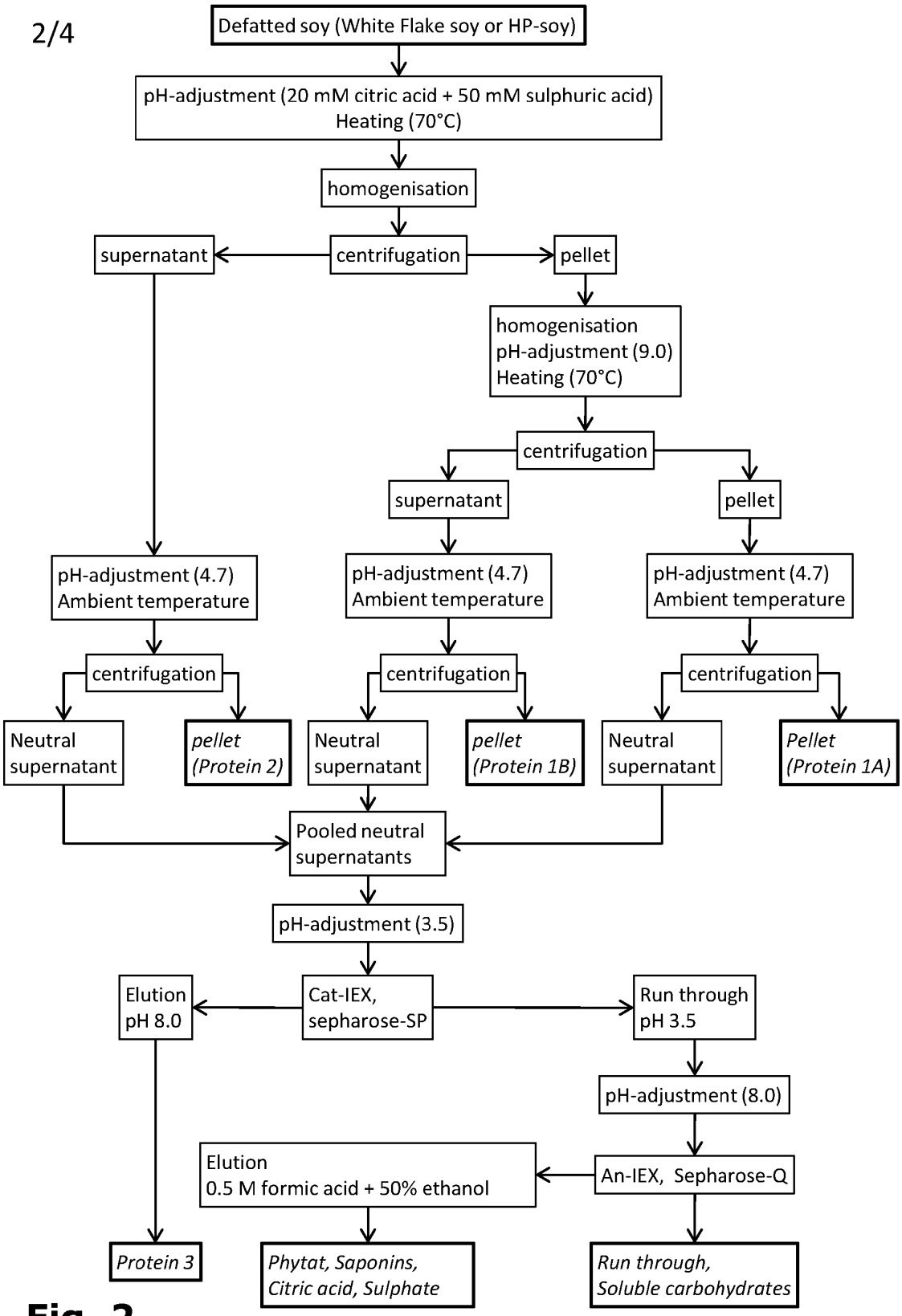
Defatted soy (White Flake soy or HP-soy)
pH-adjustment (20 mM citric acid + 50 mM sulphuric acid)
Heating (70°C)
homogenisation
supernatant
centrifugation
pellet
homogenisation
pH-adjustment (9.0)
Heating (70°C)
centrifugation
supernatant
pellet
pH-adjustment (4.7)
Ambient temperature
pH-adjustment (4.7)
Ambient temperature
pH-adjustment (4.7)
Ambient temperature
centrifugation
centrifugation
centrifugation
Neutral supernatant
pellet (Protein 2)
Neutral supernatant
pellet (Protein 1B)
Neutral supernatant
Pellet (Protein 1A)
Pooled neutral supernatants
pH-adjustment (3.5)
Elution pH 8.0
Cat-IEX, sepharose-SP
Run through pH 3.5
pH-adjustment (8.0)
Elution
0.5 M formic acid + 50% ethanol
An-IEX, Sepharose-Q
Protein 3
Phytat, Saponins, Citric acid, Sulphate
Run through, Soluble carbohydrates

Fig. 2

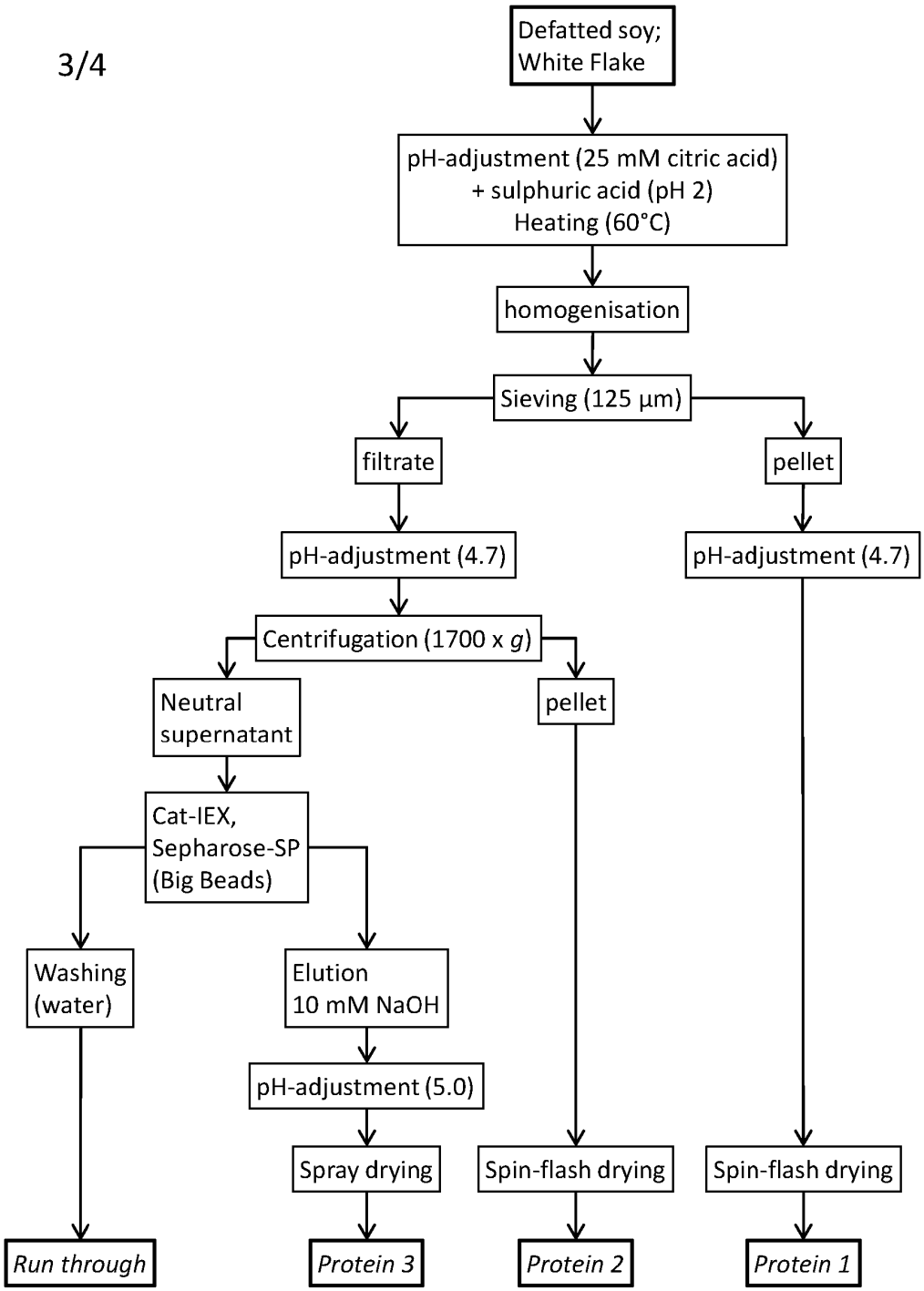
Defatted soy;
White Flake
pH-adjustment (25 mM citric acid)
+ sulphuric acid (pH 2)
Heating (60°C)
homogenisation
Sieving (125 µm)
filtrate
pellet
pH-adjustment (4.7)
pH-adjustment (4.7)
Centrifugation (1700 x g)
Neutral
supernatant
pellet
Cat-IEX,
Sepharose-SP
(Big Beads)
Washing
(water)
Elution
10 mM NaOH
pH-adjustment (5.0)
Spray drying
Spin-flash drying
Spin-flash drying
Run through
Protein 3
Protein 2
Protein 1

Fig. 3

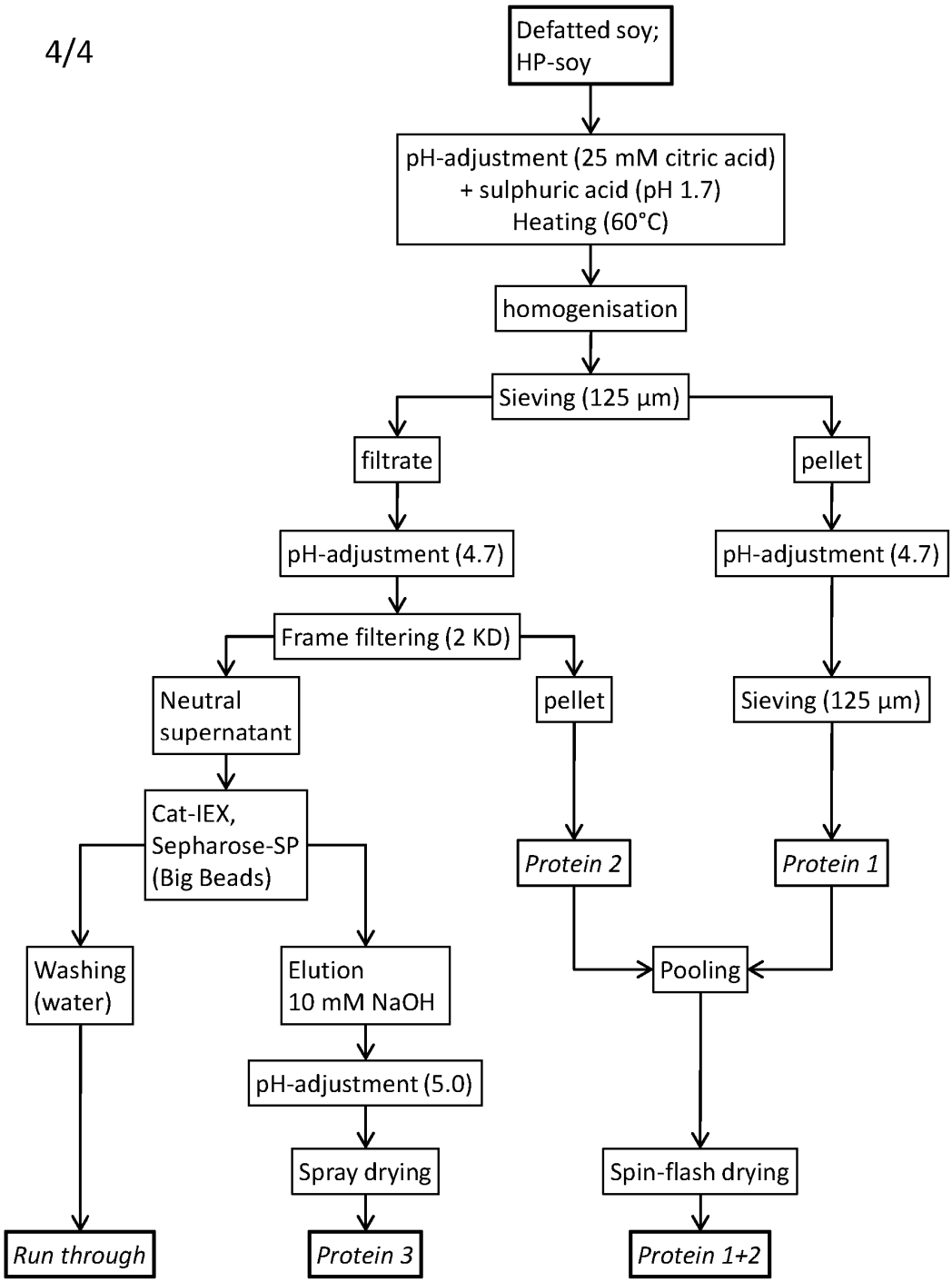
Defatted soy;
HP-soy
pH-adjustment (25 mM citric acid)
+ sulphuric acid (pH 1.7)
Heating (60°C)
homogenisation
Sieving (125 µm)
filtrate
pellet
pH-adjustment (4.7)
pH-adjustment (4.7)
Frame filtering (2 KD)
Neutral
supernatant
pellet
Sieving (125 µm)
Cat-IEX,
Sepharose-SP
(Big Beads)
Protein 2
Protein 1
Washing
(water)
Elution
10 mM NaOH
Pooling
pH-adjustment (5.0)
Spray drying
Spin-flash drying
Run through
Protein 3
Protein 1+2

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2012/050067

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23J1/00 A23J1/14 A23L1/20 A23L1/211 A23L1/216
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23J A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, FSTA, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/043438 A1 (MCN BIOPRODUCTS INC [CA]) 30 May 2003 (2003-05-30) abstract pages 1,2,9,10; claims 1,11; figures 1-4 -----	1-44
X	US 2010/063254 A1 (LOTZ MARTIN [DE] ET AL) 11 March 2010 (2010-03-11) paragraphs [0026], [0027], [0036], [0039], [0042], [0043], [0048], [0050], [0054], [0058]; claim 1 -----	1,44
A	US 4 697 004 A (PUSKI GABOR [US] ET AL) 29 September 1987 (1987-09-29) cited in the application abstract the whole document -----	1-44

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 June 2012	18/06/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	de La Tour, Camille

1

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INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2012/050067

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/DK2012/050067

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/DK2012/050067

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(74) Agents: **WILLIS, Michael** et al.; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, CA 94304-1050 (US).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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WO 2013/010037 A1

(54) Title: METHODS AND COMPOSITIONS FOR CONSUMABLES

(57) Abstract: Provided herein are methods and compositions for the production of cheese replicas. Generally the cheese replicas are produced by inducing the enzymatic curdling of non-dairy milks.

METHODS AND COMPOSITIONS FOR CONSUMABLES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. application Ser. No. 61/507,096 filed July 12, 2011, which is incorporated herein in its entirety by reference.

BACKGROUND OF THE INVENTION

[0002] Cheese making has relied on dairy milks as the major ingredient for more than 4000 years. Dairy cheese is made from curds formed from dairy milk. Dairy milks can readily be made to form curds suitable for making cheese by contacting the dairy milk with rennet (an aspartic protease which cleaves kappa-casein) at mildly acidic pH. Some cheeses, e.g., cream cheese, ricotta, cottage cheese and paneer, are made without rennet. In the absence of rennet, dairy cheese may be induced to curdle with acid (e.g., lemon juice, vinegar, etc.) or a combination of heat and acid. Acid coagulation can also occur naturally from starter culture fermentation. The strength of the curds depends on the type of coagulation. Most commercially produced cheeses use some type of rennet (animal, vegetable or microbial-derived) in their production.

[0003] The global dairy sector contributes an estimated 4 percent to the total global anthropogenic green house gas emissions. Producing 1 kg of cheddar cheese requires an average of 10,000 Liters of fresh water. Additionally, many individuals cannot digest and metabolize lactose. In these individuals enteric bacteria ferment the lactose, resulting in various abdominal symptoms, which can include abdominal pain, bloating, flatulence, diarrhea, nausea, and acid reflux. Additionally, the presence of lactose and its fermentation products raises the osmotic pressure of the colon contents. 3.4% of children in the U.S.A. are reported to have allergies to dairy milks. Many individuals choose to avoid milk for ethical or religious reasons.

[0004] Non-dairy milks, including plant-derived milks avoid many of the environmental, food sensitivity, ethical and religious problems associated with dairy milk and they can be made free of lactose, making the generation of dairy substitutes using the plant derived milks attractive. However, rennet is not an effective agent for inducing non-dairy proteins or emulsions, including plant-derived milks, including almond milk, chestnut milk, pecan milk, hazelnut milk, cashew milk, pine nut milk, and walnut milk, to curdle. Consequently, traditional cheese making techniques have not been successfully used to produce non-dairy cheese replicas.

[0005] Flavor and aroma in dairy cheese results in part from the degradation of lactose, proteins and fats, carried out by ripening agents, which include: bacteria and enzymes in the milk, bacterial cultures added during the cheese-making process, rennet, other proteases, lipases, added molds

and/or yeasts and bacteria and fungi that opportunistically colonize the cheese during ripening and aging.

[0006] Cheese replicas made principally of non-dairy ingredients are commercially available. Most of these cheese replicas include some dairy ingredients, for example, casein. Some commercially available cheese replicas contain no animal products. These include fermented cheese replicas made from nut milks from which insoluble carbohydrates have not been effectively removed, and made without using a protein crosslinking agent and several products in which a starch is a principal ingredient or containing agar, carrageenan and tofu to provide the desired texture. Most tasters consider none of the currently available cheese replicas to adequately replicate the taste, aroma and mouthfeel of dairy cheeses.

[0007] Complex carbohydrates in currently available cheese replicas made from nut milks have unfavorable effects on the texture, resulting in a product with a grainy mouthfeel and lacking the creaminess of dairy cheeses.

[0008] Starches that comprise the major gelling agent in many currently available cheese replicas lead to a relatively high carbohydrate content, which may be undesirable to consumers, for example those wishing to limit carbohydrate intake.

[0009] Because of these deficiencies, there is currently no cheese replica that is acceptable to most consumers as an alternative to traditional dairy cheeses.

[0010] Thus, it is clear that there is a great need in the art for an improved method and system for producing non-dairy cheese replicas while avoiding the shortcomings and drawbacks of the cheese replicas that have previously been available to consumers.

SUMMARY OF THE INVENTION

[0011] The invention relates to methods and compositions for non-dairy milk and cheese products, including without limitation, plant-derived milk and cheese products, as an alternative to dairy products for human consumption.

[0012] The invention provides a method for producing a non-dairy cheese by preparing an emulsion comprising proteins and fats from plants or other non-animal sources, inducing the emulsion to form a gel by enzymatic cross-linking of the proteins or denaturing the proteins, and producing a cheese-replica from the gel. In one embodiment, the emulsion contains less than 10% animal products. In one embodiment, the emulsion contains less than 8%, 7%, 6%, 5%, or 3% animal products. In one embodiment the emulsion contains no animal products. In one embodiment the inducing step of said method comprises adding an enzyme. In one embodiment of said method the enzyme used is transglutaminase. In one embodiment the enzyme used is Factor XIII (fibrin-stabilizing factor). In

one embodiment of said method the enzyme used is Keratinocyte transglutaminase (TGM1). In one embodiment the enzyme used is Tissue transglutaminase (TGM2). In one embodiment of said method the enzyme used is Epidermal transglutaminase (TGM3). In one embodiment the enzyme used is Prostate transglutaminase (TGM4). In one embodiment the enzyme used is TGM X (TGM5). In one embodiment of said method the enzyme used is TGM Y (TGM6). In one embodiment of said method the enzyme used is TGM Z (TGM7). In one embodiment, the enzyme is a transglutaminase from *Streptoverticillium mobaraense*. In one embodiment, the enzyme is similar or identical to a transglutaminase from *Streptoverticillium mobaraense*. In one embodiment, the enzyme is a lysyl oxidase. In one embodiment the emulsion is non-dairy milk. In one embodiment at least one of the plant sources are nuts. In one embodiment at least one of the plant sources are legumes. In one embodiment at least one of the plant sources are seeds. In one embodiment at least one of the plant sources are leaves. In one embodiment at least one of the plant sources are fruit from the family Fabaceae. In one embodiment at least one of the non-animal sources is a species of bacteria. In one embodiment at least one of the non-animal sources is a species of archaea. In one embodiment at least one of the non-animal sources is a species of fungus. In one embodiment at least one of the non-animal sources is a species of algae. In one embodiment the emulsion is a non-dairy milk. In one embodiment the nuts are one or more of following: almonds, cashews, brazilnuts, chestnuts, coconuts, hazelnuts, macadamia nuts, peanuts, pecans, pistachios or walnuts. One embodiment of said method has an additional step of adding a sugar to the emulsion. In one embodiment the sugar added to the emulsion is a monosaccharide. In one embodiment the sugar added to the emulsion is a disaccharide. In one embodiment said method has an additional step of inoculating with lactic acid bacteria. In the embodiment of inoculating with lactic acid bacteria the invention provides an addition step of allowing growth of the bacterial cultures. In one embodiment the method can include an additional step of adding one or more of the following *Penicillium camemberti, Geotrichum candidum, Penicilliem roqueforti, Penicillium nalgiovensis, Verticillium lecanii, Kluyveromyces lactis, Saccharomyces cerevisiae, Candida utilis, Debaryomyces hansensil, Rhodosporidum infirmominiatum, Candida jefer, Cornybacteria, Micrococcus sps., Lactobacillus sps.,Lactococcus, Staphylococcus, Halomonas, Brevibacterium, Psychrobacter, Leuconostocaceae, Streptococcus thermophilus, Pediococcus sps., or Propionibacteria.* In one embodiment the method can include an additional step of adding one or more of the following *halophilc* bacterium or *archaea.* In one embodiment the invention provides for the use of thermal denaturing and cross-linking. In one embodiment the invention provides thermal denaturing used without the addition of any enzymes. One embodiment of said method has an additional step of cutting the gel. One embodiment of said method has an additional step of draining and shaping the cut gel. One embodiment of said method

has an additional step of adding a flavoring component. In one embodiment the flavoring component is one or more species of bacteria. In one embodiment the flavoring component is lactic acid bacteria. In one embodiment the flavoring component is a mold. In one embodiment the flavoring component is yeast. In one embodiment the method includes concentrated proteins prior to preparing the emulsion. In one embodiment the concentrated proteins are each essentially homogeneous proteins. In one embodiment the method includes the additional step of concentrating proteins and fats from one or more plant sources. In one embodiment the method includes the additional step of concentrating proteins and fats from one or more non-animal sources. In one embodiment the method includes the additional step of isolating the proteins and fats from one or more plant sources. In one embodiment the method includes the additional step of isolating the proteins and fats from one or more non-animal sources. In one embodiment the method includes the additional step of purifying the proteins and fats from one or more plant sources. In one embodiment the method includes the additional step of purifying the proteins and fats from one or more non-animal sources. In one embodiment the invention provides for the concentrated proteins to come from the same essentially homogeneous plant-derived protein. In one embodiment the invention provides for the concentrated proteins to come from the same essentially homogeneous non-animal-derived protein. In one embodiment the invention provides for the concentrated proteins to come from separate plant species. In one embodiment the invention provides for the concentrated proteins to come from separate non-animal species.

