$Fe2^+$ 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.

[00395] Data analysis of heme-$Fe2^+$ 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 540nm 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.

[00396] Life-time of heme-$Fe2^+$ 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

heme-$Fe2^+$ 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 18: PREPARATION OF MEAT REPLICAS IN WHICH THE PARTICLE SIZE OF INDIVIDUAL TISSUE REPLICA UNITS IS VARIED TO CONTROL AROMA GENERATION DURING COOKING.

[00397] Muscle tissue replica and fat tissue replica were prepared separately and then combined into meat tissue replica such that the size of individual tissue replica units is varied to control aroma generation during cooking. Individual fat, muscle and connective tissue replicas were constructed in the following manner.

[00398] 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) purified were mixed with 16 ml of soybean leghemoglobin solution (6 mg/ml leghemoglobin in 20 mM potassium phosphate, 400 mM NaCl, pH 7.3) purified with following modification. Imidazole was removed from leghemoglobin by dialysis into 20mM potassium phosphate pH 7.4, 100mM NaCl and 0.1mM EDTA. Leghemoglobin was concentrated to ~ 6mg/ml and buffer exchanged into 20mM potassium phosphate pH 7.4, 100mM NaCl. . UV-VIS spectrum (250-700nm) had a characteristic signature of leghemoglobin in heme-$Fe2^+$ (reduced) state. Mixed leghemoglobin and Moong Bean 8S solution was concentrated using Amicon spin concentrators (10 kDa cut-off) to final concentration of moong bean 8S globulin 61 mg/ml, and of leghemoglobin 6.5 mg/ml. 400 mg of transglutaminase powder were added, solutions thoroughly mixed, divided into two 50 ml Falcon tubes and incubated overnight at 37°C. 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.

[00399] Connective tissue replica: Connective tissue fiber replicas were manufactured by electrospinning a solution of moong bean 8S globulin purified (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.

[00400] Fat tissue replica: Fat tissue replica was made by gelling emulsion of purified Moong Bean 8S protein and equal amounts of cocoa butter, coconut butter, olive oil and palm oil. Moong Bean 8S protein was purified. Concentration of Moong Bean 8S protein was 140 mg/ml, in 20mM K-phosphate pH 7.4, 400mM NaCl. Fat mixture was prepared by melting individual fats from solid to liquid state at 45'C for 30min. Individual fats (cocoa butter, coconut butter, olive oil and palm oil) in liquid state where then mixed at 1:1:1:1 (v/v) ratio. Protein-fat emulsion was formed by mixing 50% v/v liquid fat mixture with 7% wt/v Moong Bean 8S protein, 0.2% wt/v soybean lechitin and emulsified using hand-held homogenizer at maxium setting for 30sec followed by sonication for 1 min. After homogenization fat-protein emulsion was in single liquid phase as judged by visual observation. One fat tissue replica emulsion was stabilized by cross-linking with 4 % wt/v transglutaminase enzyme at 37°C for 12hr. Another fat tissue replica was stabilized by gelling of proteins by heating to 120°C in a water bath followed by cooling to ambient room temperature. Resulting fat tissue replicas were in a single phase. Fat tissue replica matrix formed by action of transglutaminase was softer solid than fat tissue replica matrix formed by heat/cool induced gelling.

[00401] Meat replicas with lean-to-fat ratio 85/15 were prepared by combining individual muscle, connective and fat tissues such that particle size of individual tissues in a muscle tissue 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.

[00402] 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. Fat 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 fat tissue generated aromas associated with burnt meat smell, however to varying intenisties.

[00403] 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)).

[00404] 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. We refer to this sample preparation as "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). We refer to this sample preparation as "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 80/20 (wt/wt) muscle/fat mixture.

[00405] 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.

[00406] 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 19: LEGHEMOGLOBIN CONTRIBUTION TO FLAVOR

[00407] Creation of beef flavors and aromas in non beef consumables by addition of hemeproteins. Ground chicken (90% lean, 10% fat) was strained with cheesecloth and mixed with recombinant soy leg-hemoglobin 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). Leg-hemoglobin 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 a chicken alone, chicken mixed with buffer, chicken mixed with either leg-hemoglobin or myoglobin +/- CO, or beef (90% lean, 10% fat). Judges filled out a survey to evaluate the aroma and flavor of each nugget. Adding recombinant leg-hemoglobin or myoglobin to chicken resulted in 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.

[00408] Table 2 legend: Judges rated the aroma and flavor of each nugget. 1 = chicken, 2 = chicken + faint beef, 3 = 50/50 chicken + beef, 4 = beef + faint chicken, 5 = beef. Shown 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.

	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

TABLE 2.

EXAMPLE 20: PREPARATION OF A NON-DAIRY CREAM LIQUOR

[00409] The cream liquor was made from sunflower cream fraction, rubsico and whiskey (Jameson). Sunflower cream fraction was made by blending sunflower seeds in 40mM sodium phosphate pH 8.0, 400 mM sodium chloride buffer. Seed debris was pelleted by centrifugation at 5000g for 20min, and cream fraction collected. Cream fraction was resuspended in 10mM potassium phosphate pH 7.4 buffer and collected by centrifugation at 5000g, 20 min. Rubsico was purified as described in and used as 25mg/ml stock solution in 20mM K-phosphate pH 7.0, 150mM NaCl.

[00410] Cream liquor was made as following: 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, 1.5% wt/v chocolate powder. Resulting mixture was homogenized at 5000 psi.

[00411] 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.

[00412] Beverages were served either at ambient room temperature or chilled. Resulting beverages were beige to light chocolate in color. Tasting results suggested that beverage had very creamy alcoholic flavor similar to dairy cream liquor. Chilled product was preferred. Emulsion was stable at room temperature for at least 1 week (maximum time tested).

EXAMPLE 21 –CHOCOLATE SPREAD

[00413] Chocolate spread was made from 34% (wt/wt) cane sugar, 22 % (wt/wt) cocoa powder, 19 % (wt/v) pistachio cream fraction, 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 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, 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 we refer to as the "almond skim milk"), and a lighter, creamy, opaque layer (which we refer to as the "almond cream"). Almond milk was then was pasteurized at 75°C for 16 seconds, chilled and stored at 2°C.

[00414] 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 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.

[00415] 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 pistachio cream fraction and whisked together. Resulting mixture was then poured into molds and let sit for 24hr at refrigeration and freezer temperatures.

[00416] In another example chocolate spread was made from 42% (wt/wt) cane sugar, 27 % (wt/wt) cocoa powder, 31 % (wt/v) sunflower cream fraction, 23% (v/v) almond skim milk. All ingredients and procedures apart from sunflower cream fraction were as described above.

[00417] The sunflower cream fraction was created from blending sunflowers 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, the slurry was then centrifuged. The top cream layer was then removed and is blended in the same buffer, followed by heated 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 centrifuge again, the resulting cream fraction that is very creamy, white, and neutral tasting.

[00418] In another example 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, 20 % v/v almond skim milk.

[00419] In another example 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.

[00420] In another example 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 , 20 % v/v almond skim milk.

[00421] In another example chocolate spread was made from 1.8% (wt/wt) cane sugar, 1.13% (wt/wt) cocoa powder, 88 % (wt/v) pistachio cream fraction, 9 % almond

skim milk, by whisking equal amounts of spread described above and pistachio oil bodies.

[00422] In another example chocolate spread was made from 8.5 % (wt/wt) cane sugar, 5.4 % (wt/wt) cocoa powder, 81 % (wt/v) sunflower cream fraction, 4.6 % (v/v) almond skim milk, by mixing chocolate spread described above with sunflower cream at ratio 2:1.

[00423] 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 revolved around 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.

[00424] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

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CLAIMS

WHAT IS CLAIMED IS:

1. A consumable product comprising:

a. a isolated and purified plant protein in a solution,

wherein the isolated plant protein has a solubility in the solution of at least 25 g/L.

2. The consumable product of claim 1 wherein the plant protein has a solubility of at least 1 mg/ml, wherein the temperature of the solution is 100 C, and the pH of the solution is between pH 8 and at pH 5.

3. The consumable product of claim 1 wherein the solution comprises an alcohol.

4. The consumable product of claim 1 wherein the isolated plant protein is at least 10 kDa in size.

5. The consumable product of claim 1 wherein the isolated plant protein does not negatively affect the flavor, texture, or color of a the consumable product as compared to a second consumable product which is identical to the consumable product other than that it does not comprise the isolated plant protein.

6. The consumable product of claim 1 wherein the isolated plant protein is not denatured.

7. The consumable product of claim 1 wherein the isolated plant protein retains its native conformation.

8. The consumable product of claim 1 wherein the isolated plant protein is not derived from soy.

9. The consumable product of claim 1 wherein the isolated plant protein is rubisco.

10. The consumable product of claim 1 wherein the isolated plant protein comprises a: LEA protein, dehydrin, hydrophilin, intrinsically disordered protein, or protein identified based on its ability to stay soluble after boiling at pH and salt concentrations comparable to foods.

11. The consumable product of claim 1 wherein the isolated plant protein is Moong 8S globulin.

12. The consumable product of claim 1 further comprising a second isolated and purified protein.

13. A method for making a consumable product comprising:
a. obtaining an isolated and purified plant protein
b. dissolving the isolated and purified plant protein in a solution.

14. The method of claim 13, further comprising:
a. obtaining a second isolated and purified plant protein, and
b. dissolving the second isolated and purified plant protein in the solution.