[0013] In addition, the invention provides steps for preparation to undertake the claimed methods of producing a non-animal based cheese including (a) obtaining nuts or seeds, and (b) surface sterilizing the nuts or seeds. In one embodiment the method uses nuts or seeds as the plant sources. In one embodiment the surface sterilization step is blanching procedure. In one embodiment the preparation method can also include a step for washing the nuts or seeds. In one embodiment the method of preparation can also include the step of decompounding the nuts or seeds. In one embodiment the step of decompounding the nuts or seeds is a blending procedure. In one embodiment the method of preparation can also include centrifugation. In one embodiment the method of preparation can also includes a centrifugation procedure that results in the removal of at least 85% of the suspended solids. In one embodiment the method of preparation can also includes a centrifugation procedure that results in the removal of at least 75%, 65%, 55%, or 45% of the suspended solids.

[0014] The invention provides a method comprising decompounding nuts or seeds in water, removing at least 85% of the suspended solids and adding a transglutaminase to catalyze the formation of crosslinks between proteins from the nuts or seeds.

[0015] The invention provides a method for making a non-dairy cheese replica comprising, obtaining nut milk, centrifugation of the nut milk to remove insoluble matter, and crosslinking proteins within the nut milk with transglutaminase.

[0016] In one embodiment the invention provides a composition comprising centrifuged non-dairy milk wherein at least 85% of the insoluble solids have been removed relative to the non-dairy milk prior to centrifugation.

[0017] In one embodiment the invention provides a composition comprising a non-dairy milk with less than 30% polysaccharides. In one embodiment the non-dairy milk composition has less than 10% polysaccharides. In one embodiment the non-dairy milk composition less than 1% polysaccharides. In one embodiment the non-dairy milk composition is entirely composed of ingredients derived from non-animal sources. In one embodiment the non-dairy milk composition is at least comprised of 20% protein isolated from a single plant species.

[0018] In one embodiment the invention provides for a composition comprised of non-dairy milk and a cross-linking enzyme. In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is Factor XIII (fibrin-stabilizing factor). In one embodiment of the non-dairy milk and a cross-linking enzyme composition, the enzyme is a transglutaminase from *Streptoverticillium mobaraense*. In one embodiment of the non-dairy milk and a cross-linking enzyme composition, the enzyme is similar or identical to a transglutaminase from *Streptoverticillium mobaraense*. In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is Keratinocyte transglutaminase (TGM1). In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is Tissue transglutaminase (TGM2). In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is Epidermal transglutaminase (TGM3). In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is Prostate transglutaminase (TGM4). In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is TGM X (TGM5). In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is TGM Y (TGM6). In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is TGM Z (TGM7). In one embodiment the invention provides for a composition comprised of non-dairy milk and a cross-linking enzyme. In one embodiment of the non-dairy milk and a cross-linking enzyme composition the cross-linking enzyme is lysyl oxidase. In one embodiment of the composition comprising non-dairy milk and a cross-linking enzyme, protein crosslinks are formed between glutamine and lysine side chains of respective protein constituents.

[0019] In one embodiment of the non-dairy milk and a cross-linking enzyme composition, at least 85% of the insoluble solids have been removed by centrifugation. In one embodiment of the non-dairy milk and a cross-linking enzyme composition, proteins from non-animal sources comprise at least 50% of the total mass of the composition. In one embodiment of the non-dairy milk and a cross-linking enzyme composition the starch content is less than 1% by mass. In one embodiment of the non-dairy milk and a cross-linking enzyme composition is the starch content is less than 5% by mass. One embodiment of the non-dairy milk and a cross-linking enzyme composition has less than 1% insoluble carbohydrate by mass. One embodiment the non-dairy milk and a cross-linking enzyme composition has less than 5% insoluble carbohydrate by mass. One embodiment the non-dairy milk and a cross-linking enzyme composition has less than 5% starch and less than 1% insoluble carbohydrate by mass. One embodiment the non-dairy milk and a cross-linking enzyme composition has less than 5% starch and less than 5% insoluble carbohydrate by mass. In one embodiment the non-dairy milk and a cross-linking enzyme composition has a polysaccharide content less than 1% by mass. In one embodiment the non-dairy milk and a cross-linking enzyme composition has a polysaccharide content less than 5% by mass. In one embodiment the non-dairy milk and a cross-linking enzyme composition has a carbohydrate content less than 1% by mass. In one embodiment the non-dairy milk and a cross-linking enzyme composition has a carbohydrate content less than 5% by mass. In one embodiment the non-dairy milk and a cross-linking enzyme composition has a carbohydrate content less than 10% by mass. In one embodiment of the non-dairy milk and a cross-linking enzyme composition, at least 80% of the protein content comprises a single monomeric or multimeric protein.

[0020] In some embodiments the invention provides for a composition comprising covalently crosslinked non-dairy proteins, a crosslinking enzyme, and cheese microbes. In one embodiment of the composition of covalently crosslinked non-dairy proteins, a crosslinking enzyme, and cheese microbes composition, the proteins are not from soy. In one embodiment of the composition comprising covalently crosslinked non-dairy proteins, a crosslinking enzyme, and cheese microbes, less than 5% of the proteins are from soy. In one embodiment the composition comprises less than 20% insoluble solids. In one embodiment the covalently crosslinked non-dairy proteins, a crosslinking enzyme, and cheese microbes composition also contains oils or fats isolated from non-dairy sources. In one embodiment of the composition comprising covalently crosslinked non-dairy proteins, a crosslinking enzyme, and cheese microbes the crosslinking enzyme of the composition is a transglutaminase. In one embodiment of the composition comprising covalently crosslinked non-dairy proteins, a crosslinking enzyme, and cheese microbes the crosslinking enzyme of the composition is a lysyl oxidase. In one embodiment of the composition comprising covalently

crosslinked non-dairy proteins, a crosslinking enzyme, and cheese microbes the cross-linking enzyme is Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6), TGM Z (TGM7. In one embodiment of the composition comprising non-dairy milk and a cross-linking enzyme and cheese microbes, the enzyme is a transglutaminase from *Streptoverticillium mobaraense*. In one embodiment of the composition comprising non-dairy milk and a cross-linking enzyme and cheese microbes, the enzyme is similar or identical to a transglutaminase from *Streptoverticillium mobaraense*. In one embodiment of the composition comprising non-dairy milk and a cross-linking enzyme and cheese microbes, protein crosslinks are formed between glutamine and lysine side chains of respective protein constituents. In one embodiment of the composition comprising non-dairy milk and a cross-linking enzyme and cheese microbes, protein crosslinks are formed between lysine side chains of respective protein constituents.

[0021] In some embodiments the invention provides for a cheese replica composition comprised of a gelled emulsion of non-dairy proteins and fats. In one embodiment the cheese replica composition has between 10% and 40% proteins from non-dairy sources and between 0% and 65% fats from non-dairy sources. In one embodiment the cheese replica composition further comprises a crosslinking enzyme selected from the group consisting of Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6) or TGM Z (TGM7). In one embodiment of the cheese replica composition, the enzyme is a transglutaminase from *Streptoverticillium mobaraense*. In one embodiment of the cheese replica composition, the enzyme is similar or identical to a transglutaminase from *Streptoverticillium mobaraense*. In one embodiment of the cheese replica composition, protein crosslinks are formed between glutamine and lysine side chains of respective protein constituents. In one embodiment of the cheese replica composition, protein crosslinks are formed between lysine side chains of respective protein constituents. In some embodiment of the cheese replica composition, formation of protein crosslinks is catalyzed by a lysyl oxidase.

[0022] In one embodiment the cheese replica composition further comprises cheese-making microbes. In one embodiment the cheese replica composition has one or more cheese-making microbes from the group consisting of *Penicillium camemberti, Geotrichum candidum, Penicilliem roqueforti, Penicillium nalgiovensis, Verticillium lecanii, Kluyveromyces lactis, Saccharomyces cerevisiae, Candida utilis, Debaryomyces hansensil, Rhodosporidum infirmominiatum, Candida jefer, Cornybacteria, Micrococcus sps., Lactobacillus sps., Lactococcus, Staphylococcus,*

Halomonas, Brevibacterium, Psychrobacter, Leuconostocaceae, Streptococcus thermophilus, Pediococcus sps., or *Propionibacteria.*

[0023] In some embodiments the invention provides for a cheese replica composition, wherein the cheese replica is a cheese equivalent according to a human. In some embodiments the invention provides for a cheese replica composition, wherein human subjects cannot distinguish the cheese replica from a dairy cheese. In one embodiment of the cheese replica at least 20% of the protein content comprises a single monomeric or multimeric protein. In one embodiment the cheese replica composition is made without the addition of starches or rennet or any other extrinsic proteases other than those contributed by microbial cultures. In one embodiment the cheese replica composition has a pH less than 5.5 where the acidification was accomplished solely by microbial fermentation. . In one embodiment the cheese replica composition has a pH less than 5 where the acidification was accomplished solely by microbial fermentation. In one embodiment the cheese replica composition has a pH less than 6AS$DZx where the acidification was accomplished solely by microbial In one embodiment the cheese replica composition is contains no animal products. In one embodiment the cheese replica has less than 5% by mass insoluble carbohydrates. In one embodiment the cheese replica composition contains no rennet, vinegar, or lemon juice.

[0024] In some embodiments the invention provides for a cheese replica composition made with the addition of rennet or any aspartic protease or any other type protease (e.g., serine protease) to affect or enhance the flavor and /or aroma and /or texture of the cheese replica.

[0025] In some embodiments the invention provides for a cheese replica composition made with the addition of vinegar, lemon juice or any other type of acid to affect or enhance the flavor and /or aroma and/or texture of the cheese replica.

[0026] In some embodiments the invention provides for a soft fresh cheese replica composition composed of pasteurized almond milk, pasteurized macadamia nut milk, mesophilic starter culture, transglutaminase, water, and salt. In one embodiment the soft fresh cheese replica composition includes the addition of vinegar. In one embodiment the soft fresh cheese replica composition includes the addition of microbial coagulant. In one embodiment the soft fresh cheese replica composition includes the addition of vinegar and microbial coagulant.

[0027] In some embodiments the invention provides for a salted cheese replica composition composed of pasteurized almond milk, pasteurized macadamia nut milk, mesophilic starter culture, transglutaminase, and water. In one embodiment the salted cheese replica composition includes the addition of vinegar. In one embodiment the salted cheese replica composition includes a microbial coagulant. In one embodiment the salted cheese replica composition includes vinegar and a microbial coagulant.

[0028] In some embodiments the invention provides for a soft ripened cheese replica composition composed of pasteurized almond milk, pasteurized macadamia nut milk, mesophilic starter culture, flora danica, *Geotrichum candidum, Penicillium candidum, Debaromyces hansenii,* transglutaminase, water, and salt. In one embodiment the soft ripened cheese replica composition includes vinegar. In one embodiment the soft ripened cheese replica composition includes a microbial coagulant. In one embodiment the soft ripened cheese replica composition includes vinegar and microbial coagulant.

[0029] In some embodiments the invention provides for a goat cheese replica composition composed of pasteurized almond milk, macadamia nut milk, mesophilic starter culture, transglutaminase, water, and salt. In one embodiment the goat cheese replica composition includes vinegar. In one embodiment the goat cheese replica composition includes a microbial coagulant. In one embodiment the goat cheese replica composition includes vinegar and a microbial coagulant.

[0030] In some embodiments the invention provides a method for making a cheese replica comprising removing from a non-dairy milk a proportion of insoluble solids. In some embodiments the invention provides a method for making a cheese replica comprising removing from a plant-based milk a proportion of insoluble solids. In some embodiments the invention provides a method comprising centrifuging a non-dairy milk to sediment a proportion of insoluble solids. In some embodiments about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 100% of the insoluble solids are removed.

[0031] In some embodiments the isolated or enriched proteins of the methods and compositions of the invention comprise one or more of: ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

[0032] In some embodiments the invention provides a composition comprising (A)covalently crosslinked plant-derived proteins from a set comprising ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases,

glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, and moong seed storage 8S globulin; (B) a crosslinking enzyme, and (C) cheese microbes.

[0033] In some embodiments the invention provides a composition comprising (A)covalently crosslinked plant-derived proteins from a set comprising ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, and moong seed storage 8S globulin.

INCORPORATION BY REFERENCE

[0034] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0036] **Figure 1** shows freshly cut curds prepared by the method used to produce the soft fresh cheese replicas and salted cheese replicas described in the examples.

[0037] **Figure 2** shows drained curds prepared by the method used to produce the soft fresh cheese replicas and salted cheese replicas described in the examples.

[0038] **Figure 3** shows soft fresh cheese replicas made from non-dairy milk.

[0039] **Figure 4** shows Annatto-colored cheese replicas made from non-dairy milk.

[0040] **Figure 5** shows salted cheese replica made from non-dairy milk.

[0041] **Figure 6** shows salted cheese replica with paprika and fennel pollen made from non-dairy milk.

[0042] **Figure 7** shows waxed cheese replica made from non-dairy milk.

[0043] **Figure 8** shows soft ripened cheese replica made from non-dairy milk

[0044] **Figure 9** shows goat cheese replicas made from non-dairy milk (A) with black pepper (B) with chives.

[0045] **Figure 10** shows cheese replica gels made from partially purified plant proteins and vegetable oil, cultured with mesophilic culture MA11 (Danisco): (A) depicts cultured cheese replicas made from 2% Pea albumin fraction, 3% vegetable oil, heated and crosslinked with transglutaminase; (B) depicts cultured cheese replicas made from 2% Moong bean 8S globulin fraction, 3% vegetable oil, crosslinked with transglutaminase, without prior heating; (C) depicts cultured cheese replicas made from 7.5% Pea globulin fraction, 3% vegetable oil, heated and crosslinked with transglutaminase; (D) depicts cultured cheese replicas made from 7.5% Moong bean 8S globulin fraction, 3% vegetable oil, crosslinked with transglutaminase, without prior heating of proteins.

DETAILED DESCRIPTION OF THE INVENTION

[0046] The present invention discloses methods and compositions based upon the realization that transglutaminase efficiently crosslinks the proteins in many non-dairy milks to produce a soft, moist, elastic gel (a "curdling" of non-dairy milks). This process allows the separation of the crosslinked proteins and associated fats from the "whey." The crosslinked proteins can hold a fat emulsion, and have the essential physical characteristics needed for pressing, culturing and ripening a cheese replica derived from non-dairy milk. In various embodiments the current invention includes cheese replicas principally, entirely or partially composed of ingredients derived from non-animal sources. In additional embodiments the present invention includes methods for making cheese replicas from non-animal sources. In various embodiments these results are achieved by replicating the curdling process of cheese making in non-dairy milks using enzymes.

[0047] The term a cheese "substitute" or "replica" can be any non-dairy product that serves a role as food or in food that is commonly served by traditional dairy cheese. A cheese "substitute" or "replica" can be a product that shares visual, olfactory, textural or taste characteristics of cheese such that an ordinary human observer of the product is induced to think of traditional dairy cheese.

[0048] A purified protein can be a preparation in which the cumulative abundance by mass of protein components other than the specified protein, which can be a single monomeric or multimeric protein species, is reduced by a factor of 3 or more, or a factor of 5 or more, or a factor of 10 or more relative to the source material from which the specified protein was said to be purified.

[0049] The term "homogeneous" can mean a single protein component comprises more than 90% by mass of the total protein constituents of a preparation.

[0050] The term resemble can mean one composition having characteristics recognizably similar to another composition by an ordinary human observer.

[0051] The term "indistinguishable" can mean that an ordinary human observer would not be able to differentiate two compositions based on one or more characteristics. It is possible that two compositions are indistinguishable based on one characteristic but not based on another, for example two compositions can have indistinguishable taste while having colors that are different. Indistinguishable can also mean that the product provides an equivalent function as or performs an equivalent role as the product for which it is substituting.

I. Non-dairy milks

[0052] Cheese replicas may be made using non-dairy milk prepared from nuts or plant seeds. For example almonds, cashews, brazilnuts, chestnuts, coconuts, hazelnuts, macadamia nuts, peanuts, pecans, pistachios or walnuts can provide non-dairy milk that, in various embodiments of the invention, is used to produce cheese replicas. Nuts can include so called "true nuts" as well as a wide variety of dried seeds from of plants. Any large, oily kernel found within a shell and used in food may be regarded as a nut. Plant seeds can include a wide variety embryonic plants enclosed in a seed coat. Plant seeds can include for example legumes, cereals, and gymnosperms. Non-dairy products or compositions include products or compositions where the constituent proteins, fats and/or small molecules can be isolated from or secreted by plants, bacteria, viruses, archaea, fungi, algae, or they can be made synthetically by chemical synthesis or *in vitro*. Non-dairy products are generally not derived from cows, goats, buffalo, sheep, horses, camels, or other mammals. In some embodiments non-dairy products do not contain dairy proteins. In some embodiments non-dairy products do not contain dairy fats. In some embodiments non-dairy products do not contain enzymes derived from an animal.

[0053] The nuts or seeds of the present invention can be raw. In some embodiments the nuts or seeds used to produce non-dairy milk are all raw. Alternatively some or all of the nuts or seeds used in the production of a non-dairy milk can be processed. The processed nuts or seed can be roasted, dry roasted, toasted, or baked.

[0054] Milk, or non-dairy milk, can mean an emulsion comprising proteins and fats or a solution or suspension of proteins, sometimes further comprising other solutes that might include carbohydrates, salts and other small molecules that contribute to flavor, texture, emulsion stability, protein solubility or suspension stability, or its ability to support growth of microbial cultures used in making cheese replicas, yogurt replicas, or other replicas of cultured dairy products.

[0055] The non-dairy milk can be made by a method comprising preparing the nuts or plant seeds with processing steps such as sterilizing, blanching, shocking, decompounding, centrifugation, or washing.

[0056] The non-dairy milks can be produced by decompounding the nuts or plant seeds, for example by grinding or blending or milling the nuts in a solution comprising water. In various embodiments alternative methods for decompounding the nuts or dried seeds include crushing, tumbling, crumbling, atomizing, shaving, pulverizing, grinding, milling, water eroding (for example with a water jet), or finely chopping the nuts or plant seeds. In some embodiments the decompounding step takes place in a blender. In some embodiments the decompounding is in a continuous flow grinder. In some embodiments the decompounding is in a continuous flow mill. The decompounding can be followed by a sorting, filtering, screening, or separation step. In some embodiments the decompounded nuts or seeds can be stored prior to the formation of a non-dairy milk. In some embodiments an aqueous solution is added before, during, or after the decompounding.

[0057] The nuts or seeds used in some embodiments of the invention to make non-dairy milks may have contaminants on the surface which would make a non-dairy milk unsafe or unpalatable. Accordingly the nuts or seeds can be washed prior to use. The nuts or seeds can also be sterilized to remove, reduce, or kill any contaminants on the surface of the nuts or seeds. A sterilization step can be an irradiation step, a heat step (e.g. steam sterilization, flaming, or dry heat), or a chemical sterilization (e.g, exposure to ozone). In some embodiments the sterilization step kills more than 95% of microbes on the nuts or seeds. In some embodiments the sterilization step kills more than 99% of microbes on the nuts or seeds. Blanching is a process wherein the food is exposed to hot water (e.g. boiling), removed after a brief timed interval, and finally cooled by exposure to cold water (e.g. iced or cold running water). When nuts, such as almonds or pistachios, are blanched, the skin of the nut (botanically the seed coat surrounding the embryo) softens and can be easily removed later. Accordingly, in some embodiments the invention provides for non-dairy milk compositions with a reduced percentage of components found in the skin of the nut or seed. For example the composition can have 50%, 40%, 30%, 20%, 10% or less than 5% of the seed coat remaining after the preparation process. In some embodiments the invention provides for methods for making a non-dairy milk comprising removing the seed coat.

[0058] In one embodiment the blanching procedure is as follows: Place nuts into 212°F heated water and blanch for 30 seconds. Drain nuts. Immediately move drained nuts into cold water. In some embodiments the temperature of the water is altered by 5%, 10% or 20%. In some embodiments the cold water temperature is about 0°C, 5°C, 10°C, or 20°C. In some embodiments the blanching takes about 10, 20, 30, 40, 50 seconds or about 1, 2, or 5 minutes.

[0059] In some embodiments the nuts or seeds can be hydrated, for example by immersion in water for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours. In some embodiments the hydration step lasts multiple days. In some embodiments the water contains other components such as salts or preservatives. In some embodiments the water is maintained at a constant cool temperature. In some embodiments the hydration step can occur prior to the decompounding step.

[0060] In some embodiments the nuts or seeds can be dried, for example by exposure to a low humidity and/or heated environment for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours. In some embodiments the drying step lasts multiple days. The drying step can occur prior to the decompounding step or the drying step can occur after a decompounding step.

[0061] In one embodiment the decompounding step is a blending procedure as follows: Place nuts in a clean and sanitized blender. Add clean fresh water to the blender. Turn on the blender and gradually increase speed up to maximum, blending for 5 minutes. Accumulate blended slurry in clean and sanitized ice cold container. Immediately begin rapid cooling of slurry by stirring slurry. Once the slurry has cooled to 50°F, move slurry to refrigerator to continue cooling to below 41°F. Allow slurry to rest at 36°F overnight or up to 12 hours at 36°F. In some embodiments the blending times and speeds are altered by up to 100%. In some embodiments the temperatures are altered up to 20°.

[0062] Insoluble solids may be present in milks prepared by decompounding (e.g. grinding or blending) nuts or seeds. One surprising observation of the present invention is that these insoluble materials can hinder the formation of curds from the non-dairy milks. Insoluble solids can also result in curds or cheese replicas having a texture or mouthfeel perceived as grainy or pasty in comparison to the smoother, creamier texture of dairy cheese. Accordingly provided herein are methods for removing these insoluble materials and compositions of non-dairy milks and cheese replicas with reduced amounts of the insoluble materials. Compositions of the invention can have a mouthfeel or texture indicative of the absence of insoluble solids. For example a cheese replica can have a mouthfeel indicative of the absence of insoluble solids. The insoluble materials can comprise carbohydrates.

[0063] In some embodiments the methods for producing cheese replicas includes a step for the removal of solids from non-dairy milks prior to curdling. For example, in some embodiments, the non-dairy milks are centrifuged to remove the insoluble solids. In some embodiments the present invention provides for a non-dairy cheese replica with less than 1%, 5%, 10%, 20%, 30%, 40% or 50% of insoluble solids found in the non-dairy milk before removal of the insoluble solids. In some embodiments the present invention provides for a non-dairy milk extract with less than 1%, 5%, 10%, 20%, 30%, 40% or 50% of insoluble solids found in non-dairy milk. In some embodiments the present invention provides for a non-dairy milk with 99%, 95%, 90%, 80%, 70%, 60% or 50% of insoluble solids found in non-dairy milk removed.

[0064] In one embodiment the centrifugation procedure is as follows: Pour decompounded nut or seed slurry into container. Using a centrifuge with the JS-5.0 rotor,spin at about 5000 RPM for 30 mins. In some embodiments the spin is at about 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, or 10,000 RPM for about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, or 60 minutes. In some embodiments the centrifugation speed or time is altered, for example by 20%.

[0065] In one embodiment the centrifugation procedure is as follows: Pour decompounded nut or seed slurry at a flow rate of 4-20 gallons per minute, for example, at a flow rate of 8 gallons per minute, into decanter centrifuge (such as the Flottweg S4 sedicanter), spinning at a bowl speed of between 3000 and 5000 RPM. Collect the liquid outflow for further use as a non-dairy milk, and set aside the ejected solids.

[0066] Other methods for removal of solids from non-dairy milks include, but are not limited to, straining, filtering, allowing to settle, skimming, or using coagulants and flocculants (including cations, polymer flocculants, or polyelectrolytes such as pectin, carrageenan, alginates, or carboxymethyl cellulose) to agglomerate particles for removal.