15. The method of claim 13 wherein the dissolving occurs at a temperature of less than 100 C, and a pH between pH 8 and at pH 5.

16. The method of claim 13 wherein the isolated and purified plant protein is at least partially hydrolysed when dissolved in the solution.

17. The method of claim 13 wherein the obtaining step comprises:
a. boiling a plant product,
b. filtering the boiled plant product, and
c. concentrating the isolated and purified plant protein.

18. The method of claim 13 wherein the solution is clear after the dissolving step.

19. The method of claim 13 wherein the dissolving step comprises dissolving the isolated and purified plant protein is in solution at a greater than 1% concentration.

20. The method of claim 13 wherein the isolated and purified plant protein is selected from the group consisting of rubisco, moong globulin, soy globulin, pea globulins, pea albumins, prolamins.

21. The method of claim 13 wherein the isolated and purified plant protein is lyophilized prior to the dissolving step.

22. The method of claim 13 wherein the solution is a liquid to form a high protein drink suitable for human consumption.

23. A consumable product comprising: an iron-carrying protein with an amino acid sequence with at least 70% homology to a sequence listed in Table 1; wherein the consumable product does not comprise meat.

24. The consumable product of claim 23 wherein the iron-carrying protein content is visible to the unaided human eye.

25. The consumable product of claim 23 wherein the iron-carrying protein content account for at least 0.01% of the composition.

26. The consumable product of claim 23 further comprising carbon monoxide.

27. The consumable product of claim 23 further comprising nitrites.

28. The consumable product of claim 23 further comprising: ammonium, sodium, potassium, or calcium salts.

29. A method for extending the shelf-life of a consumable product comprising:
a. obtaining a heme containing protein which is less oxidizable than myoglobin,
b. adding the heme containing protein to a consumable product.

30. The method of claim 29 wherein the iron containing protein has an amino acid sequence with at least 70% homology to a sequence listed in Table 1

31. A consumable product, comprising a gelled emulsion, said gelled emulsion comprising:

a. a protein solution;

b. a first lipid that when not in the consumable product is solid at a first temperature; and;

c. a second lipid that when not in the consumable product is liquid at the first temperature;

wherein the first and second lipids are varied in the composition to achieve a melting temperature of the combined first and second lipids in the composition that is similar to the melting temperature of lipids found in meat.

32. The consumable product of claim 31 wherein melting temperature of the combined first and second lipids in the composition is between 23°C to 27°C.

33. The consumable product of claim 31 wherein melting temperature of the combined first and second lipids in the composition is between 26°C to 30°C.

34. The consumable product of claim 31 wherein melting temperature of the combined first and second lipids in the composition is between 29°C to 33°C.

35. The consumable product of claim 31 wherein melting temperature of the combined first and second lipids in the composition is between 32°C to 36°C.

36. The consumable product of claim 31 wherein melting temperature of the combined first and second lipids in the composition is between 35°C to 39°C.

37. The consumable product of claim 31 wherein melting temperature of the combined first and second lipids in the composition is between 38°C to 42°C.

38. The consumable product of claim 31 wherein melting temperature of the combined first and second lipids in the composition is between 41°C to 45°C.

39. A method comprising:

a. obtaining a first lipid, wherein the first lipid is derivedfrom a plant source;

b. obtaining a second lipid, wherein the second lipid is derived from a plant source;

c. combining the first lipid and the second lipid to make a lipid composition with a melting temperature that is not equal to the melting temperature of either the first or second lipid; and

d. combining the lipid composition with a isolated and purified protein to make a consumable product.

40. A method comprising:

a. obtaining a first plant based fat-analogue and a second plant based fat-analogue, wherein the first and second plant based fat-analogues have different melting temperatures,

b. constructing a meat analogue composition by distributing the first and second plant based fat-analogues at non-identical locations in the composition.

41. The method of claim 40 where in the non-identical locations recapitulates the distribution of fats in a real meat.

42. A consumable comprising:

a. a plant based fat-analog with a melting temperature above room temperature and below body temperature, and

b. an isolated and purified protein.

43. A fat tissue replica, comprising a:

a. a protein composition, the protein composition comprising an isolated and purified non-animal protein;

b. fat droplets, the fat droplets comprising non-animal lipids; and

c. a three-dimensional matrix , the matrix comprising fibers derived from non-animal sources, wherein the fat droplets and protein solution are dispersed in said three-dimensional matrix, and wherein said three-dimensional matrix stabilizes a structure of the fat tissue replica.

44. The fat tissue replica of claim 43 wherein the fat droplets have a median size of less than 100 microns.

45. The fat tissue replica of claim 43 wherein a fat content of the fat tissue replica is greater than 50% by weight.

46. The fat tissue replica of claim 43 wherein when cooked at least 30% of the lipids are released from the matrix.

47. The fat tissue replica of claim 43 wherein the fat tissue replica has a tensile strength equivalent to that of an animal fat.