[0067] Centrifugation step can result in a “cream layer” and a “skim layer”. The cream layer is an emulsion comprising fats, proteins and water. The skim layer is a solution comprising proteins in water,. In some embodiments, the cream layer and skim layer are separated by centrifugal separation. In some embodiments, the cream layer and skim layer are separated by centrifugal separation in a Flotwegg ac1500 or GEA ME55. In some embodiments the cream layer and the skim layer are incompletely separated. The skim layer and the cream layer can be separated from the insoluble solids in a separation process. In some embodiments the skim layer and the cream layer are stored separately. The non-dairy milk can comprise the skim layer. The non-dairy milk can comprise the cream layer. Typically the skim layer and the cream layer are combined to form the non-dairy milk. The non-dairy milk can be up to 100% cream layer. In some embodiments the ratio

of cream layer to skim layer in the non-dairy milk is about 100:1, 90:1, 80:1, 70:1, 60:1, 50:1, 40:1, 30:1, 20:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:20, 1:30, 1:40, 1:50, or 1:60. In some embodiments the methods described herein comprise measuring the amount of skim layer and cream layer being added to a non-dairy milk.

[0068] In one embodiment the separation procedure is as follows: Remove cream layer from centrifuged bucket and place in new container that is buried in an ice bath. Move the liquid layer (skim layer) from the bucket into a container that is ice cold. Keep skim and cream ice cold at all times.

[0069] In another embodiment the separation procedure is as follows: Flow the non-dairy milk, from which insoluble solids have been substantially removed by passage through a decanter centrifuge, into a separator centrifuge, for example, a Flotwegg ac1500 or GEA ME55. In some embodiments, the cream layer and skim layer are separated by centrifugal separation in the separator centrifuge. In some embodiments the separated skim and cream layers are kept refrigerated.

[0070] In some embodiments the non-dairy milk is pasteurized or sterilized. The pasteurization can be high-temperature, short-time (HTST), "extended shelf life" (ESL) treatment, or ultra-high temperature (UHT or ultra-heat-treated). In some embodiments the pasteurization procedure is as follows: Pasteurize blended non-dairy milk at 164°F -167°F for 16 seconds. Using controlled chilling system, bring non-dairy milk temperature down rapidly and store in a refrigerator at 36°F.

[0071] Cheese replicas may also be made using non-dairy milk prepared using proteins isolated, enriched, or purified from a plant, or microbial source or synthesized *in vitro*. For example ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco) can isolated from a plant source and included in a non-dairy milk for the production of a cheese replica. For example 8S globulins can isolated from a Moong beans and included in a non-dairy milk for the production of a cheese replica. For example Pea globulins can isolated from Pea seeds and included in a non-dairy milk for the production of a cheese replica. For example Pea albumins can isolated from peas and included in a non-dairy milk for the production of a cheese replica. Isolated, enriched, or purified plant proteins can be combined with one or more oils or fats also isolated from plant sources, in a colloidial suspension, solution or emulsion to form the non-dairy milk for making a cheese replica. In some embodiments the isolated non-dairy proteins are combined with non-non-dairy oils or fats to form a non-dairy milk. In some embodiments multiple isolated, enriched or purified plant proteins are used to make a non-dairy milk. Without being bound by theory the non-dairy milks derived from isolated, enriched, or purified proteins may reduce the problems which can be caused by insoluble solids obtained in a slurry derived from a nut or seed. Without being bound by theory the non-dairy milks derived from isolated, enriched, or purified proteins may reduce the problems which can be caused by proteins

with undesirable properties, such as lipoxygenases or proteases. Additional non-limiting examples of proteins that may be isolated, enriched, or purified from a plant source for the production of a non-dairy milk for cheese replica production include: seed-storage proteins from any seed, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin. Non-limiting examples of oils that may be incorporated in a non-dairy milk include: corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, 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.

[0072] In some instances the proteins used to make the non-dairy milk may be purified proteins. Accordingly, in some embodiments the total protein content of the cheese replica can, in some

embodiments, consist of greater than 25%, 50%,75%, or 90%, of a purified protein, for example any single monomeric or multimeric protein.

[0073] Non-dairy milk compositions can also contain sugars or other fermentable carbon sources and other nutrients. Without being bound by theory these sugars or nutrients can favor growth of the cheese culture microbes or serve as substrates for production of lactic acid or other organic acids by the cheese culture microbes. For example, in some embodiments, the non-dairy milk comprises glucose. In other embodiments, the non-dairy milk comprises fructose. In other embodiments, the non-dairy milk comprises sucrose. In other embodiments, the non-dairy milk comprises high-fructose corn syrup. In other embodiments, the non-dairy milk comprises sugar-cane extract. In other embodiments, the non-dairy milk comprises a fruit juice. In other embodiments, the non-dairy milk comprises sugar-cane extract. In other embodiments, the non-dairy milk comprises agave syrup. In other embodiments, the non-dairy milk comprises molasses. Molasses can be cane molasses or beet molasses or made from other non-dairy sources. In other embodiments the non-dairy milk can comprises treacle, honey, refined sugars, or syrup (e.g high fructose corn syrup). In some embodiments fuel for glycolosis, or a component (or intermediate) of the glycolysis pathway, can be included in the compositions. Oligosaccharides can be a part of the non-dairy milk. Disaccharides (eg., maltose, sucrose) can be part of a non-dairy milk. Monosaccharides (e.g. fructose, glucose, or galactose) can be a part of the non-dairy milk. In some embodiments the sugars are added as an addition step. In some embodiments the sugars are not derived from the same organism as the proteins and fats in the composition.

[0074] In some embodiments a sugar in the composition of the invention is not treacle, honey, refined sugars, or syrup (e.g high fructose corn syrup). In some embodiments a sugar in the composition of the invention is not a monosaccharide (e.g. fructose, glucose, or galactose). In some embodiments a sugar in the composition of the invention is not a disaccharide. In some embodiments a sugar in the composition of the invention is not an oligosaccharide.

[0075] Non-dairy milk can, in some embodiments, contain one or more organic acids, such as lactic acid, or acetic acid, for example to adjust the pH and/or produce the characteristic sour taste of cheese. These organic acids may be used in addition to or as an alternative to microbial cultures. Accordingly, in some embodiments the non-dairy milk comprises an organic acid. The organic acid can be one or more of: lactic acid, acetic acid, citric acid, malonic acid, malic acid, propionic acid.

[0076] In some instances the compositions of the invention do not contain any animal products. In some embodiments the compositions of the invention use no components that are derived from animals. In some embodiments the compositions of the invention contain no fats from animals. In some embodiments the compositions of the invention contain no proteins from animals. In some

embodiments the compositions of the invention contain no enzymes from animals. In some embodiments the compositions of the invention contain no dairy products.

[0077] In one embodiment the invention provides a composition free of or substantially free of one or more of the following: dairy products, animal products, agar, carrageenan, or tofu.

[0078] In some embodiments the compositions have less than 20%, less than 15%, less that 10%, less than 5%, less than 1% or less than 0.5% starch content. In some embodiments no refined starch (e.g. cornstarch, tapioca, wheat, or potato starch) is added to the compositions of the invention.

[0079] In some embodiments the non-dairy milk contains no artificial flavoring or coloring.

[0080] In some instances the non-dairy milk does contain some animal products. For example, in some embodiments, non-dairy milk comprises enzymes obtained from animals. In some embodiments, the non-dairy milk contains dairy products. In some embodiments animal fats are included in the non-dairy milk. In some embodiments the non-dairy milk can comprise enzymes (e.g. proteases and lipases), and/or microbes (e.g. flactic bacteria, yeast and mold). Without being bound by theory these enzymes and or microbes can be added to non-dairy milk formulations to produce desirable flavor and aroma compounds.

[0081] Enzymes, including proteases and lipases, and microbes, including lactic bacteria, yeast and mold, can be added to non-dairy milk formulations to produce desirable flavor and aroma compounds.

[0082] In some embodiments the invention provides a method comprising isolating or purifying a single non-dairy protein, mixing the isolated non-dairy protein with a non-dairy fat source, and adding a microorganism selected from the group consisting of *Penicillium camemberti, Geotrichum candidum, Penicilliem roqueforti, Penicillium nalgiovensis, Verticillium lecanii, Kluyveromyces lactis, Saccharomyces cerevisiae, Candida utilis, Debaryomyces hansensil, Rhodosporidum infirmominiatum, Candida jefer, Cornybacteria, Micrococcus sps., Lactobacillus sps.,Lactococcus, Staphylococcus, Halomonas, Brevibacterium, Psychrobacter, Leuconostocaceae, Streptococcus thermophilus, Pediococcus sps*., or *Propionibacteria.* In some embodiments at least 1 kg of the plant protein is isolated or purified from a single non-dairy source. In some embodiments the fat is isolated from another non-dairy source.

[0083] In some embodiments the invention provides for a composition comprising an isolated or purified non-dairy protein and a polynucleotide identifiable as derived from one or more of *Penicillium camemberti, Geotrichum candidum, Penicilliem roqueforti, Penicillium nalgiovensis, Verticillium lecanii, Kluyveromyces lactis, Saccharomyces cerevisiae, Candida utilis, Debaryomyces hansensil, Rhodosporidum infirmominiatum, Candida jefer, Cornybacteria, Micrococcus sps., Lactobacillus sps.,Lactococcus, Staphylococcus, Halomonas, Brevibacterium, Psychrobacter,*

Leuconostocaceae, Streptococcus thermophilus, Pediococcus sps., or Propionibacteria. In some embodiments the cheese replicas comprise *Penicillium camemberti, Geotrichum candidum, Penicilliem roqueforti, Penicillium nalgiovensis, Verticillium lecanii, Kluyveromyces lactis, Saccharomyces cerevisiae, Candida utilis, Debaryomyces hansensil, Rhodosporidum infirmominiatum, Candida jefer, Cornybacteria, Micrococcus sps., Lactobacillus* sps., Lactococcus, *Staphylococcus, Halomonas, Brevibacterium, Psychrobacter, Leuconostocaceae, Streptococcus thermophilus, Pediococcus sps*., or *Propionibacteria*. In some embodiments compositions of the invention comprise *Penicillium, Geotrichum, Saccharomyces, Kluyveromyces,* or *Debaryomyces*.

II. "Curdling" the non-dairy milk

[0084] Generally the cheese-replicas are made by causing cross linking or denaturation of proteins in non-dairy milks. These processes replicate the curdling process of traditional dairy cheese making.

[0085] Crosslinking is typically induced using an enzyme that creates covalent crosslinks between polypeptide chains. In some embodiments cross-linking enzymes are used in a cross-linking step to curdle the non-dairy milk. In various embodiments, transglutaminase is the cross-linking enzyme used to induce the curdling of non-dairy milk replicas. In some embodiments the crosslinking enzyme used to induce curdling in non-dairy milk replicas is a lysyl oxidase. In one embodiment, the invention provides for a method of inducing the curdling of a non-dairy milk comprising obtaining a non-dairy milk and adding a cross-linking enzyme to the non-dairy milk. In some embodiments the cross-linking enzyme is transglutaminase. In some embodiments the cross-linking enzyme is not derived from an animal source. In some embodiments the curdling process does not use rennet.

[0086] In some embodiments between 0.1 and 20 units (U) of transglutaminase is added per 1 mL of non-dairy milk. In some embodiments about 0.1, 0.5, 1, 1.5, 2, 2.5, 3, 5, 7, 10, 15, or 20 U transglutaminase is added per 1 mL of non-dairy milk. In some embodiments the transglutaminase is followed by heated incubation, for example in a 100°F water bath. The heated incubation can be at a temperature optimized for the enzyme function. In some embodiments the temperature is about 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120 or 125 °F.

[0087] Transglutaminases are a family of enzymes that catalyze the formation of a covalent bond between a free amine and the gamma-carboxyl group of glutamine thereby linking proteins together. For example, transgluaminases catalyze crosslinking of e.g., lysine in a protein or peptide and the gamma-carboxamide group of a protein- or peptide- glutamine residue. The covalent bonds formed by transglutaminase exhibit high resistance to proteolytic degradation.

[0088] Many types of transglutaminase can be used in various embodiments of the invention. Acceptable transglutaminases include, but are not limited to, *Streptoverticillium mobaraense* transglutaminase, an enzyme is similar or identical to a transglutaminase from *Streptoverticillium mobaraense*, other microbial transglutaminases, transglutaminases produced by genetically engineered bacteria, fungi or algae, Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6), TGM Z (TGM7), or lysyl oxidase. Accordingly, in some embodiments an enzyme selected from the group consisting of a transglutaminase is used to crosslink a non-dairy milk protein. In some embodiments the transglutaminase is *Streptoverticillium mobaraense* transglutaminase, other microbial transglutaminases, transglutaminases produced by genetically engineered bacteria, fungi or algae, Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6), TGM Z (TGM7), or lysyl oxidase In some embodiments the present invention provides for a composition comprising a non-dairy milk and *Streptoverticillium mobaraense* transglutaminase, an enzyme is similar or identical to a transglutaminase from *Streptoverticillium mobaraense*, other microbial transglutaminases, transglutaminases produced by genetically engineered bacteria, fungi or algae, Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6) and/or TGM Z (TGM7). In some embodiments the enzyme used for cross-linking is not Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6) , TGM Z (TGM7), or lysyl oxidase.

[0089] In some embodiments the invention provides for a composition comprising a non-dairy milk, wherein the composition is free of Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6) and/or TGM Z (TGM7).

[0090] Transglutaminases can be produced by *Streptoverticillium mobaraense* fermentation in commercial quantities or extracted from animal tissues. Additionally, the transglutaminase (TGM) of the present invention may be isolated from bacteria or fungi, expressed in bacteria or fungi from a synthetic or cloned gene, Factor XIII, keratinocyte transglutaminase, tissue transglutaminase, epidermal transglutaminase, prostate transglutaminase, TGM X, TGM Y, TGM Z, or another member of the transglutaminase family. In some particular embodiments transglutaminase is

obtained from commercial sources, for example in the form of Activa™ from Ajinmoto Food Ingredients LLC.

[0091] In some embodiments the compositions of the invention have a detectable amount of a nucleic acid from the genome of a cell that encoded and produced an enzyme, for example a transglutaminase. For example a cheese replica can have a detectable amount of *Streptoverticillium mobaraense* DNA. This detectable amount can, for example, be a small amount of DNA that has carried over to the cheese replica from the production of transglutaminase by *Streptoverticillium mobaraense*.

[0092] In some embodiments the cross-linking of proteins in non-dairy milk is induced by transglutaminase. In some embodiments the cross-linking of proteins in non-dairy milk is induced by a lysyl oxidase. Various non-dairy milks have been crosslinked with transglutaminase. In some embodiments the invention provides a composition comprising a non-dairy milk and transglutaminase. In some embodiments the invention provides a composition comprising a curdled non-dairy milk and transglutaminase. In some embodiments the invention provides a cheese replica containing transglutaminase. In some embodiment the invention provides a cheese replica with a detectable amount of degraded transglutaminase. In some embodiment the invention provides a cheese replica with a detectable amount of improperly folded transglutaminase. In some embodiment the invention provides a cheese replica with a detectable amount of transglutaminase by-product. In some embodiment the invention provides a cheese replica with a detectable peptide which is identifiable as portion of a transglutaminase used in the manufacturing process for making the cheese replica. In some embodiment the invention provides a cheese replica with one or more detectable peptides containing a crosslink between a lysine and glutamine residue. For example the crosslinked glutamine and lysine residues may be detectable by mass spectroscopy. In some embodiment the invention provides a cheese replica with one or more detectable peptides containing a crosslink between two lysine residues. For example the crosslinked lysine residues may be detectable by mass spectroscopy.

[0093] Denaturation can be used instead of, or in addition to, the cross-linking enzyme for curdling the non-dairy milk. For example, heat denaturation of a non-dairy milk followed by cooling of the mixture can result in a curd-like gel as, for example, described in example 7. Accordingly, the present invention provides for non-dairy milk curds comprising denatured non-dairy proteins. The present invention also provides for methods for making a non-dairy milk curd by heating a non-dairy milk to a temperature of about between 30-35, 35-40, 40-45, 45-50, 50-55, 55-60, 60-65, 65-70, 70-75, 75-80, 80-85, 90-95, 95-100 °C for about 10, 20, 30, 40, 50, 60 seconds or about 1, 2, 3, 4, 5, 6,

7, 8, 9, or 10 minutes followed by cooling. In some embodiments non-dairy milks are curdled using both enzymatic cross-linking with transglutaminase and heat denaturation.

[0094] Additional denaturization procedures are possible in additional embodiments of the invention. Acids, solvents, chaotropic agents, or disulfide bond reducers can be used to denature the proteins in the non-dairy milk. In one embodiment urea is added to the non-dairy milk to form curds.

III. Cheese replica production from the curdled non-dairy milk

[0095] Cheese replicas can be produced from the curdled non-dairy milk. Accordingly, the invention provides methods for producing cheese replicas from curdled non-dairy milk, as well as cheese replica compositions.

[0096] To create the cheese replicas one might use elements of the traditional cheese making process. Cheese replicas can be produced using one or more steps including cutting, draining, forming, pressing, waxing, aging, scalding, smoking, salting or ripening.

[0097] The curd and whey may be separated in a traditional way. For example, the cheese may be scalded to create a replica of traditional cheddar or other hard cheese, or, the whey and curd might be separated through a cloth drain.

[0098] The cheese replicas may be ripened in a way similar to traditional cheese. For example, surface mould may be allowed to grow to create a rind. In order to create a rind or color, the process may introduce certain bacteria to the cheese replicas in the ripening process. By way of example only, *Brevibacterium linens* can be introduced to produce an orange color and pungent aroma to the cheese replica.

[0099] In some embodiments, the cheese replica can be a salted or salt-washed cheese replica. The salting process can preserve the cheese replica and/or add flavor. In some embodiments the salt is chosen based on the geographic location of where the salt is originally obtained. In some embodiments salt is added to the cut curds. In some embodiments salt is contacted with the outside surface of the cheese replica, for example by rubbing. In some embodiments the cheese replica is contacted with or soaked in brine.

[00100] In some embodiments, the cheese replica can be a soft fresh cheese produced by procedures exemplified by example 3.

[00101] In some embodiments, the cheese replica can be a salted or salt-washed cheese produced by procedures exemplified by example 4.

[00102] In some embodiments, the cheese replica can be a soft ripened or molded-rind cheese produced by procedures exemplified by example 5.

[00103] In some embodiments, the cheese replica can be a goat cheese replica produced by procedures exemplified by example 6.

[00104] In some embodiments, the cheese replica can have edible materials added (e.g. herbs, pepper, spices) on its surface to enhance flavor or add to the visual appeal of the product, as exemplified by examples 4 and 6. In some embodiments the edible materials are embedded in the cheese replica.

[00105] In some embodiment the cheese replica can be shaped. For example the cheese replica can be shaped in a basket or a mold. In some embodiments the cheese replica is pressed, for example with a weight. The pressing can help expel any additional liquid from the cheese replica.

[00106] In some embodiments the production of the cheese replica includes a waxing step. In one embodiment the waxing procedure is as follows: Cut food-grade paraffin wax into ½-inch pieces. Place in double boiler and heat wax to 210°F. Place cheese replicas in standard freezer for fifteen minutes to reduce temperature of cheese replicas to 33°F. Using 3-grams of melted wax per piece, brush wax onto cheese replicas one side at a time. Placed waxed cheese replicas onto clean waxed paper on aging racks. Age waxed cheese replicas in aging room at 36°F with 75% humidity, for example for six months. In some embodiments the aging room is between 33-70°F. In some embodiments the humidity of the aging room is altered to aid in rind formation. In some embodiments the waxed cheese is stored for years, for example for 2 years or more.

[00107] In some embodiments the production of the cheese replica includes a smoking step. In some embodiments the cheese replica is cold smoked. In some embodiments the cheese replica is smoked at the curd stage or prior to the curd stage. In some embodiments the cheese replica is smoked after the cheese replica is formed. In some embodiment the smoking procedure is as follows: Soak wood chips for six hours. Drain chips of all water and place in smoking unit. Ignite smoker and as soon as chips have fully ignited, snuff out flames to create smoke-filled unit. Place cheese replicas on racks in smoker for five minutes per side. Remove from smoker and place on cooling racks. Place cheese replicas in cooling room for 24 hours, at 36°F. In various embodiments smoking times and cooling times and temperatures will be adjusted according to the particular cheese replica and particular desired taste profile.

[00108] In some instances the cheese replica is exposed to or injected with mold or yeast. In some embodiments the cheese replica is exposed to a particular bacterial strain or strains.

[00109] When the cheese replica is made without any dairy ingredients, the production process requires no dairy farming. Accordingly, the process for making cheese replicates is suitable for locations where keeping animals or storing milk is difficult or costly. In some embodiments the cheese replicas are made in non-dairy producing regions. This will reduce the need for shipping the

products. So in some embodiments the cheese replica is a product that is made in the same locality as its eventual consumption. For example the cheese replica is made within 10, 20, 50, or 100 miles of its point of sale. The cheese replica production process may also be useful in remote areas where dairy farming is difficult or impossible. For example the cheese replica production process could occur on an island, aboard an oil platform, or aboard a space station.

[00110] The cheese replicas may further contain microbes useful for cheese making. So in some embodiments the cheese replicas may be made by a process involving culturing with any microbes used for cheese making. The cheese replicates may be made by a process involving fermentation. In any process, the proteins may be crosslinked by treatment with an enzyme, which may be from the family of transglutaminase. The cheese replicas may be created from curds formed by a treatment of a protein-containing liquid with a transglutaminase, or other enzymes that promote gelling, or they may be prepared by a process involving heat denaturation of one or more of the protein components in the solution, suspension or emulsion to induce formation of a gel.

[00111] In some embodiments the cheese replicas comprise *Penicillium, Geotrichum, Saccharomyces, Kluyveromyces,* or *Debaryomyces.*

IV. Non-dairy cheese replicas

[00112] The cheese replicas can be made for consumption by either humans or other animals or both. In some instances the cheese replicas are used to feed domesticated animals. For example, a cheese replica of the present invention can be fed to a dog. In one particular embodiment, the cheese replica contains a veterinary medication In another embodiment the cheese replica comprises a treat for a domestic animal. In some embodiments the domestic animal treat is shaped like traditional a wedge of cheese. In some embodiments the animal treat is artificially colored to resemble dairy cheese, e.g. cheddar cheese.

[00113] The cheese replicas include products similar to conventional cheese, yogurt and other dairy products. Cheese replica can be made from a liquid extract of nuts or other fruits or seeds. The current invention also includes cheese replicas made from proteins and fats isolated and enriched or purified from one or more plant. The current invention also includes cheese replicas made from proteins and fats isolated and enriched or purified from one or more non-dairy source.

[00114] In some embodiments the cheese replica has less than 1%, 5%, 10%, 20%, 25% or 30% polysaccharides.

[00115] In some embodiments the cheese replica has less than 1%, 5%, 10%, 20%, 25% or 30% polysaccharides because of centrifugal separation.

[00116] In some embodiments the cheese replica has less than 1%, 5%, 10%, 20%, 25% or 30% polysaccharides because of the use of purified proteins.

[00117] The cheese replicas may be suitable for consumption by humans or animals who are unable to eat certain animal products, such as a person who is lactose-intolerant or allergic to dairy. Accordingly, in some embodiments the cheese-replica does not contain lactose. The cheese replicas might contain enough protein or other nutrients to be nutritionally equivalent to traditional cheese or other animal products.