48. A connective tissue replica, comprising: a protein content comprising one or more isolated and purified proteins assembled into fibrous structures.

49. The connective tissue replica of claim 48 wherein the fibrous structures are stabilized by a cross-linking agent.

50. A connective tissue replica, comprising: a three-dimensional matrix comprisingfibers derived from non-animal source, and an isolated and purified plant protein.

51. The connective tissue replica of claim 50 wherein said fibers have an average diameter of between 10 and 300 microns.

52. The connective tissue replica of claim 50 wherein a tensile strength of the connective tissue replica is about equivalent to the tensile strength of connective tissue.

53. A meat replica, comprising:

a. a muscle replica unit, comprising a first isolated and purified protein;

b. a connective tissue replica unit, comprising a second isolated and purified proteins; and

c. a fat tissue replica unit comprising a proteincomposition with fat droplets suspended therein.

54. The meat replica of claim 53 wherein each of said muscle tissue, fat tissue, and connective tissue replica units are purposefully located within the meat replica to approximate the structure of meat.

55. The meat replica of claim 53 further comprising a heme protein.

56. The meat replica of claim 53 wherein the composition

a. does not contain meat,

b. looks and smells like meat before the composition is cooked; and

c. looks and smells like meat when the composition is cooked.

57. The meat replica of claim 56 wherein the replicated meat is ground beef.

58. The meat replica of claim 53 wherein the muscle tissue unit is 10mm3 or less.

59. The meat replica of claim 52 wherein the connective tissue unit is 10mm3 or less.

60. The meat replica of claim 53 wherein the fat tissue unit is 10mm3 or less.

61. A method of preparing a food or beverage product, comprising adding one or more isolated and purified proteins to said food or beverage product.

62. The method of claim 60 wherein said one or more isolated and purified proteins does not negatively affect the flavor, texture, or color of said food or beverage product.

63. A method for imparting a beef like flavor on a consumable comprising:

a. obtaining a consumable composition

b. adding to the consumable composition an iron-carrying protein with an amino acid sequence with at least 70% homology to a sequence listed in Table 1,

wherein after cooking the composition has a more beef like flavor than the compositon would have without the addition step.

64. A method for the production of a consumable comprising:

a. isolating and purifying proteins from plant sources.

b. recombining the proteins into an artificial mixture,

c. producing a consumable comprising the artificial mixture.

65. The method of claim 63 wherein the artificial mixture is gel.

66. The method of claim 63 wherein the recombining comprises enzymatic crosslinking.

67. The method of claim 63 wherein the recombining comprises thermal denaturation.

68. The method of claim 63 wherein the producing comprises extruding the artificial mixture to form fibers.

69. A liquid composition comprising:

a. an isolated and purified protein that does not alter the visual appearance or taste of the liquid as compared to the liquid without the addition of isolated and purified proteins.

70. The liquid composition of claim 68 wherein the beverage has an improved mouthfeel compared to a beverage without the isolated and purified protein.

71. The composition of claim 69 wherein the beverage contains ethanol.

72. The composition of claim 69 wherein the beverage is a cream liquor that contains no animal products further comprising: a non-dairy lipid emulsion.

73. A muscle tissue analogue comprising :

a. isolated plant based protein

b. a heme protein

c. a crosslinking enzyme and

d. NaCl,

wherein the final protein concentration is between 50 and 100 mg/ml.

74. A fat tissue replica comprising:

a. an emulsion comprising isolated plant protein and isolated plant lipids;

b. plant based fibers

wherein the fat replica has a tensile strength greater than 20 kPa.

75. A meat replica comprising:

a. a plant protein

b. a fat tissue replica that is solid a room temperature, and

c. a plant based lipid that is a liquid at room temperature.

76. A method for extending the shelf life of a meat free consumable product, wherein the product comprises a plant-derived heme protein, the method comprising a step of perfusing the product with carbon monoxide gas.

77. A method for extending the shelf life of a meat free consumable product, wherein the product comprises a plant-derived heme protein, the method comprising a step of reducing heme-Fe3+ to heme-Fe2+.

78. A method for making a meat replica comprising:

a. combining at an 80:20 ratio: a plurality of muscle replica pieces and a plurality of fat replica pieces, wherein an average individual volume of the pieces is at least 1mm3; and

b. grinding the combined composition of step a to make a fine size particle mix.

79. A method for making a poultry composition taste like beef comprising adding a heme protein to the poultry composition.

80. A chocolate flavored spread comprising:

a. sugar

b. cocoa powder, and

c. a cream fraction from a plant based milk.

ABSTRACT OF THE DISCLOSURE

Methods and compositions for the production of non-meat consumable products are desctibed herein. A meat substitute is described which is constructed from a muscle analogue, a fat analogue, and a connective tissue analogue.