[00118] The cheese replicas may contain less fat, less saturated fat or less cholesterol than traditional cheese or animal products, and may be suitable for a healthier diet. So in some embodiments provided herein is a method for distributing cheese replicas comprising the steps of informing a lactose intolerant person of the cheese replica, receiving an order for a cheese replica, and making the cheese replica available to the lactose intolerant person.

[00119] In some embodiments the lactose intolerant person is informed through advertising. In some embodiments the lactose intolerant person is informed by their personal physician. In some embodiments the order for the cheese replica is sent by the lactose intolerant person. In some embodiments the lactose intolerant person receives the cheese replica via a distributor that places the order. In some embodiments the informing and receiving steps take place on a network, for instance a computer network and are enacted with specialized computer software stored on a computer readable media. In some embodiments the method further comprises producing a cheese replica of the present invention and supplying it to lactose intolerant person.

[00120] The cheese replicas are, in some embodiments, designed to replicate the experience of eating cheese. The look, texture, and taste of the cheese replicas can be such that it is similar or indistinguishable from cheese. The invention provides for methods for determining whether a animal or human can distinguish the cheese replicas from cheese. In some embodiments a property of the compositions of the invention is that an animal, for example a human, will identify the composition as cheese. In some embodiments the human identifies the composition of the invention as equivalent to cheese. In some embodiments one or more properties of cheese are equivalent according to an ordinary human's perception. Such properties include the properties that can be tested listed below. In some embodiments an ordinary human identifies a cheese replica of the present invention as more cheese like than cheese substitutes found in the art.

[00121] One method to determine whether the cheese replica is comparable to cheese is to a) define the properties of cheese and b) determine whether the cheese replicas has similar properties. Properties of cheese that can be tested include mechanical properties such as hardness, cohesiveness, brittleness, chewiness, gumminess, viscosity, elasticity, and adhesiveness. Properties of cheese or a

cheese replica that can be tested also include geometric properties such as particle size and shape, and particle shape and orientation. Additional properties can include moisture content and fat content. The properties of the cheese replica 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" or "spreadable" to describe viscosity; "plastic" or "elastic" to describe elasticity; "sticky," "tacky" or "gooey" to describe adhesiveness; "gritty," "grainy" or "coarse" or "heterogeneous" 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. In one embodiment a group of people can be asked to rate a certain cheese, for instance cheddar, according to properties which describe the cheese. These ratings can be used as an indication of the properties of the cheese. The cheese replicas of the present invention can then be compared to the properties of traditional dairy cheese to determine how similar the cheese replica is to the dairy cheese. In some instances the properties of the cheese replicas are then altered to make the cheese replica more similar to the cheese. In some embodiments, the cheese replica has a property or properties that are rated similar to cheese according to human evaluation. In some embodiments the cheese replica is indistinguishable from real cheese to a human. In some embodiments the cheese replica is distinguishable by some properties from real cheese to a human.

[00122] In some embodiments the cheese replica is compared to real cheese 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. In some embodiments the cheese replica is a product that causes similar olfactometer readings to those of a particular target cheese. In some embodiments the cheese replica is a product that causes nearly similar , but slightly different olfactometer readings to those of a particular target cheese.

[00123] Gas chromatography–mass spectrometry (GCMS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GCMS can, in some embodiments, be used to evaluate the properties of a cheese replica. For example volatile chemicals can be isolated from the head space around cheese. These chemicals can be identified using GCMS. A profile of the volatile chemicals in the headspace around cheese 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 cheese replicas. The GCMS could be used to refine the cheese replica. In some embodiments the cheese replica has a GCMS profile similar to that of cheese. In some embodiments the cheese replica has a GCMS profile identical to that of cheese.

[00124] The cheese replicas may be modified to fit into traditional cheese-types, including: fresh, bloomy, semi-soft, washed, firm, hard and blue. There may be additional processes to modify the cheese replicas and fit them into any cheese-type category. These categories also include different presentations of the cheese replicas, for example: sliced, shredded, block, with rind, without rind, wet or dry. The cheese replicas may be aged, or a method may be created to mimic the taste of traditionally-aged dairy cheese. The cheese replicas may be classified in taste the same way traditional cheese is. For example, they may be tart, tangy, smooth, creamy, buttery, fluffy, rich, earthy, pungent, aromatic, eggy, fruited, sharp, dry, caramelly, grainy, punchy or complex, among other descriptions. The invention includes methods to modify the cheese replicas to fit into any category or taste description.

[00125] The cheese replicas may be modified to have or not have a rind, may be coated in wax, and may have craters or veins typical to blue cheese. The cheese replicas may be spreadable, such as cream cheese. The cheese replicas can contain flavor additives, for example: truffle, mushrooms, nuts, herbs, chives, and other flavors.

[00126] The cheese replicas may be meltable, and have other properties of conventional cheese.

[00127] The cheese replicas may also be similar in consistency and taste to traditional yogurt or cottage cheese. The cheese replicas might be flavored with fruits, sweeteners, or other flavor additives. The cheese replicas might contain bacteria to aid with digestion or other health additives. The cheese replicas may be modified to have an appropriate or appealing consistency.

[00128] In various embodiments the cheese replicas may be used in all cooking and recipes as a substitute for traditional cheese. In some embodiments the cheese replicas may be used in cooking and recipes as a flavor substitute for traditional cheese. In some embodiments the cheese replicas may be used in cooking and recipes as a functional substitute for traditional cheese.

EXAMPLES

[00129] **EXAMPLE 1**

[00130] THE EFFECTIVENESS OF TRANSGLUTAMINASE IN CURDLING VARIOUS NUT MILKS WAS COMPARED.

[00131] Cashews, almonds, macadamia nuts, brazil nuts were used to produce a slurry, by adding fresh water in a 3:1 weight ratio to the nuts, then blending in a Vitamix 4500 blender at high

speed for 2 minutes. Because we found in preliminary experiments that the insoluble solids in the milks at this stage (slurry) inhibited formation of optimal creamy curds and produced a cheese replica with grainy mouth feel the slurry was subjected to centrifugation at 10,000 G for 15 minutes at 4°C to remove insoluble solids. The liquid supernatant, consisted of two layers, a lighter, creamy, opaque layer and a slightly denser clear to translucent aqueous layer. Liquid supernatant layers were recovered and blended together and the pelleted solids were discarded. The blended supernatants are referred to as "nut milks" (a non-dairy milk). Each of the 4 resulting nut milks and each pair-wise combination at a 1:1 blend, were evaluated for curdling in the presence of transglutaminase (TG): 30 U TG was added to 15 mls of each nut milk or blend in a 20 ml glass vial, followed by incubation in a 100°F water bath. After 5 hours all but the pure cashew nut milk had formed sturdy curds, sufficient to retain their form when the glass vial was inverted. The cashew milk and cashew brazil nut milks formed soft curds. The macadamia and almond milks formed the most solid curds. Control samples without added transglutaminase remained liquid under these conditions.

[00132] **EXAMPLE 2**

[00133] PREPARATION OF ALMOND AND MACADAMIA NUT MILKS

[00134] Blanching (surface sterilization of the nuts)**:**

[00135] **Note:** Almonds and macadamia nuts were blanched separately.

[00136] A 30 gallon tilt skillet filled to ½ -capacity with fresh water was heated to 212°F. 25 pounds of either almonds or macadamia nuts were dumped into the boiling water bath and left for 30 seconds. The nuts were recovered by draining into a colander then immediately immersed in fresh ice-cold water in a second tilt skillet to rapidly cool them. After rapid cooling the nuts were recovered by draining in a colander and spread on sheet trays to dry.

[00137] Macadamia nuts proceeded directly to the blending process. Almonds were first hydrated as described below before blending.

[00138] Hydration of Almonds:

[00139] The blanched almonds were transferred to Cambro buckets and fresh water was added to a level two inches above the level of the nuts. The buckets were then placed at 36°F for up to 16 hours. After hydration, the nuts were recovered by draining into a colander, rinsed with fresh water, then proceeded to the blending step.

[00140] Blending of nuts:

[00141] Note: Almonds and macadamia nuts were blended separately.

[00142] One pound batches of nuts were placed in a Vitamix blender (model Vitaprep 3), with one liter of fresh water and blended for 5 minutes, gradually increasing the blender speed to high. The blended slurries from each batch were collected in a stainless steel bain-marie sitting in a large container of ice and stirred with a frozen cooling stick to cool. Once the accumulated slurry cooled to 50°F, the bain-marie was placed in a refrigerator at 36°F for up to 12 hours.

[00143] Separation of skim and cream:

[00144] Sets of four 1800 ml aliquots of the blended slurry were centrifuged at 5000 RPM / 7480 G at 4°C for 30 minutes in a Beckman-Coulter Avanti J-HC Centrifuge with a JS-5.0 rotor, resulting in separation of the blend into 3 layers, a dense pellet of insoluble solids, a clear to translucent aqueous layer (which we refer to as the "skim"), and a lighter, creamy, opaque layer (which we refer to as the "cream"). The cream layer was carefully collected from each bucket using a spoon, and placed in a pan sitting in a container of ice. The viscous aqueous layer (skim) from each bucket was carefully poured into a ½-Cambro sitting in a container of ice. The accumulated cream and skim layers were kept at 36°F until the pasteurization step. Typical yields were about 0.77 lbs of cream and 0.95 lbs of skim per lb of macadamia nuts and about 0.29 lbs of cream and 1.62 lbs of skim per lb of almonds.

[00145] Blending

[00146] Almond skim and cream were combined in a ratio specified by the recipe for the specific cheese replica being produced (Table 1), and blended with an immersion blender. Macadamia skim and cream were combined in a ratio specified by the recipe for the specific cheese replica being produced (Table 1), and blended with an immersion blender. We hereafter refer to each blended mixture of skim and cream as "non-dairy milk." The two non-dairy milks were then blended in a ratio specified by the recipe for the specific cheese replica being produced (Table 1).

[00147] Pasteurization:

[00148] The non-dairy milk blend was pasteurized at 167°F for 16 seconds after which the non-dairy milk temperature was rapidly reduced to 50°C in 12 seconds by a controlled chilling system. The non-dairy milk temperature was then further reduced by placing containers in an ice bath at 36°F. Pasteurized non-dairy milks were stored at 36°F. Non-dairy milks were discarded if the pH of the non-dairy milk dropped below pH 6.0 during storage, indicating spoilage.

[00149] **EXAMPLE 3**

[00150] PRODUCTION OF A SOFT FRESH CHEESE REPLICA.

[00151] The ingredients and amounts needed for one batch (about one and a half pieces) of soft fresh cheese replica are listed in Table 2. The recipe can be scaled up or down proportionately.

[00152] Soft fresh cheese replica was prepared by the following procedure:

non-dairy

[00153] Bring pasteurized non-dairy milk formula (see Table 2) to 80°F in a water bath.

[00154] Sprinkle the mesophilic starter culture (see Table 2) onto the non-dairy milk formula and allow the culture to hydrate for five minutes without stirring. Gently stir in the starter culture with a spatula for two minutes. Hold at 80°F for one hour.

[00155] Increase the water bath temperature to begin to bring the non-dairy milk temperature to 100°F.

[00156] Optional step: Add microbial "rennet" and/or distilled vinegar (see Table 2), folding gently with a spatula. Hold for 15 minutes.

[00157] Dilute the hydrated transglutaminase (see Table 2) with a small amount of warm non-dairy milk formula and then add it to the non-dairy milk formula, folding gently with a spatula for two minutes. Allow formula to reach 100°F.

[00158] Remove the container of non-dairy milk formula from the water bath and cover with plastic wrap and aluminum foil. Allow the non-dairy milk formula to coagulate for twelve hours at room temperature.

[00159] Cut curd into ½ inch dice pieces. (**Figure 1**). Allow curd to re-mat for 10 minutes. Pour the coagulated formula (curd and whey) into a draining bag and measure its weight. Hang bag and allow curd to drip for a minimum of 20 minutes until proper viscosity and density of curd is achieved. The drained curd should weigh about 60% of original formula weight.

[00160] Place curd in a mixing bowl. Add cheese salt (see Table 2). Gently whisk the curd for ten minutes, or blend for five minutes with a Hobart mixer on low to medium speed.

[00161] Place 13.75 ounces of curd (enough to yield eight ounces after completion of draining and brining) into a micro-perforated mold (**Figure 2**). Drain at room temperature for one hour without the follower in place. Then place the follower in the mold and add 600 grams of weight to the follower. Refrigerate at 36°F for 24 hours.

[00162] Preheat saturated brine to 50°F. Fully immerse the cheese replica, still in its mold, into the preheated brine for 1/2 hour. After brining, place mold on draining rack and return it to 36°F for 24 hours.

[00163] Remove cheese replica from the mold. Place on draining mat and return it to 36°F for 24 hours.

[00164] The soft fresh cheese replica **(Figure 3)** is now ready for packaging and shipment. Packaging process includes nitrogen flush and airtight heat-sealing.

[00165] **EXAMPLE 4**

[00166] PRODUCTION OF A SALTED CHEESE REPLICA.

[00167] The ingredients and amounts needed for one batch (about one and a half pieces) of salted cheese replica are listed in Table 2. The recipe can be scaled up or down proportionately.

[00168] Bring pasteurized non-dairy milk formula (see Table 2) to 80°F in a water bath.

[00169] Sprinkle the mesophilic starter culture (see Table 2) onto the non-dairy milk formula and allow the culture to hydrate for five minutes without stirring. Gently stir in starter culture with a spatula for full two minutes. Hold at 80°F for one hour.

[00170] Increase water bath temperature to begin to bring non-dairy milk temperature to 100°F.

[00171] Optional: Add microbial "rennet" and/or distilled vinegar (see Table 2), folding gently with a spatula. Hold for 15 minutes.

[00172] Dilute the hydrated transglutaminase (see Table 2) with a small amount of warm non-dairy milk formula and then add it to the non-dairy milk formula, folding gently with a spatula for two minutes. Allow formula to reach 100°F.

[00173] Remove container of non-dairy milk formula from the water bath and cover. Allow the non-dairy milk formula to coagulate for twelve hours at room temperature.

[00174] Cut curd into ½ inch dice pieces. Allow curd to re-mat for 10 minutes. Pour the coagulated formula (curd and whey) into a draining bag and measure its weight. Hang bag and allow curd to drip for a minimum of 20 minutes until proper viscosity and density of curd is achieved. The drained curd should weigh about 60% of original formula weight.

[00175] Place curd in mixing bowl. Optional: For the classic presentation of a cheddar cheese replica (**Figure 3**), annatto– a natural vegetable dye, is added to create the yellow cheddar color. The percentage of annatto evenly distributed throughout the curd is 0.08%. Gently whisk curd for ten minutes, or blend for five minutes with a Hobart mixer on low to medium speed.

[00176] Place 16.3 ounces of curd (enough to yield eight ounces after completion of draining and brining) into a micro-perforated mold (see **Figure 1**). Drain at room temperature for one hour without the follower in place. Then place the follower in the mold and add 600 grams of weight to the follower. Refrigerate at 36°F for 24 hours.

[00177] Preheat saturated brine to 50°F. Fully immerse the cheese replica, still in its mold, into the preheated brine for ½ hour. After brining, place mold on draining rack and return it to 36°F for 24 hours.

[00178] Remove the cheese replica from the mold. Place the cheese replica on a draining mat and return it to 36°F for 24 hours.

[00179] Move the cheese replica into a drying room for seven days at 55°F, with 55% humidity, and minimal airflow. Turn the cheese replica and place on a fresh draining mat daily.

[00180] After the seven-day drying period, remove the draining mats and place the salted cheese replica (**Figures 4 and 5)** directly on an aging rack.

[00181] If the cheese replica is to be waxed or smoked, return the cheese replica to the drying room for two additional weeks at 55°F, with 55% humidity and minimal airflow. Then follow the procedure for waxing or smoking.

[00182] If the cheese replica is to be rolled in paprika and fennel pollen mixture, follow the steps for the paprika & fennel pollen process.

[00183] Paprika and fennel pollen process:

[00184] Brush the salted cheese replica with cold water using a pastry brush. Roll the cheese replica in 1.5 grams of a mixture of 10-parts organic paprika and 1-part fennel pollen. Place the coated cheese replica on an aging rack and return it to the drying room for two weeks at 55°F, with 55% humidity and minimal airflow, turning daily.

[00185] Following the drying period, move the cheese replica to 36°F for 24-hours. The paprika-fennel pollen cheese replica (**Figure 6**) is now ready for packaging and shipment.

[00186] Waxing Process:

[00187] Cut food-grade paraffin wax into ½-inch pieces and heat to 210°F in a double boiler.

[00188] Place the cheese replica in a freezer to reduce its temperature to 33°F. Using three grams of melted wax per piece of cheese replica, brush wax onto cheese replica one side at a time. Place waxed-side down onto waxed paper and then continue brushing remainder of the cheese replica including sides and top. Placed waxed cheese replica (**Figure 7**) onto clean waxed paper on an aging rack.

[00189] Age the waxed cheese replica in aging room at 36°F with 75% humidity for up to six months. The waxed cheese replica is now ready for packaging and shipment.

[00190] Smoking Process:

[00191] Soak equal parts apple and cherry wood chips for six hours. Drain chips of all water and place in a smoking unit. Ignite smoker and as soon as chips have fully ignited, snuff out flames to create a smoke-filled unit. Place salted cheese on rack into smoker for five minutes per side. Remove cheese from smoker and place on cooling rack.

[00192] Place the cheese at 36°F for 24 hours. Smoked cheese is now ready for vacuum aging or packaging and shipment.

[00193] **EXAMPLE 5**

[00194] PRODUCTION OF A SOFT RIPENED CHEESE REPLICA.

[00195] The ingredients and amounts needed for one batch (about one and a half pieces) of soft ripened cheese replica are listed in Table 2. The recipe can be scaled up or down proportionately.

[00196] Bring pasteurized non-dairy milk formula (see Table 2) to 90°F in a water bath.

[00197] Sprinkle lactose-free preparations of Florica Danica, Mesophilic Starter, Geotrichum Candidum, Penicillium Candidum, and Debaromyces hansenii (see Table 2) onto the milk formula. Optional: Add microbial "rennet." (see Table 2). Let milk sit for 5 minutes. Gently fold in and top stir with a spatula for two minutes. Hold at 90°F for 90 minutes.

[00198] Increase water bath temperature to start to bring non-dairy milk temperature up to 100°F.

[00199] Optional: Add in distilled vinegar (see Table 2), folding gently with spatula. Hold 15 minutes.

[00200] Dilute the hydrated transglutaminase (see Table 2) with a small amount of warm non-dairy milk formula and then add it to the non-dairy milk formula, folding gently with a spatula for two minutes. Allow formula to reach 100°F.

[00201] Remove container of non-dairy milk formula from the water bath and cover with plastic wrap and aluminum foil. Allow the non-dairy milk formula to coagulate for twelve hours at room temperature.

[00202] Cut curd into ½ inch dice pieces. Allow curd to re-mat for 10 minutes. Pour the coagulated formula (curd and whey) into a draining bag and measure its weight. Hang bag and allow curd to drip for a minimum of 20 minutes until proper viscosity and density of curd is achieved. The drained curd should weigh about 60% of original formula weight.

[00203] Place curd in mixing bowl. Add cheese salt. Gently whisk curd for ten minutes, or blend for five minutes with a Hobart mixer on low to medium speed.

[00204] Place 17.64 ounces (500 grams) of curd (enough to yield eight ounces after completion of draining and brining) into a micro-perforated mold (**Figure 8**) on a draining mat. Drain at room temperature for one hour without the follower in place. Then place the follower in the mold just

touching the cheese replica. Do not add any additional weight to the follower. Refrigerate at 36°F for 24 hours.

[00205] Preheat saturated brine to 50°F. Fully immerse the cheese replica, still in its mold, into the preheated brine for ½ hour. After brining, place mold on draining rack and return it to 36°F for 24 hours.

[00206] Remove the cheese replica from the mold. Place on draining mat and return it to 36°F for 24 hours.

[00207] Transfer the cheese from 36°F to dry yeasting room for three days at 60°F, with 75% humidity.

[00208] After three days, transfer cheese from a draining mat to an aging mat. Place the mat on an aging rack, and move to ripening room at 50°F, with 90% humidity and continuous airflow. Turn the cheese replica and replace mat daily.

[00209] After seven days, transfer the cheese replica directly onto an aging rack, allowing maximum aeration for seven more days, or until mold coverage is complete.

[00210] After the cheese replica has thoroughly molded (**Figure 8**), move it to 36 °F for sixteen hours. Wrap cheese in perforated paper and place in a wooden box for shipment.

[00211] **EXAMPLE 6**

[00212] PRODUCTION OF A GOAT CHEESE REPLICA.

[00213] The ingredients and amounts needed for one batch (about one and a half pieces) of goat cheese replica are listed in Table 2. The recipe can be scaled up or down proportionately.

[00214] Bring pasteurized milk formula (see Table 2) to 80°F in a water bath.

[00215] Sprinkle the mesophilic starter culture (see Table 2) onto the non-dairy milk formula and allow the culture to hydrate for five minutes without stirring. Gently stir in starter culture with a spatula for full two minutes. Hold at 80°F for one hour.

[00216] Increase water bath temperature to begin to bring non-dairy milk temperature to 100°F.

[00217] Optional: Add microbial "rennet" and/or distilled vinegar (see Table 2), folding gently with a spatula. Hold for 15 minutes.

[00218] Dilute the hydrated transglutaminase (see Table 2) with a small amount of warm non-dairy milk formula and then add it to the non-dairy milk formula, folding gently with a spatula for two minutes. Allow formula to reach 100°F.

[00219] Remove container of non-dairy milk formula from the water bath and cover with plastic wrap and aluminum foil. Allow the non-dairy milk formula to coagulate for twelve hours at room temperature.

[00220] Cut curd into ½ inch dice pieces. Allow curd to re-mat for 10 minutes. Pour the coagulated formula (curd and whey) into a draining bag and measure its weight. Hang bag and allow curd to drip for a maximum of 24 hours until proper viscosity and density of curd is achieved. The drained curd should weigh about 50% of original formula weight.

[00221] Place curd in mixing bowl. Add cheese salt (see Table 2). Using Hobart mixer on high speed, mix and aerate completely.

[00222] Place sixteen ounces of curd (enough to yield eight ounces after completion of draining and brining) into a cylindrical goat cheese mold (**Figure 9**) and stand the mold upright on a draining mat. Refrigerate at 36°F for 24 hours allowing the cheese replica to drain.

[00223] Preheat saturated brine to 50°F. Fully immerse the cheese replica, still in its mold, horizontally into the preheated brine for ½ hour.

[00224] After brining, stand mold on draining mat placed at 36°F for 24 hours. Turn mold and change mat every twelve hours for two days.

[00225] Slide shaped goat cheese replica out of mold directly onto a bed of three grams of coarse ground pepper. Gently roll the cheese replica in pepper, covering evenly and completely. Place the cheese replica on waxed paper and return to 36°F for 48 hours.

[00226] The goat cheese replica (**Figure 10**) is now ready for packaging and shipment. Packaging includes Cryovac sealing process.

[00227] **EXAMPLE 7**

[00228] CULTURED GELS MADE WITH EMULSIONS OF PURIFIED PLANT PROTEINS AND FATS.

[00229] Procedure

[00230] Mixtures of purified or partially purified protein + glucose + oil were assembled and mixed with a vortex mixer to create an emulsion. Emulsions were either treated with transglutaminase (Ajinomoto Activa TI at a final concentration of 2% or 4%, as noted) or first heated to 85˚C, rapidly cooled on ice and then treated with transglutaminase. Mesophilic starter culture (Danisco Choozit MA11) was added to a concentration of 0.01% (wt/vol) concomitantly with transglutaminase to allow cultures to ferment, acidify and impart flavors and aroma to the gels. Reactions were incubated overnight at 30˚C before evaluating for gel formation and aroma.

[00231] Protein purification methods:

[00232] 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 -20C until further use. Prior to their use in experiments, the precipitates were resuspended in 50mM KPhosphate buffer pH 7.4 + 0.5M NaCl and dialyzed overnight against the same buffer to remove ammonium sulfate. The dialyzed solution was then centrifuged at 12000 g for 20min to remove precipitate and then used in experiments. Protein composition at individual fractionation steps was monitored by SDS-PAGE and protein concentrations were measured by standard UV-Vis methods.

[00233] (i) Pea-albumins: Dry flour of green peas was used as a source of pea albumins. The flour was resuspended in 10 volumes of 50mM sodium acetate buffer pH 5 and stirred for 1hr. The slurry was centrifuged at 8000 g for 20 minutes and the supernatant was collected. To this crude protein extract, solid ammonium sulfate was added to 50% saturation. The solution was stirred for 1hour and then centrifuged. To the supernatant from this step, ammonium sulfate was added to bring to 90% saturation. After stirring for 1 hour, the solution was stirred and then centrifuged to collect the pea albumin proteins in the pellet. The pellet was stored at -20˚C until further use.

[00234] (ii) Pea-globulins: Dry green pea flour was used to extract pea globulin proteins. The flour was resuspended in 10 volumes of 50mM KPhosphate buffer pH 8 + 0.4M NaCl and stirred for 1hr. After 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.

[00235] (iii) Moong bean 8S globulins: Moong bean flour was used to extract 8S globulins by first resuspending 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.

[00236] Gel formation was tested with the following protein fractions:

[00237] (i) Pea-albumins (90% ammonium sulfate fraction from green pea dry seed flour)

[00238] (ii) Pea-globulins (80% ammonium sulfate fraction from green pea dry seed flour)

[00239] (iii) Moong bean 8S globulin (90% ammonium sulfate fraction from moong flour)

[00240] The protein solutions were dialyzed into 50mM KPhosphate buffer pH 7-7.4 + 0.5M NaCl and used at concentrations between 8mg/ml and 75mg/ml (0.8-7.5%) as described below.

[00241] Canola oil was used to make emulsions. Stock solutions of transglutaminase (Ajinomoto) were prepared at 40% in 50mM KPhosphate pH 7. Mesophilic starter culture (MA011 from Danisco) was used as the cheese bacterial culture; the freeze-dried culture was resuspended at a 10% in 50mM KPhosphate buffer and used immediately in the experiments. Glucose (1%) was used as sugar source for growth of mesophilic cultures; stock solution was prepared at 40% in water.

[00242] Two conditions were used to form gels:

[00243] (i) 'Set 1': 2% protein + 3% oil + 4% transglutaminase; the rest water. Emulsify by vortex mixing.

[00244] (ii) 'Set 2': 7.5% protein + 3% oil + 2% transglutaminase; the rest water. Emulsify by vortex mixing.

[00245] Both gel compositions listed above were tested for gel formation catalyzed by transglutaminase, with and without heat denaturation of the protein component. All reactions (total volume of each, 1ml) were first assembled in 1.5 ml micro-centrifuge tubes with protein + oil+ sugar components. Emulsions were made by vortexing the samples for 30sec. Samples to be heat denatured were immediately placed in a water bath and heated at 85°C for 30minutes. These samples were subsequently rapidly cooled on ice for 20 minutes. Transglutaminase and starter culture were added to all samples and mixed. Samples that formed gels upon heat/cool step were vortexed to mix the enzyme and cultures in. All samples were removed from the reaction tubes and evaluated for gel consistency and aroma after overnight incubation at 30°C.

[00246] Aroma evaluation:

[00247] The following gels were tested for aroma (smell by 4 individuals):

[00248] Set 1: pea-albumin (heat/cool), moong (heat/cool), moong (no heat/cool),

[00249] Set 2: pea globulin (heat/cool), moong (no heat/cool)

[00250] All four individuals reported yogurt-like/fermented/sour aroma in the moong (set 1, no heat/cool) gels. They reported similar smells but to a lower degree in the moong (set2, no heat/cool) and pea globulin (set 2, heated) gel samples. For pea-albumins (set 1, heated) and moong (set 1,

heated), very faint or no aroma was reported. One of the four testers reported the aroma from moong (set 2, no heat/cool) - to be unpleasant.

[00251] Gel firmness evaluation: (**Figure 10**)

[00252] 'Set 1': all samples formed gels. In general these gels were weak and disintegrated easily. Pea albumins (heat/cooled) were slightly firmer in this regard.

[00253] 'Set 2': All samples with exception of pea-globulins (no heat/cool) formed gels. Gels were stronger compared to Set 1 and held their shape even after removal from the tubes. Gels formed without heat/cool.

Table 1: MILK FORMULAS FOR SOFT FRESH, SALTED, SOFT RIPENED AND GOAT CHEESE REPLICAS

Type of cheese replica	milk component	Amount (grams)
Soft fresh, salted or goat		
	Almond milk (per 800 ml batch).	
	Almond skim	725.0
	Almond Fat	75.0
	Macadamia nut milk (per 660 ml batch)	
	Macadamia nut skim	328.5
	Macadamia nut fat	337.5
Soft ripened		
	Almond milk (per 800 ml batch).	
	Almond skim	725.0
	Almond Fat	75.0
	Macadamia nut milk (per 660 ml batch)	
	Macadamia nut skim	291.0
	Macadamia nut fat	375.0

Table 2: INGREDIENTS FOR ONE BATCH OF CHEESE REPLICA

Ingredients	Amount (grams)
Soft Fresh Cheese Replica:	
Pasteurized almond milk	800.0
Pasteurized macadamia nut milk	666.0
Mesophilic starter culture (MAO11 direct set)	0.5
Transglutaminase (900 units in 15 mls of water)	15.0
Cheese salt (internal)	2.5
Salted Cheese Replica	
Pasteurized almond milk	800.0
Pasteurized macadamia nut milk	666.0
Mesophilic starter culture (MAO11 direct set)	0.5
Transglutaminase (900 units in 15 mls of water)	15.0
Soft Ripened Cheese Replica	
Pasteurized almond milk	800.0
Pasteurized macadamia nut milk	666.0
Mesophilic starter culture (MAO11 direct set)	0.3
Flora Danica	0.2
Geotrichum candidum	0.1
Penicillium candidum	0.1
Debaromyces hansenii	0.1
Transglutaminase (733 units in 15 mls of water)	15.0
Cheese salt (internal)	4.2
Goat Cheese Replica	
Pasteurized almond milk	800.0
Pasteurized macadamia nut milk	666.0
Mesophilic starter culture (MAO11 direct set)	0.5
Transglutaminase (900 units in 15 mls of water)	15.0
Cheese salt (internal)	2.5
Optional Ingredients	
Distilled white vinegar	5.3
Microbial "rennet:"	0.2

Notes:

"Transglutaminase" here refers to Activa TI, 100U/g, from Ajinomoto
"Mesophilic starter culture" here refers to Danisco Choozit lyophilized MA 11
"Geotrichum candidum" here refers to Danisco Choozit GEO17 LYO 10D
"Debaromyces hansenii" here refers to Chr. Hansen Debaryomyces hansenii LAF3
"Flora Danica" here refers to Chr. Hansen "Flora Danica freeze-dried lactic culture for direct vat set"
"Penicillium candidum" here refers to "Penicillium candidum FD PC A1" from Chr. Hansen
Microbial "rennet" here refers to FROMASE 50, protease from Rhyzomicrobium mucor

CLAIMS

WHAT IS CLAIMED IS:

1. A method comprising:

(a) preparing an emulsion comprising proteins and fats from plant sources

(b) inducing the emulsion to form a gel by enzymatic cross-linking of the proteins or denaturing the proteins, and

(c) producing a cheese-replica from the gel.

2. The method of claim 1, wherein the emulsion contains less than 10% animal products.

3. The method of claim 1, wherein the emulsion contains no animal products.

4. The method of claim 1, wherein the inducing step comprises adding an enzyme.

5. The method of claim 4, wherein the enzyme is transglutaminase.

6. The method of claim 5, wherein the transglutaminase is selected from the group consisting of: a transglutaminase from *Streptoverticillium mobaraense*, an enzyme is similar or identical to a transglutaminase from *Streptoverticillium mobaraense*, Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6), or TGM Z (TGM7).

7. The method of claim 1, wherein the emulsion is a non-dairy milk.

8. The method of claim 1, wherein at least one of the plant sources are nuts.

9. The method of claim 8, wherein the nuts are selected from one or more of following group: almonds, cashews, brazilnuts, chestnuts, coconuts, hazelnuts, macadamia nuts, peanuts, pecans, pistachios or walnuts.

10. The method claim 1, wherein the preparation includes the steps of

(a) obtaining nuts or seeds, and

(b) surface sterilizing the nuts or seeds,

wherein the nuts or seeds comprise the plant sources.

11. The method of claim 10, wherein the surface sterilization is blanching.

12. The method of claim 8, wherein the method further comprises washing the nuts or seeds.

13. The method of claim 10, wherein the preparation further comprising decompounding the nuts or seeds.

14. The method of claim 13, wherein the decompounding is blending.

15. The method of claim 10, further comprising centrifuging the sample.

16. The method of claim 12, wherein the centrifuging removes at least 85% of the suspended solids.

17. The method of claim 1, further comprising adding a sugar to the emulsion.

18. The method of claim 17, wherein the sugar is a monosaccharide.

19. The method of claim 17, wherein the sugar is a disaccharide.

20. The method of claim 1, further comprising inoculating with lactic acid bacteria.

21. The method of claim 20, further comprising incubating to allow growth of the bacterial cultures.

22. The method of claim 1, further comprising cutting the gel.

23. The method of claim 22, further comprising draining and shaping the cut gel.

24. The method of claim 1, further comprising adding a flavoring component.

25. The method of claim 24, wherein the flavoring component is a lactic acid bacteria, mold or yeast.

26. The method of claim 1, further comprising adding one or more of *Penicillium camemberti, Geotrichum candidum, Penicilliem roqueforti, Penicillium nalgiovensis, Verticillium lecanii, Kluyveromyces lactis, Saccharomyces cerevisiae, Candida utilis, Debaryomyces hansensil, Rhodosporidum infirmominiatum, Candida jefer, Cornybacteria, Micrococcus sps., Lactobacillus*

sps.,Lactococcus, Staphylococcus, Halomonas, Brevibacterium, Psychrobacter, Leuconostocaceae, Streptococcus thermophilus, Pediococcus sps., or *Propionibacteria.*

27. The method of claim 1 further comprising adding a halophilc bacterium or achaea.

28. The method of claim 1, wherein at least one of the plant sources is a legume.

29. The method of claim 1, wherein the proteins are concentrated prior to preparing the emulsion.

30. The method of claim 29, wherein the concentrated proteins are each essentially homogeneous proteins.

31. The method of claim 1, further comprising concentrating, isolating, or purifying the proteins and fats from one or more plant sources.

32. The method of claim 30, wherein the concentrated proteins are all the same essentially homogeneous plant-derived protein.

33. The method of claim of claim 30, wherein each concentrated protein is from a separate plant species.

34. A method comprising decompounding nuts or seeds in water, removing at least 85% of the suspended solids and adding a transglutaminase to catalyzes the formation of crosslinks between proteins from the nuts or seeds.

35. A method for making a non-dairy cheese replica comprising, obtaining nut milk, centrifugation of the nut milk to remove insoluble matter, and crosslinking proteins within the nut milk with transglutaminase.

36. The method of claim 1, wherein thermal denaturing is used in addition to enzymatic cross-linking.

37. The method of claim 1, wherein thermal denaturing is used without the addition of any enzymes.

38. A composition comprising a centrifuged non-dairy milk wherein at least 85% of the insoluble solids have been removed relative to the non-dairy milk prior to centrifugation.

39. A composition comprising a non-dairy milk with less than 30% polysaccharides.

40. The composition of claim 38, with less than 10% polysaccharides.

41. The composition of claim 38, with less than 1% polysaccharides.

42. The composition of claim 38, wherein the composition is entirely composed of ingredients derived from non-animal sources.

43. The composition of claim 38, wherein the composition is at least comprised of 20% protein isolated from a single plant species.

44. A composition comprising a non-dairy milk and a cross-linking enzyme.

45. The composition of claim 44, wherein the cross-linking enzyme is selected from the group consisting of: a transglutaminase from *Streptoverticillium mobaraense*, an enzyme is similar or identical to a transglutaminase from *Streptoverticillium mobaraense*, Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6), TGM Z (TGM7), or lysyl oxidase.

46. The composition of claim 44, wherein at least 85% of the insoluble solids have been removed by centrifugation.

47. The composition of claim 44, wherein proteins from non-animal sources comprise at least 50% of the total mass of the composition.

48. The composition of claim 44, wherein the composition has a starch content less than 1% by mass.

49. The composition of claim 44, wherein the composition has a starch content less than 5% by mass.

50. The composition of claim 44, wherein the composition has less than 1% insoluble carbohydrate by mass.

51. The composition of claim 44, wherein the composition has less than 5% insoluble carbohydrate by mass.

52. The composition of claim 43, wherein the composition has less than 5% starch and less than 1% insoluble carbohydrate by mass.

53. The composition of claim 44, wherein the composition has less than 5% starch and less than 5% insoluble carbohydrate by mass.

54. The composition of claim 44, wherein the composition has a polysaccharide content less than 1% by mass.

55. The composition of claim 44, wherein the composition has a polysaccharide content less than 5% by mass.

56. The composition of claim 44, wherein the composition has a carbohydrate content less than 1% by mass.

57. The composition of claim 44, wherein the composition has a carbohydrate content less than 5% by mass.

58. The composition of claim 44, wherein the composition has a carbohydrate content less than 10% by mass.

59. The composition of claim 44, wherein the composition has at least 80% of the protein content from a single monomeric or multimeric protein.

60. A composition comprising covalently crosslinked plant-derived proteins, a crosslinking enzyme, and cheese microbes.

61. The composition of claim 60, wherein the plant derived proteins are not soy based.

62. The composition of claim 60, wherein the water content of the composition is less than 5%.

63. The composition of claim 60, with less than 20% insoluble solids.

64. The composition of claim 60, further comprising oils or fats isolated from plant sources.

65. The composition of claim 60, wherein the crosslinking enzyme is selected from the group consisting of:atransglutaminase from *Streptoverticillium mobaraense*, an enzyme is similar or

identical to a transglutaminase from *Streptoverticillium mobaraense*, Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6) , TGM Z (TGM7), or lysyl oxidase.

66. The composition of claim 60, wherein the crosslinks are formed between glutamine and lysine side chains of respective protein constituents.

67. A cheese replica comprising a gelled emulsion of plant-derived proteins and fats.

68. The composition of claim 67, comprising between 10% and 40% proteins from plant sources and between 0% and 65% fats from plant sources.

69. The composition of claim 67, further comprising a crosslinking enzyme selected from the group consisting of: a transglutaminase from *Streptoverticillium mobaraense*, an enzyme is similar or identical to a transglutaminase from *Streptoverticillium mobaraense*, Factor XIII (fibrin-stabilizing factor), Keratinocyte transglutaminase (TGM1), Tissue transglutaminase (TGM2), Epidermal transglutaminase (TGM3), Prostate transglutaminase (TGM4), TGM X (TGM5), TGM Y (TGM6), TGM Z (TGM7), or lysyl oxidase.

70. The composition of claim 67, further comprising cheese-making microbes.

71. The composition of claim 67, wherein the cheese-making microbes are selected from one or more of: *Penicillium camemberti, Geotrichum candidum, Penicilliem roqueforti, Penicillium nalgiovensis, Verticillium lecanii, Kluyveromyces lactis, Saccharomyces cerevisiae, Candida utilis, Debaryomyces hansensil, Rhodosporidum infirmominiatum, Candida jefer, Cornybacteria, Micrococcus sps., Lactobacillus sps.,Lactococcus, Staphylococcus, Halomonas, Brevibacterium, Psychrobacter, Leuconostocaceae, Streptococcus thermophilus, Pediococcus sps.,* or *Propionibacteria.*

72. The method of claim 67, further comprising adding a halophilc bacterium or achaea.

73. A cheese replica composition, wherein the cheese replica is a cheese equivalent according to a human.

74. The cheese replica of claim 73, comprising protein, wherein at least 20% of the protein content comprises a single monomeric or multimeric protein.

75. The cheese replica of claim 73, made without the addition of starches or rennet or any other extrinsic proteases other than those contributed by microbial cultures.

76. The cheese replica of claim 73, with a pH less than 5.5 where the acidification was accomplished solely by microbial fermentation.

77. The cheese replica of claim 73, without animal products.

78. The cheese replica of claim 73, with less than 5% by mass insoluble carbohydrates.

79. The cheese replica of claim 73, made without rennet, vinegar, or lemon juice.

80. A cheese replica made with the addition of rennet or any aspartic protease or any other type protease (e.g., serine protease) to affect or enhance the flavor and /or aroma and /or texture of the cheese replica.

81. A cheese replica made with the addition of vinegar, lemon juice or any other type of acid to affect or enhance the flavor and /or aroma and/or texture of the cheese replica.

82. A soft fresh cheese replica comprising:

(a) pasteurized almond milk,
(b) pasteurized macadamia nut milk,
(c) mesophilic starter culture,
(d) transglutaminase,
(e) water, and
(f) cheese salt.

83. A salted cheese replica comprising

(a) pasteurized almond milk,
(b) pasteurized macadamia nut milk,
(c) mesophilic starter culture,
(d) transglutaminase, and
(e) water.

84. A soft ripened cheese replica comprising:

(a) pasteurized almond milk,
(b) pasteurized macadamia nut milk,
(c) mesophilic starter culture,

(d) flora danica,
(e) Geotrichum candidum,
(f) Penicillium candidum,
(g) Debaromyces hansenii
(h) transglutaminase,
(i) water, and
(j) cheese salt.

85. A goat cheese replica comprising:
(a) pasteurized almond milk
(b) macadamia nut milk,
(c) mesophilic starter culture,
(d) transglutaminase,
(e) water, and
(f) cheese salt.

86. The composition of claims 82-85 further comprising distilled white vinegar.

87. The composition of claims 82-85 further comprising microbial coagulant.

88. The composition of claims 82-85 further comprising white vinegar and microbial coagulant.

89. The method of claim 4, wherein the enzyme is lysyl oxidase.

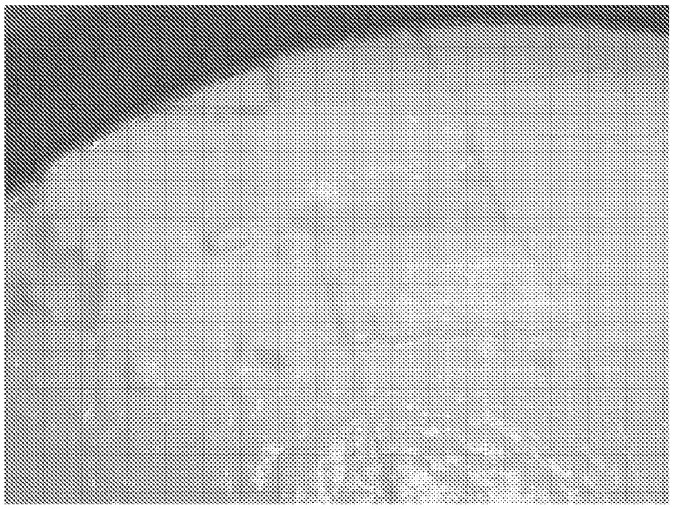


Figure 1.

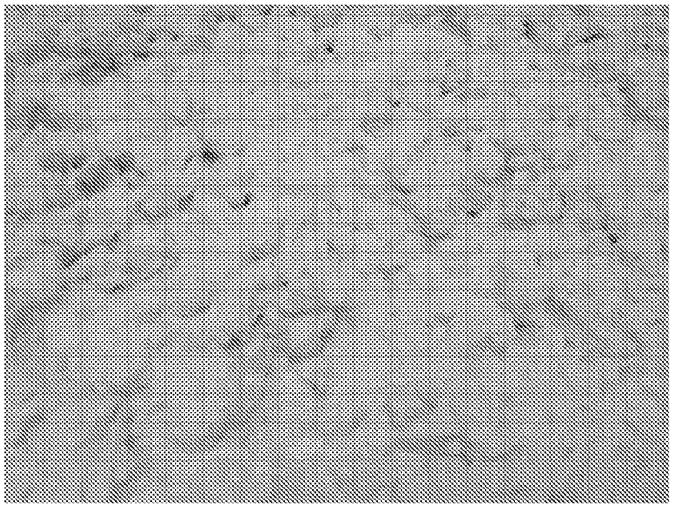
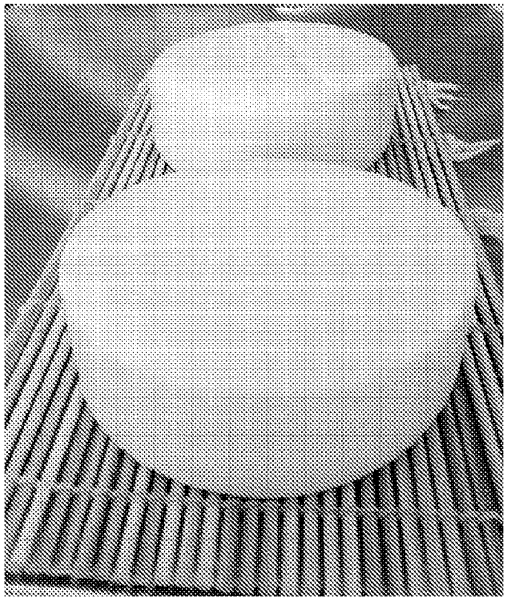


Figure 2.



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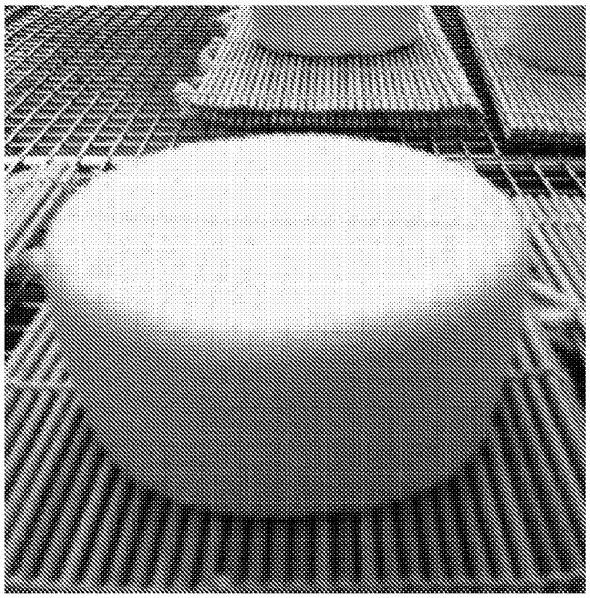
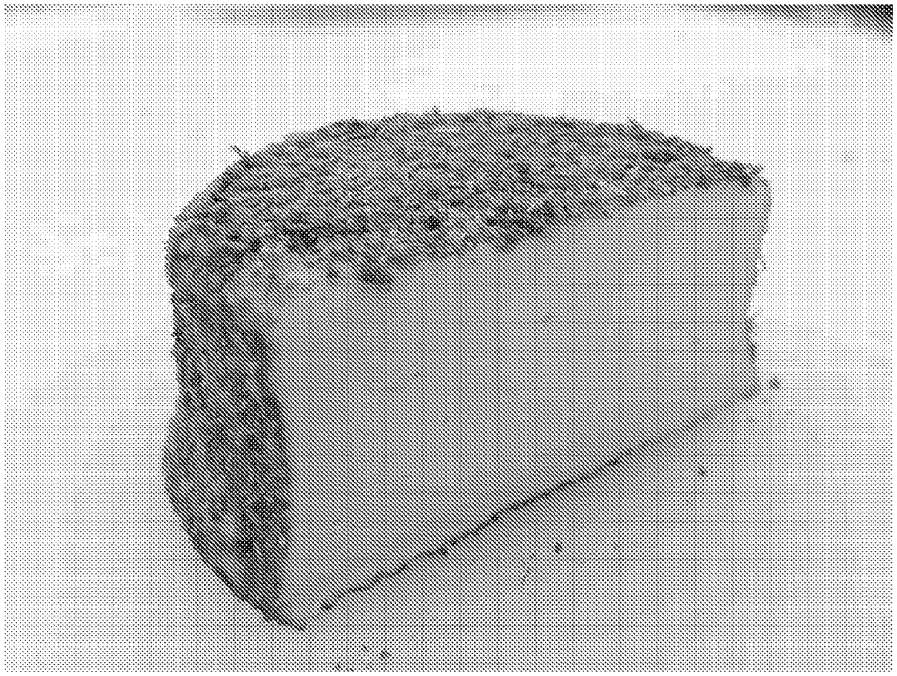


Figure 4.

Figure 5.



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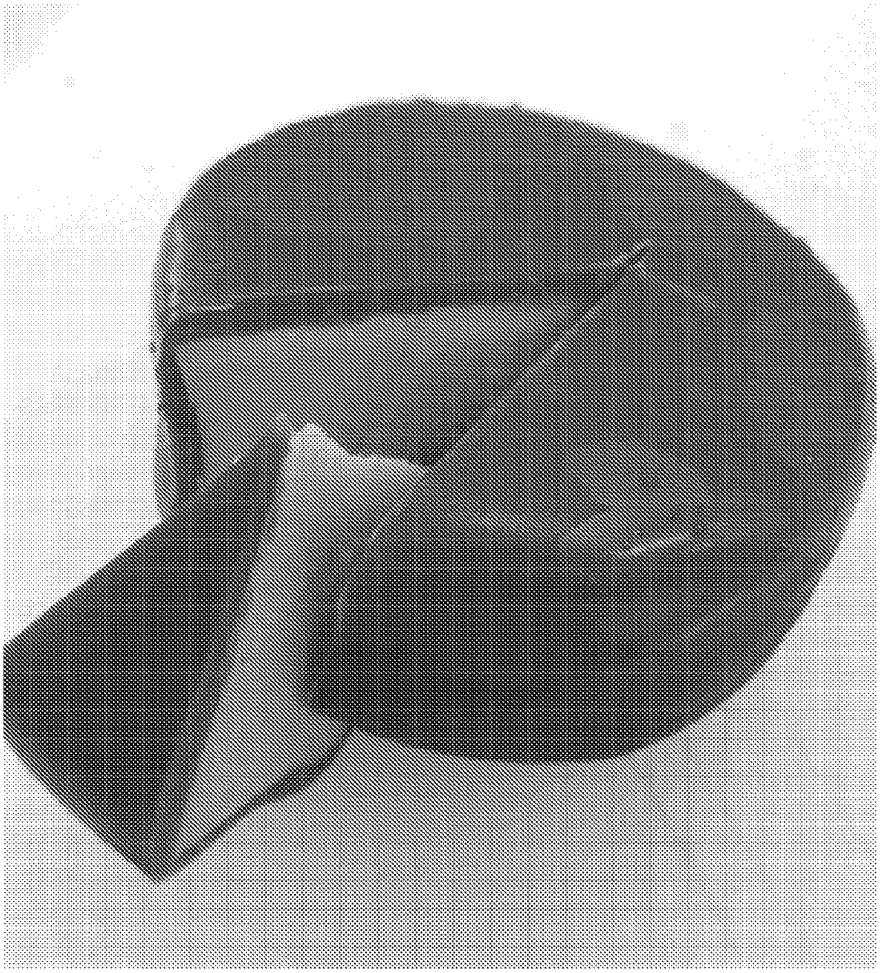

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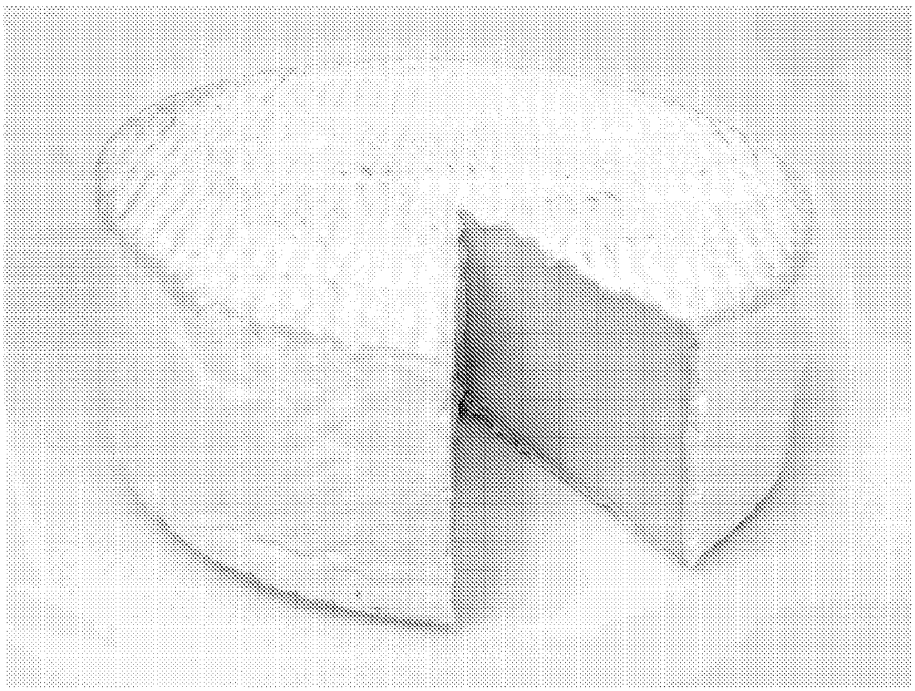

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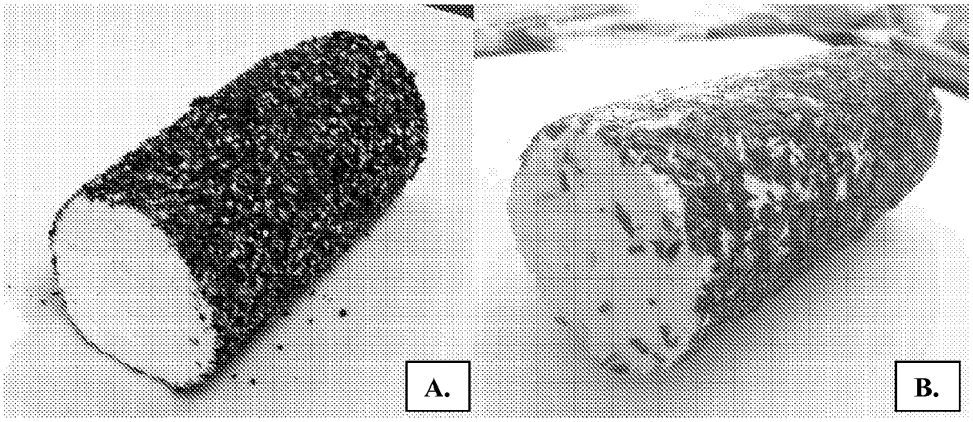

Figure 9.

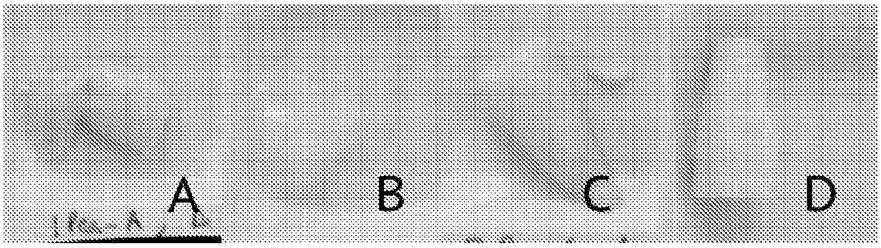

Figure 10.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/46552

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A23L 1/052; A23D 7/00; C12P 13/00 (2012.01)
USPC - 426/573, 426/602, 426/632
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC - 426/573, 426/602, 426/632

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 426/573, 426/602, 426/632; 435/128

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Lyrical Foods, Casino, Voccola, Varadan, transglutaminase, cheese, replica, imitation, plant, nut, soy, milk, extract, lysyl oxidase,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,055,310 A (Nonaka et al.) 18 October 1991 (18.10.1991), especially col 1, ln 35-36; col 2, ln 9-59; col 4, ln 15-46;	1-33 and 89
Y	US 4,678,676 A (Ishizuka et al.) 07 July 1987 (07.07.1987), especially col 2, ln 50 to col 3, ln 2; col 3, ln 45-56;	1-33 and 89
Y	US 2008/0299254 A1 (Kim et al.) 04 December 2008 (04.12.2008), especially para [0013]	20-21 and 25-26
Y	US 2011/0064847 A1 (McCready et al.) 17 March 2011 (17.03.2011), especially para [0004], [0026	8-16 and 33
Y	US 5,807,601 A (CARPENTER et al.) 15 September 1998 (15.09.1998) abstract; col 2, ln 1-5	8-16 and 33
Y	US 2003/0224476 A1 (CHOU) 4 December 2003 (04.12.2003) abstract; para [0003]; [0008]; [0042]; [0050]; [0176]	89
Y	US 2008/0292749 A1 (GOODWINS et al.) 27 November 2008 (27.11.2008) para [0021]; [0029]; [0034]	27

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
31 October 2012 (31.10.2012)

Date of mailing of the international search report
19 NOV 2012

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer:
Lee W. Young
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/46552

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-33 and 89, directed to a method comprising: (a) preparing an emulsion comprising proteins and fats from plant sources; (b) inducing the emulsion to form a gel by enzymatic cross-linking of the proteins or denaturing the proteins; and (c) producing a cheese-replica from the gel.

Group II: claims 34, 38, 40-43 and 52, directed to a method comprising decompounding nuts or seeds in water, removing at least 85% of the suspended solids and adding a transglutaminase to catalyzes the formation of crosslinks between proteins from the nuts or seeds.

- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-33 and 89

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/46552

Continuation of Box III: Lack of Unity of Invention

Group III: claims 35-37, directed to a method for making a non-dairy cheese replica comprising, obtaining nut milk, centrifugation of the nut milk to remove insoluble matter, and crosslinking proteins within the nut milk with transglutaminase.

Group IV: claim 39, directed to a composition comprising a non-dairy milk with less than 30% polysaccharides

Group V: claims 44-51 and 53-59 directed to a composition comprising a non-dairy milk and a cross-linking enzyme.

Group VI: claims 60-66, and 82-85, directed to a composition comprising covalently crosslinked plant-derived proteins, a crosslinking enzyme, and cheese microbes.

Group VII: claims 67-72, directed to a cheese replica comprising a gelled emulsion of plant-derived proteins and fats.

Group VIII: claims 73-79, directed to a cheese replica composition, wherein the cheese replica is a cheese equivalent according to a human.

Group IX: claim 80, directed to a cheese replica made with the addition of rennet or any aspartic protease or any other type protease (e.g., serine protease) to affect or enhance the flavor and/or aroma and /or texture of the cheese replica.

Group X: claim 81, directed to a cheese replica made with the addition of vinegar, lemon juice or any other type of acid to affect or enhance the flavor and/or aroma and/or texture of the cheese replica.

The inventions listed as Groups I - X do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of the claims of Groups I-X are indicated above in the Group descriptions.

There is no common technical element shared by all of the above groups. Groups I and VII-X are related to a cheese replica. Groups II-V are related to a non-dairy milk, which, in Groups II and III may be a nut milk. Groups I-III, V, and VI share the common technical element of a crosslinking enzyme, which, in Groups I-III and VI may be a transglutaminase. These common technical elements do not represent an improvement over the combined prior art of US 5,055,310 A to Nonaka et al. (hereinafter "Nonaka") and US 2005/0220937 A1 to Ito et al. (hereinafter "Ito"), as follows: Nonaka teaches a shelf-stable soybean curd product which is prepared by reacting a non-dairy milk (soy milk) with a solidifying agent and a transglutaminase (abstract); wherein the milk is produced by separating (filtering) plant solids from the liquid (col 9, ln 24-35). Although Nonaka does not specifically recite wherein the milk may also be a nut milk, it would have been obvious to a person of ordinary skill in the art to substitute a nut milk in place of the soy milk, as both were plant milk products in common use at the time of the invention. Further, Ito teaches producing a cheese-like food product (para [0058], [0071], [0072]) by using a transglutaminase (para [0059]) to enable crosslinking (shaping) of a soybean paste foodstuff (para [0059]), wherein the foodstuff comprises a non-dairy milk product (soy milk; para [0071]). It would have been obvious to a person skilled in the art to combine the teaching of Nonaka regarding the formation of a solid non-dairy milk product directly from the liquid with the teaching of Ito regarding the formation of a cheese replica made from plant material in order to make a cheese replica directly from the non-dairy milk without undue experimentation. A person of ordinary skill in the art would have desired to form a non-dairy cheese replica in a manner similar to dairy cheese making, while avoiding the use of allergenic dairy products to provide a cheese replica to users unable to consume dairy products.

Therefore, the inventions of Groups I-X lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
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61/572,205 12 July 2011 (12.07.2011) US

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(54) Title: METHODS AND COMPOSITIONS FOR CONSUMABLES

(57) Abstract: Provided herein are methods and compositions related to plant based meat substitutes which have properties similar to meat.

METHODS AND COMPOSITIONS FOR CONSUMABLES

This application claims priority to U.S. application Ser. No. 61/572,205 filed July 12, 2011, which is incorporated herein in its entirety by reference.

BACKGROUND OF THE INVENTION

[0001] Animal farming has a profound negative environmental impact. Currently it is estimated that 30% of Earth's land surface is dedicated to animal farming and that livestock account for 20% of total terrestrial animal biomass. Due to this massive scale animal farming accounts for more than 18% of net greenhouse gas emissions. Animal farming may be the largest human source of water pollution, and animal farming is by far the world's largest threat to biodiversity. It has been estimated that if the worlds human population could shift from a meat containing diet to a diet free of animal products, 26% of Earth's land surface would be freed for other uses. Furthermore the shift to a vegetarian diet would massively reduce water and energy consumption.

[0002] The consumption of meat has a profound negative impact on human health. The heath benefits of a vegetarian diet are well established. If the human population would shift to a vegetarian diet the cost savings in health care would be significant.

[0003] Hunger is a worldwide problem, yet the world's 4 major commodity crops (soybeans, maize, wheat, and rice) already supply more than 100% of the human population's requirements for calories and protein, including every essential amino acid.

[0004] Plant based meat substitutes have largely failed to cause a shift to a vegetarian diet. 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.

[0005] Disclosed herein are improved methods and compositions which more accurately replicate the characteristics that consumers value in the preparation and consumption of meat and which overcome the shortcomings and drawbacks of current meat substitutes.

SUMMARY OF THE INVENTION

[0006] In some aspects, the invention provides a meat substitute composition comprising a protein content, wherein one or more isolated and purified proteins accounts for 10% or more of said protein content by weight, wherein said meat substitute composition accurately mimics the taste, texture, or color of a meat product derived from animal sources.

[0007] In one embodiment, the meat substitute composition accurately mimics the color of said meat product in its raw state and in a cooked state after cooking.

[0008] In another embodiment, the one or more isolated and purified proteins accounts for 25% or more of said protein content by weight.

[0009] In another embodiment, the one or more isolated and purified proteins accounts for 50% or more of said protein content by weight.

[0010] In another embodiment, the one or more isolated and purified proteins accounts for 75% or more of said protein content by weight.

[0011] In another embodiment, the one or more isolated and purified proteins accounts for 90% or more of said protein content by weight.

[0012] In another embodiment, gluten does not account for 10% or more of said protein content by weight.

[0013] In another embodiment, each of said isolated, purified proteins is separately isolated and purified.

[0014] In another embodiment, the meat substitute composition comprises 1-7 isolated and purified proteins.

[0015] In another embodiment, said 1-7 isolated and purified proteins are each isolated from different plant species.

[0016] In some embodiments the meat substitute comprises less than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 individual proteins isolated from one or more plant species.

[0017] In another embodiment, said protein content comprises no more than trace amounts of any other proteins derived from the one or more plant species.

[0018] In another embodiment, said one or more isolated and purified proteins are selected from the group consisting of leghemoglobin, non-symbiotic hemoglobin, hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

[0019] In another embodiment, said one or more isolated and purified proteins are not isolated from an animal.

[0020] In another embodiment, said one or more isolated and purified proteins are isolated from a single plant source.

[0021] In another embodiment, said one or more isolated and purified proteins are isolated from multiple plant sources.

[0022] In another embodiment, wherein said one or more isolated, purified proteins are isolated from a genetically modified organism.

[0023] In some embodiments, said genetically modified organism is a genetically modified bacteria or yeast organism.

[0024] In some embodiments, said isolated, purified protein has been formed into fibers.

[0025] In particular embodiments, said fibers resemble skeletal muscle fibers.

[0026] In yet more particular embodiments, said fibers are asymmetric fibers.

[0027] In some embodiments, the meat substitute composition further comprises one or more isolated and purified iron-containing proteins.

[0028] In some embodiments, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins,.

[0029] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAEK LFALVRDSAGQLKASGTVVADAALGSVHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDELS RAWEVAYDELAAAIKKA.

[0030] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 2. SEQ ID NO 2: MIDQKEKELI KESWKRIEPN KNEIGLLFYA NLFKEEPTVS VLFQNPISSQ SRKLMQVLGI LVQGIDNLEG LIPTLQDLGR RHKQYGVVDS HYPLVGDCLL KSIQEYLGQG FTEEAKAAWT KVYGIAAQVM TAE. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 80% homology to SEQ ID NO 2. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 90% homology to SEQ ID NO 2. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 98% homology to SEQ ID NO 2.

[0031] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 3. SEQ ID NO 3: MRKQPTVFEK LGGQAAMHAA VPLFYKKVLA DDRVKHYFKN TNMEHQAKQQ EDFLTMLLGG PNHYKGKNMA EAHKGMNLQN SHFDAIIENL AATLKELGVS DQIIGEAAKV IEHTRKDCLG K. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 80% homology to SEQ ID NO 3. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 90% homology to SEQ ID NO 3. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 98% homology to SEQ ID NO 3.

[0032] In some embodiments, the isolated and purified proteins are assembled into one or more gels.

[0033] In some embodiments, the meat substitute composition further comprises one or more fats.

[0034] In particular embodiments, said one or more fats are derived from a plant source.

[0035] In another aspect, the invention provides a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources.

[0036] In some embodiments, said indicator is a visual indicator that accurately mimics the color transition of a meat product during said cooking progression.

[0037] In one embodiment, said color transition is from red to brown.

[0038] In one embodiment, said color transition is from pink to white or tan.

[0039] In one embodiment, said visual indicator transitions from a translucent to opaque color during said cooking progression.

[0040] In some embodiments, the indicator is an olfactory indicator that indicates cooking progression.

[0041] In one embodiment, said olfactory indicator is one or more volatile odorants released during cooking.

[0042] In some embodiments, said indicator comprises one or more isolated, purified iron-containing proteins.

[0043] In particular embodiments, said one or more isolated, purified iron-containing proteins is in a reduced state before cooking.

[0044] In one embodiment, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

[0045] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1:
MVAFTEKQDALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAEK
LFALVRDSAGQLKASGTVVADAALGSVHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDELS
RAWEVAYDELAAAIKKA.

[0046] In some embodiments, said one or more isolated and purified iron-containing proteins are not isolated from an animal. In some embodiments compositions of the invention do not contain any proteins from an animal.

[0047] In particular embodiments, said one or more isolated and purified iron-containing proteins are isolated from one or more plant sources.

[0048] In yet more particular embodiments, said one or more isolated, purified proteins are isolated from the root nodules, roots, seeds, leaves, or stems of said one or more plant sources.

[0049] In other particular embodiments, said one or more plant sources are soy or pea plants.

[0050] In one embodiment, said one or more plant sources comprises one or more plants of the legume family.

[0051] In some embodiments, said one or more isolated and purified iron carrying proteins in a reduced or oxidized state has a similar UV-VIS profile to a myoglobin protein derived from an animal source when in an equivalent reduced or oxidized state.

[0052] In a particular embodiment, the difference between the peak absorbance wavelength of said one or more isolated and purified iron-containing proteins and the peak absorbance wavelength of myoglobin derived from an animal source is less than 5%.

[0053] In some embodiments, said one or more isolated, purified proteins are isolated from a genetically modified organism.

[0054] In one embodiment, said genetically modified organism is a genetically modified bacteria or yeast organism.

[0055] In some embodiments, the meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[0056] In particular embodiments, the meat substitute product additionally contains less than 1% wheat gluten.

[0057] In a more particular embodiment, said meat substitute product contains no wheat gluten.

[0058] In other particular embodiments, said meat substitute product contains no soy protein isolate.

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

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

[0061] In a more particular embodiment, said meat substitute product contains less than 5% carbohydrates.

[0062] In other particular embodiments, said meat substitute product contains no tofu.

[0063] In some embodiments, said meat substitute product contains no tofu, and no wheat gluten.

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

[0065] In some embodiments, said meat substitute product contains no animal products and less than 5% carbohydrates.

[0066] In some embodiments, said meat substitute product contains less than 1% cellulose.

[0067] In some embodiments, said meat substitute product contains less than 5% insoluble carbohydrates.

[0068] In some embodiments, said meat substitute product contains no soy protein, and less than 1% cellulose.

[0069] In some embodiments, said meat substitute product contains no soy protein, and less than 5% insoluble carbohydrates.

[0070] In some embodiments, said meat substitute product contains no wheat gluten, and less than 1% cellulose.

[0071] In some embodiments, said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.

[0072] In another aspect, the invention provides a muscle tissue replica comprising a protein content, wherein said protein content comprises one or more isolated and purified proteins, wherein said muscle

tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source.

[0073] In some embodiments, said one or more isolated and purified proteins accounts for at least 50% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 40% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 30% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 20% of said protein content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 10% of said protein content by weight.

[0074] In some embodiments, said one or more isolated and purified proteins accounts for at least 50% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 40% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 30% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 20% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 10% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 5% of said composition content by weight. In some embodiments, said one or more isolated and purified proteins accounts for at least 1% of said composition content by weight.

[0075] In some embodiments, said protein content is derived from one or more non-animal sources.

[0076] In particular embodiments, said one or more non-animal sources is a plant source.

[0077] In other particular embodiments, said one or more non-animal sources is a genetically modified yeast or bacteria.

[0078] In some embodiments, each of said one or more isolated proteins is isolated and purified separately.

[0079] In some embodiments, said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin. .

[0080] In one embodiment, said seed storage protein is moong bean 8S protein.

[0081] In some embodiments, said protein content is suspended in a gel.

[0082] In some embodiments, said protein content is in the form of a gel.

[0083] In one embodiment, said gel comprises an isolated, purified cross-linking enzyme.

[0084] In some embodiments, said isolated, purified cross-linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.

[0085] In a particular embodiment, said isolated, purified cross-linking enzyme is transglutaminase.

[0086] In some embodiments, said protein content has been assembled into fibers.

[0087] In particular embodiments, said fibers are arranged isotropically.

[0088] In one embodiment, said fibers are asymmetric fibers.

[0089] In some embodiments, the muscle tissue replica further comprises one or more isolated and purified iron-containing proteins.

[0090] In some embodiments, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

[0091] In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTEKQDALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAEK LFALVRDSAGQLKASGTVVADAALGSVHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDELS RAWEVAYDELAAAIKKA. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 80% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 90% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 95% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 98% homology to SEQ ID NO 1.

[0092] In particular embodiments, the muscle tissue replica comprises a protein content, wherein (i) one isolated and purified protein that is not an iron-containing protein accounts for 40-95% of said protein content, (ii) one or more isolated and purified iron-containing proteins accounts for 1-20% of said protein content, and (iii) one or more cross-linking reagents accounts for 0.1-35% of said protein content.

[0093] In one embodiment, said protein content accounts for 5-50% of said replica by weight or by weight/volume.

[0094] In one embodiment, said one isolated and purified protein is moong bean 8S protein.

[0095] In one embodiment, said one or more isolated and purified iron-containing proteins is leghemoglobin.

[0096] In one embodiment, said one or more cross-linking reagents is transglutaminase.

[0097] In some embodiments, the muscle tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[0098] In particular embodiments, the muscle tissue replica additionally contains less than 1% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 5% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 10% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 0.1% wheat gluten.

[0099] In a more particular embodiment, said muscle tissue replica contains no wheat gluten.

[00100] In other particular embodiments, said muscle tissue replica contains no soy protein isolate.

[00101] In other particular embodiments, said muscle tissue replica contains no soy protein concentrate.

[00102] In other particular embodiments, said muscle tissue replica contains no soy protein.

[00103] In a more particular embodiment, said muscle tissue replica contains less than 5% carbohydrates.

[00104] In other particular embodiments, said muscle tissue replica contains no tofu.

[00105] In some embodiments, said muscle tissue replica contains no tofu, and no wheat gluten.

[00106] In some embodiments, said muscle tissue replica contains no soy protein, and no wheat gluten.

[00107] In some embodiments, said muscle tissue replica contains no animal products and less than 5% carbohydrates.

[00108] In some embodiments, said muscle tissue replica contains less than 1% cellulose.

[00109] In some embodiments, said muscle tissue replica contains less than 5% insoluble carbohydrates.

[00110] In some embodiments, said muscle tissue replica contains no soy protein, and less than 1% cellulose.

[00111] In some embodiments, said muscle tissue replica contains no soy protein, and less than 5% insoluble carbohydrates.

[00112] In some embodiments, said muscle tissue replica contains no wheat gluten, and less than 1% cellulose.

[00113] In some embodiments, said muscle tissue replica contains no wheat gluten, and less than 5% insoluble carbohydrates.

[00114] In some embodiments, the muscle tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[00115] In particular embodiments, the meat replica contains less than 1% wheat gluten. In particular embodiments, the meat replica contains less than 5% wheat gluten. In particular embodiments, the meat replica contains less than 10% wheat gluten. In particular embodiments, the meat replica contains less than 0.1% wheat gluten.

[00116] In a more particular embodiment, the meat replica contains no wheat gluten.

[00117] In other particular embodiments, the meat replica contains no soy protein isolate.

[00118] In other particular embodiments, the meat replica contains no soy protein concentrate.

[00119] In other particular embodiments, the meat replica contains no soy protein.

[00120] In a more particular embodiment, the meat replica contains less than 5% carbohydrates.

[00121] In other particular embodiments, the meat replica contains no tofu.

[00122] In some embodiments, the meat replica contains no tofu, and no wheat gluten.

[00123] In some embodiments, the meat replica contains no soy protein, and no wheat gluten.

[00124] In some embodiments, the meat replica contains no animal products and less than 5% carbohydrates.

[00125] In some embodiments, the meat replica contains less than 1% cellulose. In some embodiments, the meat replica contains less than 0.1% cellulose. In some embodiments, the meat replica contains less than 10% cellulose. In some embodiments, the meat replica contains less than 5% cellulose.

[00126] In some embodiments, the meat replica contains less than 5% insoluble carbohydrates.

[00127] In some embodiments, the meat replica contains no soy protein, and less than 1% cellulose.

[00128] In some embodiments, the meat replica contains no soy protein, and less than 5% insoluble carbohydrates.

[00129] In some embodiments, the meat replica contains no wheat gluten, and less than 1% cellulose.

[00130] In some embodiments, the meat replica contains no wheat gluten, and less than 5% insoluble carbohydrates.

[00131] In another aspect, the invention provides a fat tissue replica comprising a gelled emulsion, said gelled emulsion comprising a protein solution with fat droplets suspended therein.

[00132] In some embodiments, said fat droplets are derived from a non-animal source.

[00133] In some embodiments, said fat droplets are comprised of one or more plant oils.

[00134] In some embodiments, said one or more plant oils is selected from the group consisting of corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, 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.

[00135] In one embodiment, said one or more plant oils is rice bran oil or canola oil.

[00136] In some embodiments, said protein solution comprises one or more isolated, purified proteins.

[00137] In some embodiments, said one or more isolated, purified proteins accounts for 75% or more of the protein in said protein solution.

[00138] In some embodiments, said one or more isolated, purified proteins are derived from a non-animal source.

[00139] In some embodiments, said non-animal source is a plant source.

[00140] In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

[00141] In some embodiments, each of said one or more isolated proteins is isolated and purified separately.

[00142] In some embodiments, said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin. .

[00143] In some embodiments, said one or more isolated, purified proteins is an albumin protein, a seed storage protein, or pea globulin protein.

[00144] In particular embodiments, said albumin protein is isolated pea albumin protein.

[00145] In some embodiments, said seed storage protein is moong bean 8S protein.

[00146] In some embodiments, said gelled emulsion comprises a protein solution comprising 1-3 isolated and purified proteins, wherein said solution accounts for 30-70% of the volume of said emulsion; a plant oil, wherein said plant oil accounts for 30-70% of the volume of said emulsion; and an isolated, purified cross-linking enzyme, wherein said cross-linking enzyme accounts for 0.5-5% of said emulsion by wt/volume; wherein said plant oil is emulsified in said protein solution, wherein said emulsion is formed into a gel by said cross-linking enzyme.

[00147] In other embodiments said gelled emulsion comprises a protein solution comprising 1-3 isolated and purified proteins, wherein said solution accounts for 1-30% of the volume of said emulsion; a plant oil, wherein said plant oil accounts for 70-99% of the volume of said emulsion; and an isolated, purified cross-linking enzyme, wherein said cross-linking enzyme accounts for 0.5-5% of said emulsion by

wt/volume; wherein said plant oil is emulsified in said protein solution, wherein said emulsion is formed into a gel by said cross-linking enzyme.

[00148] In some embodiments, the fat replica further comprises a cross-linking enzyme.

[00149] In some embodiments, said cross-linking enzyme is transglutaminase.

[00150] In some embodiments, one of said 1-3 isolated and purified proteins is moong bean 8S protein, pea albumin protein, or pea globulin protein.

[00151] In particular embodiments, said plant oil is rice bran oil or canola oil.

[00152] In some embodiments, the fat tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[00153] In particular embodiments, the fat tissue replica additionally contains less than 1% wheat gluten.

[00154] In a more particular embodiment, said fat tissue replica contains no wheat gluten.

[00155] In other particular embodiments, said fat tissue replica contains no soy protein isolate.

[00156] In other particular embodiments, said fat tissue replica contains no soy protein concentrate.

[00157] In other particular embodiments, said fat tissue replica contains no soy protein.

[00158] In a more particular embodiment, said fat tissue replica contains less than 5% carbohydrates.

[00159] In other particular embodiments, said fat tissue replica contains no tofu.

[00160] In some embodiments, said fat tissue replica contains no tofu, and no wheat gluten.

[00161] In some embodiments, said fat tissue replica contains no soy protein, and no wheat gluten.

[00162] In some embodiments, said fat tissue replica contains no animal products and less than 5% carbohydrates.

[00163] In some embodiments, said fat tissue replica contains less than 1% cellulose.

[00164] In some embodiments, said fat tissue replica contains less than 5% insoluble carbohydrates.

[00165] In some embodiments, said fat tissue replica contains no soy protein, and less than 1% cellulose.

[00166] In some embodiments, said fat tissue replica contains no soy protein, and less than 5% insoluble carbohydrates.

[00167] In some embodiments, said fat tissue replica contains no wheat gluten, and less than 1% cellulose.

[00168] In some embodiments, said fat tissue replica contains no wheat gluten, and less than 5% insoluble carbohydrates.

[00169] In another aspect, the invention provides a connective tissue replica, comprising a protein content comprising one or more isolated, purified proteins, wherein said protein content has been assembled into structures approximating the texture and visual appearance of connective tissue or skin.

[00170] In some embodiments, said protein content is derived from non-animal source.

[00171] In some embodiments, said non-animal source is a plant source.

[00172] In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

[00173] In some embodiments, said one or more isolated proteins account for 50% or more of said protein content by weight.

[00174] In some embodiments, said one isolated and purified protein accounts for 90% or more of said protein content by weight.

[00175] In some embodiments, each of said one or more isolated proteins is isolated and purified separately.

[00176] In some embodiments, said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

[00177] In some embodiments, said one or more isolated and purified proteins is a prolamin family protein.

[00178] In some embodiments, said one or more isolated and purified proteins is zein.

[00179] In some embodiments, said protein content is suspended in a gel.

[00180] In some embodiments, said gel comprises an isolated, purified cross-linking enzyme.

[00181] In some embodiments, said isolated, purified cross-linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.

[00182] In some embodiments, said isolated, purified cross-linking enzyme is transglutaminase.

[00183] In some embodiments, said protein content is formed into a fiber.

[00184] In some embodiments, said fiber is produced by an extrusion process.

[00185] In some embodiments, said fiber is stabilized by protein crosslinks.

[00186] In some embodiments, fiber contains an isolated, purified cross-linking enzyme.

[00187] In some embodiments, said isolated, purified cross-linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.

[00188] In some embodiments, said isolated, purified cross-linking enzyme is transglutaminase.

[00189] In another aspect, the invention provides a meat substitute product, comprising a muscle replica; a fat tissue replica; and a connective tissue replica; wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat.

[00190] In some embodiments, the meat substitute product comprises two or more of said muscle replica, fat tissue replica, and connective tissue replica.

[00191] In some embodiments of the meat substitute product, said muscle replica accounts for 40-90% of said product by weight, said fat tissue replica accounts for 1-60% of said product by weight, and said connective tissue replica accounts for 1-30% of said product by weight.

[00192] In some embodiments, the meat substitute product comprises 60-90% water; 5-30% protein content; and 1-20% of a fat or fat replica; wherein said protein content comprises one or more isolated, purified plant proteins.

[00193] In some embodiments, said protein content is derived from non-animal source.

[00194] In some embodiments, non-animal source is a plant source.

[00195] In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

[00196] In some embodiments, 50% or more of said protein content by weight are one or more isolated purified proteins.

[00197] In some embodiments, each of said one or more isolated proteins is isolated and purified separately from different plant species.

[00198] In some embodiments, one or more of said isolated proteins is selected from the group consisting of: hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin. .

[00199] In some embodiments, the meat substitute product further comprises one or more isolated and purified iron-containing proteins.

[00200] In some embodiments, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins,. In some embodiments, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. [SEQ ID NO 1:
MVAFTEKQDALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAEK
LFALVRDSAGQLKASGTVVADAALGSVHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDELS
RAWEVAYDELAAAIKKA]

[00201] In some embodiments, the meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[00202] In particular embodiments, the meat substitute product additionally contains less than 1% wheat gluten.

[00203] In a more particular embodiment, said meat substitute product contains no wheat gluten.

[00204] In other particular embodiments, said meat substitute product contains no soy protein isolate.

[00205] In other particular embodiments, said meat substitute product contains no soy protein concentrate.

[00206] In other particular embodiments, said meat substitute product contains no soy protein.

[00207] In a more particular embodiment, said meat substitute product contains less than 5% carbohydrates.

[00208] In other particular embodiments, said meat substitute product contains no tofu.

[00209] In some embodiments, said meat substitute product contains no tofu, and no wheat gluten.

[00210] In some embodiments, said meat substitute product contains no soy protein, and no wheat gluten.

[00211] In some embodiments, said meat substitute product contains no animal products and less than 5% carbohydrates.

[00212] In some embodiments, said meat substitute product contains less than 1% cellulose.

[00213] In some embodiments, said meat substitute product contains less than 5% insoluble carbohydrates.

[00214] In some embodiments, said meat substitute product contains no soy protein, and less than 1% cellulose.

[00215] In some embodiments, said meat substitute product contains no soy protein, and less than 5% insoluble carbohydrates.

[00216] In some embodiments, said meat substitute product contains no wheat gluten, and less than 1% cellulose.

[00217] In some embodiments, said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.

[00218] In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by an animal.

[00219] In some embodiments, said one or more isolated, purified iron-containing proteins is derived from a non-animal source.

[00220] In some embodiments, said non-animal source is a plant source.

[00221] In some embodiments, said plant source comprises one or more plants of the legume family.

[00222] In some embodiments, said one or more plants of the legume family is a soy or pea plant.

[00223] In some embodiments, said non-animal source is a genetically modified yeast or bacteria.

[00224] In some embodiments, said iron-containing protein is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

[00225] In one embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. [SEQ ID NO 1:

MVAFTEKQDALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAEK LFALVRDSAGQLKASGTVVADAALGSVHAQKAVTDPQFVVVKEALLKTIKAAVGDKWSDELS RAWEVAYDELAAAIKKA]

[00226] In some embodiments, the food product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

[00227] In particular embodiments, the food product additionally contains less than 1% wheat gluten.

[00228] In a more particular embodiment, said food product contains no wheat gluten.

[00229] In other particular embodiments, said food product contains no soy protein isolate.

[00230] In other particular embodiments, said food product contains no soy protein concentrate.

[00231] In other particular embodiments, said food product contains no soy protein.

[00232] In a more particular embodiment, said food product contains less than 5% carbohydrates.

[00233] In other particular embodiments, said food product contains no tofu.

[00234] In some embodiments, said food product contains no tofu, and no wheat gluten.

[00235] In some embodiments, said food product contains no soy protein, and no wheat gluten.

[00236] In some embodiments, said food product contains no animal products and less than 5% carbohydrates.

[00237] In some embodiments, said food product contains less than 1% cellulose.

[00238] In some embodiments, said food product contains less than 5% insoluble carbohydrates.

[00239] In some embodiments, said food product contains no soy protein, and less than 1% cellulose.

[00240] In some embodiments, said food product contains no soy protein, and less than 5% insoluble carbohydrates.

[00241] In some embodiments, said food product contains no wheat gluten, and less than 1% cellulose.

[00242] In some embodiments, said food product contains no wheat gluten, and less than 5% insoluble carbohydrates.

[00243] In another aspect, the invention provides a method of making a meat substitute composition, comprising isolating and purifying one or more proteins; and assembling said one or more proteins into a physical organization that approximates the physical organization of meat.

[00244] In another aspect, the invention provides a method of making a muscle tissue replica, comprising isolating and purifying one or more proteins; and assembling said one or more proteins into a physical organization that approximates the physical organization of skeletal muscle.

[00245] In another aspect, the invention provides a method of making a fat tissue replica, comprising isolating and purifying one or more proteins; preparing a solution comprising one or more proteins; emulsifying one or more fats in said solution; and stabilizing said solution into a gelled emulsification with one or more cross-linking reagents.

[00246] In another aspect, the invention provides a method of making a connective tissue replica, comprising isolating and purifying one or more proteins; and precipitating said one or more proteins, wherein said precipitating results in said one or more proteins forming physical structures approximating the physical organization of connective tissue.

[00247] In some embodiments, said precipitating comprises solubilizing said one or more proteins in a first solution; and extruding said first solution into a second solution, wherein said one or more proteins is insoluble in said second solution, wherein said extruding induces precipitation of said one or more proteins.

[00248] In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by an animal.

[00249] In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by humans.

[00250] In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by an animal. In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by humans.

[00251] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[00252] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[00253] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[00254] Figure 1 depicts a comparison of aligned GCMS profiles (in mirrored orientations) of otherwise identical muscle-replica samples cooked in the presence of leghemoglobin (LHb, Right side profile) or Ferric ion (Left side profile). In this illustration, the vertical axis represents retention time in the gas chromatography separation step, and the horizontal axis represents m/z ratios of ions produced by fragmentation of the corresponding separated volatile compounds. Selected compounds differentially represented in the two samples are labeled on the Right of the figure.

[00255] Figure 2 depicts a portion of the root of a pea plant (Pisum sativum) with the root nodules sliced open to demonstrate the red color conferred by leghemoglobin contained therein. The sliced open rood nodule appears red.

[00256] Figure 3 depicts leghemoglobin isolated from 1 oz of pea roots. The red color commonly attributed to meat is evident in the color photo.

[00257] Figure 4 shows that leghemoglobins from different species are homologs and have similar color properties. In Figure 4, panel A shows an SDS_PAGE gels of lysed root-nodules of three legume plant species (1) Fava bean (2) English Pea (3) Soybean. Arrows mark respective leghemoglobins. Panel B

shows the similarity of UV-VIS spectral profile of leghemoglobins from two different plant species (Favabean and Soybean).

[00258] Figure 5 shows a comparison of reduced (heme iron 2+) and oxidized (heme iron 3+) soybean leghemoglobin (Figure 5 panel A) and equine heart muscle myoglobin (Figure 5 panel B) showing similarity of UV-VIS absorption profiles of two proteins. We purified soybean leghemoglobin from soybean root-nodules using here described protocol. Purified equine myoglobin was purchased from SigmaAldrich. Soybean leghemoglobin (Figure 5 panel A) and equine myoglobin (Figure 5 panel B) were reduced with 1mm sodium hydrosulfite. Shown are UV-VIS absorption spectra of heme Fe3+ (blue line – the higher peak in figures 5 and 6) and heme Fe2+ (red line) of soybean leghemoglobin (Figure 5 panel A) and equine myoglobin (Figure 5 panel B). Insets show a zoom-in of UV-VIS spectra in 450nm to 700nm region. (Figure 5 panel C) Images of 10ul liquid droplet of a 40 mg/ml solution of soybean leghemoglobin in the heme-Fe3+ state (left droplet) showing characteristic rusty red color and a 40mg/ml solution of soybean leghemoglobin solution in the heme-Fe2+ state (right droplet) showing characteristic red color of and (right image) corresponding samples of equine myoglobin.

[00259] Figure 6 depicts examples of successful reduction of leghemoglobin heme iron with sodium hydrosulfite and titanium citrate. In Figure 6 the UV-VIS spectrogram of purified soybean leghemoglobin in which the heme iron is in the oxidized (+3) state is represented by the blue curves in each panel (the blue curves have the higher peaks on the main graphs). The red curves in each panel represent the UV-VIS spectra of the same leghemoglobin species after reduction to the (+2) state (red lines) by addition of (Panel A) 1mM sodium hydrosulfite or (Panel B) 0.24 % (wt/v) titanium citrate in 20mM potassium phosphate pH 7.3, 100mM sodium chloride buffer. The Insets show a zoom-in of UV-VIS spectra in 450 -700 nm region. For this example, leghemoglobin was purified from soybean root nodules using 60/90% ammonium sulfate fractionation and exchanged into 20mM potassium phosphate pH 7.4, 100mM sodium chloride buffer. Sodium hydrosulfite stock solution was prepared by dissolving 100mM sodium hydrosulfite in1mM sodium hydroxide in water. Titanium citrate stock solution was prepared from 20 % (wt/v) Ti-chloride in hydrochloric acid by mixing it with 0.2M sodium citrate (1:10 v/v). pH was adjusted using sodium carbonate to pH 7.0.

[00260] Figure 7 depicts an example of the leghemoglobin purification flow from soybean root nodules. The figure shows SDS-PAGE fractionation of different soybean leghemoglobin purification steps (Lane 1) Soybean root-nodule lysate; (Lane 2) Soybean root-nodule lysate purified by 60/90 % (wt/v) ammonium sulfate fractionation. Shown is the protein content of 90% ammonium sulfate fractionated protein pellet resuspended in 20mm potassium phosphate pH 7.4, 100mM sodium chloride, 1mM EDTA buffer; Proteins from 90% ammonium sulfate pellet were further purified by anion-exchange chromatography (FFQ GE Healthcare) in 20mM potassium phosphate ph 7.4, 100mM sodium chloride. Leghemoglobin collected in the flowthrough is shown in Lane 3. Anion-exchange flowthrough was fractionated using size-exclusion chromatography (Sephacryl S-100 GE Healthcare) and resulting leghemoglobin fraction is shown in Lane 4. Leghemoglobin content at different purification steps was determined by determining the fraction of leghemoglobin band on SDS-PAGE gel in a respective sample

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

[00261] Figure 8 shows stained SDS-PAGE gel analysis of (A) soybean leghemoglobin expressed and purified using recombinant protein technology and (B) soybean leghemoglobin purified from soybean root nodules. (A) Recombinant Soybean leghemoglobin A carrying His-tag and TEV protease His-tag removal site was expressed in E.coli BL21 strain and purified using His-tag affinity chromatography (Talon resin, CloneTech). The left lane contains molecular weight standards, the right lane contains purified recombinant soybean leghemoglobin A (arrow). Expected molecular weight of the recombinant soybean leghemoglobin A is 17.1kDa. (B) SDS-PAGE gel of purified Soybean leghemoglobin from root nodules. The left lane contains molecular weight standards, the right lane contains purified soybean leghemoglobin A (arrow). Mass spectrometry analysis of purified material determined that all four soybean leghemoglobin isoforms are present, and are full length (data not shown). Expected molecular weights (MW) of soybean leghemoglobin isoforms range from MW15.4 to 15.8kDa.

[00262] Figure 9 shows an example of 6 cubes of a commercial meat analog (Quorn chicken analog), about 1cm on a side, 4 of which (Left and lower right) have been soaked in a solution of about 10 mg/ml soybean leghemoglobin in 20 mM Potassium phosphate pH 7.4 and 100 mM NaCl; the remaining two (Upper right) were soaked in the same buffer without leghemoglobin. A deep pink color of the leghemoglobin-infused cubes is apparent in color photos contrasting the pale tan color of the un-infused cubes.

[00263] Figure 10 shows the 4 leghemoglobin-infused cubes of Quorn chicken analog in the process of cooking in a pan at 350°C. The two lower cubes have been turned over to expose the grilled surface, which has turned brown. In the upper two cubes that the heated portion has turned grey-brown, while the cooler top surface retains its pink color. In some embodiments the consumable is injected with a heme containing solution, for instance a leghemoglobin solution, until the consumable is the color of uncooked meat.

[00264] Figure 11 depicts 43 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 37 ml of leghemoglobin solution (46.5 mg/ml leghemoglobin and 20mg/ml of other soybean root nodule protein) in 20 mM potassium phosphate, 100 mM NaCl, pH 7.3). 20 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly mixed, divided into two 50 ml Falcon tubes and incubated overnight at room temperature. Final protein concentrations were 65 mg/ml for moong bean protein, 18 mg/ml of leghemoglobin, 91 mg/ml total protein.

[00265] Figure 12 depicts "White" muscle analog prepared by mixing 43ml moong bean protein solution (150mg/ml) with 45ml of 11.7 mg/ml solution of leghemoglobin and 0.8 % (wt/v) of transglutaminase solution. Final protein concentrations were 63 mg/ml for moong bean protein, 5.2 mg/ml of leghemoglobin, 68 mg/ml total protein.

[00266] Figure 13 depicts a fat tissue analog based on moong beans and prepared in eppendorf tubes formed an opaque gel of off-white color, smooth uniform texture, with no visible discernible liquid that was not incorporated into the gel. The gel was freely standing, elastic and springy. The gel has a slight, pleasant aroma and a mild and pleasant flavor. The taste is mildly salty.

[00267] Figure 14 depicts at tissue analog based on pea globulin and prepared in eppendorf tubes very similar to moong bean-based fat analog, except that it gave up a little of oil upon compression.

[00268] Figure 15 shows connective-tissue analog strands that were created using a 1:3 ratio in 70% ethanol, loaded into a syringe with a 23 gauge needle (ID 0.337 mm). The solution was slowly extruded from the bottom of a 5 inch-high vessel into an excess of 5 M NaCl solution. The ethanol-zein solution being less dense than the NaCl solution, floated upward, drawing out a fibrous stand of solidifying zein. The NaCl was constantly stirred as the strands began to develop to assist in the strand lengthening. The strands bunch together and become a hard, dense mass.

[00269] Figure 16 depicts a ground beef prototype patty was made by combining 62% (wt/wt) muscle analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "white muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (Figure 16 panel A). A ground beef prototype patty was made by combining 62% muscle analog (62% "dark muscle analog" and 38% "white muscle analog), 29% fat tissue analog (from moong bean seed 8S protein and rice bran oil), 5% connective tissue analog (Figure 16 panel B). A ground beef prototype patty was made by combining 71 % (wt/wt) muscle tissue analog (composed of 60% "white" muscle analog, 40 % "dark" muscle analog), 23% fat tissue (from pea seed globulin proteins and canola oil) (Figure 16 panel C). A ground beef prototype patty was made by combining 67% "White" muscle analog, with 28% fat tissue analog (from pea globulins and rice bran oil), (Figure 16, panel D)

[00270] Figure 17 depicts a ground beef patty analog was made by combining 62% (wt/wt) muscle tissue analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog. The panel on the left shows the patty before cooking and the panel on the right shows the same patty after cooking for about 2 minutes. Observers described the aroma of the cooking ground beef replica as distinctly "beefy".

DETAILED DESCRIPTION OF THE INVENTION

[00271] Methods and compositions for the production of consumables are described herein. The consumables can be for animal consumption. For example the consumable can be food fit for human consumption. The consumable can be approved by suitable regulatory authorities. The consumables can be sold in grocery stores or prepared in restaurants, schools, hospitals, military facilities, prisons, shelters, long-term care facilities, similar to already existing human foods. The consumables could also be food for domestic animals. For instance, dog food could be produced according to the present inventions. The consumables may also be food for wild animals. For instance, the consumables could be provided to non-domesticated predatory animals.

[00272] The consumables of the present invention can compete with, supplement or replace animal based foods. For instance the consumables can be meat replicas made entirely from plant sources. The

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

[00273] The consumable may be made entirely from plant based sources. In some instances the consumable can be made from organic sources. The consumables may also be made from a combination of plant based sources and animal based sources. For instance, the consumable may be a ground beef product supplemented with plant based products of the invention.

[00274] The consumables can be made from local products. For instance the consumables can be made from plants grown within a certain radius of the eventual consumer. That radius could be 1, 10, 100, or 1000 miles for example. So, in some embodiments, the invention provides a method for producing a meat replica which does not contain products which have been shipped over 1, 10, 100, or 1000 miles prior to producing the meat replica.

[00275] The present invention provides methods for producing consistent properties from the consumables when they are produced from various sources. So, for example, a plant based meat replica produced from local plants in Iowa, USA, will have substantially similar taste, odor, and texture as a plant based meat replica produced from local plants in Lorraine, France. This consistency allows for methods for advertising locally grown foods with consistent properties. The consistency can arise from the concentration or purification of similar components at different locations. These components can be combined in predetermined ratios to insure consistency. In some embodiments a high degree of characteristic consistency is possible using components (e.g. isolated or concentrated proteins and fats) which come from the same plant species. In some embodiments a high degree of characteristic consistency is possible using components (e.g. isolated or concentrated proteins and fats) which come from the different plant species. In some embodiments the same proteins can be isolated from different plant species. In some embodiments the invention provides for a method comprising isolating similar plant constituents from plant sources in different locations, assembling in both locations compositions provided herein, and selling the compositions, wherein the compositions assembled and sold at different the geographic locations have consistent physical and chemical properties. In some embodiments the isolated constituents are from different plant populations in different locations. In some embodiments one or more of the isolated constituents are shipped to the separate geographic locations.

[00276] The consumables may require fewer resources to produce than consumables produced from domesticated animals. Accordingly, the present invention provides for meat replicates which require less water or energy to produce than meat. For example a consumable can require less than about 10, 50, 100, 200, 300, 500, or 1000 gallons of water per pound of consumable. For comparison beef can require over 2000 gallons of water per pound of meat.

[00277] The consumable may require less land are to produce than a meat product with similar protein content. For example the consumable may require 30% or less of the land area required to produce a meat product with similar protein content.

[00278] 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.

[00279] 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.

[00280] 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.

[00281] The consumable may provide alternatives to animal products or combinations of animal products whose consumption is forbidden by religious beliefs. For example, the consumable may be a kosher pork chop.

[00282] 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.

[00283] 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.

[00284] The consumables are, in some embodiments, designed to replicate the experience of eating meat. The look, texture, and taste of the consumable can be such that it is similar or indistinguishable from

meat. The invention therefore provides in certain embodiments methods for determining whether an animal or human can distinguish the consumable from meat.

[00285] One method to determine whether the consumable is comparable to meat is to a) define the properties of meat and b) determine whether the consumable has similar properties. Properties of meat that can be tested include mechanical properties such as hardness, cohesiveness, brittleness, chewiness, gumminess, viscosity, elasticity, and adhesiveness. Properties of meat that can be tested also include geometric properties such as particle size and shape, and particle shape and orientation. Additional properties can include moisture content and fat content. These properties can be described using terms such as "soft," "firm" or "hard" describe hardness; "crumbly," "crunchy," "brittle," "chewy," "tender," "tough," "short," "mealy," "pasty," or "gummy," to describe cohesiveness; "thin" or "viscous" to describe viscosity; "plastic" or "elastic" to describe elasticity; "sticky," "tacky" or "gooey" to describe adhesiveness; "gritty," "grainy" or "course" to describe particle shape and size; "fibrous," "cellular" or "crystalline" to describe particle shape and orientation, "dry," "moist," "wet," or "watery" to describe moisture content; or "oily" or "greasy" to describe fat content. So, in one embodiment a group of people can be asked to rate a certain meat, for instance ground beef, according to properties which describe the meat. These ratings can be used as an indication of the properties of the meat. The consumables of the present invention can then be compared to the meat to determine how similar the consumable is to the meat. In some instances the properties of the consumables are then altered to make the consumable more similar to the meat. So, in some embodiments, the consumable is rated similar to meat according to human evaluation. In some embodiments the consumable is indistinguishable from real meat to a human.

[00286] In some embodiments, subjects asked to identify the consumable identify it as a form of meat. In some embodiments one property of the compositions of the invention is that an animal, for example a human, will identify the composition as a meat. In some embodiments the human identifies the composition of the invention as having properties equivalent to meat. In some embodiments one or more properties of meat are equivalent according to a human's perception. Such properties include the properties that can be tested. In some embodiments a human identifies a consumable of the present invention as more meat like than meat substitutes found in the art.

[00287] In embodiments an experiment can demonstrate that consumable s acceptable to consumers. A panel can be used to screen a variety of consumables described herein. A number of human panelists can tested multiple consumable samples, namely, natural meats vs. the consumable compositions described herein. Variables such as fat content can be standardized for example to 20% fat using lean and fat meat mixes. Fat content can be determined using the Babcock for meat method (S. S. Nielson, Introduction to the Chemical Analysis of Foods (Jones & Bartlett Publishers, Boston, 1994)). Mixtures of ground beef and consumables of the invention prepared according to the procedure described herein can be formulated.

[00288] 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.

[00289] The results of this experiment can indicate significant differences ($p<0.05$) or similarities between the traditional meats and the compositions of the invention.

[00290] 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.

[00291] Consumables of the invention can also have similar physical characteristics as traditional meat. In one embodiment the force required to pierce a 1 inch thick structure (e.g. a patty) made of a consumable of the invention with a fixed diameter steel rod is not significantly different than the force required to pierce a 1 inch thick similar meat structure (e.g. a ground beef patty) with a similar fixed diameter steel rod. Accordingly, the invention provides for consumables with similar physical strength characteristics to meat.

[00292] In some embodiments composition of the invention have a similar cook loss characteristic as meat. In one embodiment a consumable of the invention with a similar fat and protein content as ground beef has the same reduction in size when cooked as real ground beef. Similar similarities in size loss profiles can be achieved for various compositions of consumables described herein matched to various meats.

[00293] In some embodiments the consumable is compared to real meat based upon olfactometer readings. In various embodiments the olfactometer can be used to assess odor concentration and odor thresholds, odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of appreciation, or relative intensity of odors. In some embodiments the olfactometer allows the training and automatic evaluation of expert panels. So in some embodiments the consumable is a product that causes similar or identical olfactometer readings. In some embodiments the similarity is sufficient to be beyond the detection threshold of human perception.

[00294] 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 could be used to refine the consumable.

[00295] Characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions molecules including amino acids, fats and sugars which are found in plants as well as meat. Therefore in some embodiments the consumable is tested for similarity to meat during or after cooking. In some embodiments human ratings, human evaluation, olfactometer readings, or GCMS measurements, or combinations thereof, are used to create an olfactory map of cooked meat. Similarly, an olfactory map of the consumable, for instance a meat replica, can be created. These maps can be compared to assess how similar the cooked consumable it so meat. In some embodiments the olfactory map of the consumable during or after cooking is similar to or indistinguishable from that of cooked or cooking meat. In some embodiments the similarity is sufficient to be beyond the detection threshold of human perception.

[00296] In one aspect, the invention provides a meat substitute product (alternatively referred to herein as "consumable") that is substantially or entirely composed of ingredients derived from non-animal sources, yet recapitulates key features associated with the cooking and consumption of an equivalent meat product derived from animals. The equivalent meat product can be a white meat or a dark meat. The equivalent meat product can be derived from any animal. Non-limiting examples of animals used to derive the equivalent meat product include farmed animals such as, e.g., cattle, sheep, pig, chicken, turkey, goose, duck, horse, dog or game animals (whether wild or farmed) such as, e.g., rabbit, deer, bison, buffalo, boar, snake, pheasant, quail, bear, elk, antelope, pigeon, dove, grouse, fox, wild pig, goat, kangaroo, emu, alligator, crocodile, turtle, groundhog, marmot, possum, partridge, squirrel, raccoon, whale, seal, ostrich, capybara, nutria, guinea pig, rat, mice, vole, any variety of insect or other arthropod, seafood such as, e.g, fish, crab, lobster, oyster, muscle, scallop, abalone, squid, octopus, sea urchin, tunicate and others. Many meat products are typically derived from skeletal muscle of an animal but it is understood that meat can also come from other muscles or organs of the animal. In some embodiments, the equivalent meat product is a cut of meat derived from skeletal muscle. In other embodiments, the equivalent meat product is an organ such as, e.g., a kidney, heart, liver, gallbladder, intestine, stomach, bone marrow, brain, thymus, lung, tongue. Accordingly, in some embodiments the compositions of the present invention are consumables similar to skeletal muscle or organs.

[00297] In some aspects, the present invention provides meat substitute products comprising one or more of a first composition comprising a muscle tissue replica, a second composition comprising an adipose tissue replica, and/or a third composition comprising a connective tissue replica, wherein the one or more compositions are combined in a manner that recapitulates the physical organization of meat. In other aspects, the present invention provides compositions for a muscle tissue replica (herein referred to as "muscle replica"), an adipose tissue replica (herein referred to as "fat replica"), and a connective tissue replica (herein referred to as "connective tissue replica"). In some embodiments, the compositions and meat substitute products are principally or entirely composed of ingredients derived from non-animal sources. In alternative embodiments, the muscle, fat, and/or connective tissue replica, or the meat substitute products comprising one or more of said replicas, are partially derived from animal sources but supplemented with ingredients derived from non-animal sources. In yet other alternative embodiments,

the invention provides meat products substantially derived from animal sources but which are supplemented with one or more of a muscle tissue replica, a fat replica, and/or a connective tissue replica, wherein said replicas are derived substantially or entirely from non-animal sources. A non-limiting example of such a meat product is an ultra-lean ground beef product supplemented with a non-animal derived fat replica which improves texture and mouthfeel while preserving the health benefits of a consumable low in animal fat. Such alternative embodiments result in products with properties that more closely recapitulate key features associated with preparing and consuming meat but which are less costly and associated with a lesser environmental impact, less animal welfare impact, or improved health benefits for the consumer.

[00298] The physical organization of the meat substitute product can be manipulated by controlling the localization, organization, assembly, or orientation of the muscle, fat, and/or connective tissue replicas described herein. In some embodiments the product is designed in such a way that the replicas described herein are associated with one another as in meat. In some embodiments the consumable is designed so that after cooking the replicas described herein are associated with one another as in cooked meat. In some embodiments, one or more of the muscle, fat, and/or connective tissue replicas are combined in a manner that recapitulate the physical organization of different cuts or preparations of meat. In an example embodiment, the replicas are combined in a manner that approximates the physical organization of natural ground meat. In other embodiments, the replicas are combined in a manner that approximates different cuts of beef, such as, e.g., ribeye, filet mignon, London broil, among others.

PROTEINS AND PROTEIN SOURCES

[00299] In some embodiments, any of the meat substitute products, muscle tissue replica, fat replica, or connective tissue replica, comprise one or more isolated, purified proteins. In some embodiments, the meat substitute products are comprised of one or more of a muscle replica, a fat replica, and/or connective tissue replica which comprise one or more isolated, purified proteins. In other embodiments, the muscle replica, fat replica, and/or connective tissue replica comprises one or more isolated, purified proteins. In some embodiments, about 0.1%,, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component is comprised of one or more isolated, purified proteins. For the purposes of this document, "purified protein" will refer to a preparation in which the cumulative abundance by mass of protein components other than the specified protein, which can be a single monomeric or multimeric protein species, is reduced by a factor of 2 or more, 3 or more, 5 or more, 10 or more, 20 or more, 50 or more, 100 or more or 1000 or more relative to the source material from which the specified protein was isolated.

[00300] In some embodiments, the one or more isolated, purified proteins are derived from non-animal sources. Non-limiting examples of non-animal sources include plants, funghi, bacteria, archaea, genetically modified organisms such as genetically modified bacteria or yeast, chemical or in vitro synthesis. In particular embodiments, the one or more isolated, purified proteins are derived from plant

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

[00301] Proteins that are abundant in plants can be isolated in large quantities from one or more source plants and thus are an economical choice for use in any of the muscle, fat, connective tissue replicas, or meat substitute products. Accordingly, in some embodiments, the one or more isolated proteins comprises an abundant protein found in high levels in a plant and capable of being isolated and purified in large quantities. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70% of the total protein content of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5-40%, about 10-50%, about 20-60%, or about 30-70% of the total protein content of the source plant. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50% of the total weight of the dry matter of the source plant. In some embodiments, the abundant protein comprises about 0.5-5%, about 1-10%, about 5-20%, about 10-30%, about 15-40%, about 20-50% of the total weight of the dry matter of the source plant.

[00302] In particular embodiments, the one or more isolated proteins comprises an abundant protein that is found in high levels in the leaves of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80% of the total protein content of the leaves of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%, about 20%-60%, or about 30-70% of the total protein content of the leaves of the source plant. In particular embodiments, the one or more isolated proteins comprise ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco activase). Rubisco is a particularly useful protein for meat replicas because of its high solubility and an amino acid composition with close to the optimum proportions of essential amino acids for human nutrition. In particular embodiments, the one or more isolated proteins comprise ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase). In particular embodiments, the one or more isolated proteins comprise a vegetative storage protein (VSP).

[00303] In some embodiments, the one or more isolated proteins includes an abundant protein that is found in high levels in the seeds of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or 90% or more of the total protein content of the seeds of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5%-40%, about 10%-60%, about 20%-60%, or about 30-70% or >70% of the total protein content of the seeds of the source plant. Non-limiting examples of proteins found in high levels in the seeds of plants are seed storage proteins, e.g., albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins

[00304] In some embodiments, the one or more isolated proteins includes proteins that interact with lipids and help stabilize lipids in a structure. Without wishing to be bound by a particular theory, such proteins may improve the integration of lipids and/or fat replicas with other components of the meat substitute product, resulting in improved mouthfeel and texture of the final product. A non-limiting example of a lipid-interacting plant protein is the oleosin family of proteins. Oleosins are lipid-interacting proteins that are found in oil bodies of plants. Other non-limiting examples of plant proteins that can stabilize emulsions include seed storage proteins from Great Northern Beans, albumins from peas, globulins from peas, 8S globulins from Moong bean, 8S globulins from Kidney bean.

MUSCLE REPLICAS

[00305] A large number of meat products comprise a high proportion of skeletal muscle. Accordingly, the present invention provides a composition derived from non-animal sources which replicates or approximates key features of animal skeletal muscle. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources which replicates or approximates animal skeletal muscle. Such a composition will be labeled herein as "muscle replica". In some embodiments, the muscle replica and/or meat substitute product comprising the muscle replica are partially derived from animal sources. In some embodiments, the muscle replica and/or meat substitute product comprising the muscle replica are entirely derived from non-animal sources.

[00306] Many meat products comprise a high proportion of striated skeletal muscle in which individual muscle fibers are organized mainly in an isotropic fashion. Accordingly, in some embodiments the muscle replica comprises fibers that are to some extent organized isotropically. In some embodiments the fibers comprise a protein component. In some embodiments, the fibers comprise about 1%, about 2%, about 5%, about 10%, about 15%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99% or more of a protein component.

[00307] In some embodiments, the protein component comprises one or more isolated, purified proteins. For example the one or more isolated, purified protein can comprise the 8S globulin from Moong bean seeds, or the albumin or globulin fraction of pea seeds. These proteins provide examples of proteins with favorable properties for constructing meat replicas because of their ability to form gels with textures

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

[00308] 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.

[00309] 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.

[00310] In another aspect, the invention provides methods for making a muscle replica. In some embodiments, the composition is formed into asymmetric fibers prior to incorporation into the consumable. In some embodiments these fibers replicate muscle fibers. In some embodiments the fibers are spun fibers. In other embodiments the fibers are extruded fibers. Accordingly, the present invention provides for methods for producing asymmetric or spun protein fibers. In some embodiments, the fibers are formed by extrusion of the protein component through an extruder. Methods of extrusion are well known in the art, and are described in US6379738, US3693533, US20120093994, which are herein incorporated by reference.

[00311] In some embodiments extrusion can be conducted using an MPF19 twin-screw extruder (APV Baker, Grand Rapids, Mich.) with a cooling die. The cooling die can cool the extrudate prior to return of the extrudate to atmospheric pressure, thus substantially inhibiting expansion or puffing of the final

product. In the MPF19 apparatus, dry feed and liquid can be added separately and mixed in the barrel. Extrusion parameters can be, for example: screw speed of 200 rpm, product temperature at the die of 150 C., feed rate of 23 g/min, and water-flow rate of 11 g/min. Product temperature can be measured during extrusion by a thermocouple at the end of the extrusion barrel. Observations can be made on color, opacity, structure, and texture for each collected sample. Collected samples can be optionally dried at room temperature overnight, then ground to a fine powder (<60 mesh) using a Braun food grinder. The pH of samples can be measured in duplicate using 10% (w/v) slurries of powdered sample in distilled water.

[00312] FAT REPLICA

[00313] Animal fat is important for the experience of eating cooked meat. Accordingly, the present invention provides a composition derived from non-animal sources which recapitulates key features of animal fat. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources which recapitulates animal fat. Such a composition will be labeled herein as a "fat replica". In some embodiments, the fat replica and/or meat substitute product comprising the fat replica are partially derived from animal sources.

[00314] In some embodiments the meat substitute product has a fat component. In some embodiments the fat content of the consumable is 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, or 60% fat. In some embodiments, the fat replica comprises a gel with droplets of fat suspended therein. In some embodiments, the gel is a soft, elastic gel comprising proteins and optionally carbohydrates. In particular embodiments, the proteins used in the gel are plant or microbial proteins. In some embodiments, the proteins used in the fat replica might include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microorganisms, or synthesized in vitro. The proteins may be artificially designed to emulate physical properties of animal fat. The proteins may be artificially designed to emulate physical properties of animal fat.

[00315] The fat droplets used in some embodiments of the present invention can be from a variety of sources. In some embodiments, the sources are non-animal sources. In particular embodiments, the sources are plant sources. Non-limiting examples of oils include corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, 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.

[00316] In some embodiments, fat droplets are derived from pulp or seed oil. In other embodiments, the source may be yeast or mold. For instance, in one embodiment the fat droplets comprise triglycerides derived from *Mortierella isabellina*.

[00317] 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.

[00318] In some embodiments, the fat replica comprises a protein component comprising one or more isolated, purified proteins. The purified proteins contribute to the taste and texture of the meat replica. In some embodiments purified proteins can stabilize emulsified fats. In some embodiments the purified proteins can form gels upon denaturation or enzymatic crosslinking, which replicate the appearance and texture of animal fat. Examples and embodiments of the one or more isolated, purified proteins are described herein. In particular embodiments, the one or more isolated proteins comprise a protein isolated from the legume family of plants. Non-limiting examples of legume plants are described herein, although variations with other legumes are possible. In some embodiments, the legume plant is a pea plant. In some embodiments the isolated purified proteins stabilize emulsions. In some embodiments the isolated purified proteins form gels upon crosslinking or enzymatic crosslinking. In some embodiments, the isolated, purified proteins comprise seed storage proteins. In some embodiments, the isolated, purified proteins comprise albumin. In some embodiments, the isolated, purified proteins comprise globulin. In a particular embodiment, the isolated, purified protein is a purified pea albumin protein. In another particular embodiment, the isolated, purified protein is a purified pea globulin protein. In another particular embodiment the isolate purified protein is a Moong bean 8S globulin. In another particular embodiment, the isolated, purified protein is an oleosin. In another particular embodiment, the isolated, purified protein is a caloleosin. In another particular embodiment, the isolated, purified protein is Rubisco. In some embodiments, the protein component comprises about 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or more of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises about 0.1-5%, about 0.5-10%, about 1-20%, about 5-30%, about 10-50%, about 20-70%, or

about 30-90% or more of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises a solution containing one or more isolated, purified proteins.

[00319] 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 . In particular embodiments, the cross-linking enzyme is transglutaminase.

[00320] In another aspect, the invention provides methods for making a fat replica. In some embodiments, the fat droplets are suspended in a gel. In some embodiments the present invention provides for methods for producing droplets of fat suspended in the gel. The fat can isolated and homogenized. For example an organic solvent mixture can be used to help mix a lipid. The solvent can then be removed. At this point the lipid can be frozen, lyophilized, or stored. So in some aspects the invention provides for a method for isolating and storing a lipid which has been selected to have characteristics similar to animal fat. The lipid film or cake can then be hydrated. The hydration can utilize agitation or temperature changes. The hydration can occur in a precursor solution to a gel. After hydration the lipid suspension can be sonicated or extruded to further alter the properties of the lipid in the solution.

[00321] In some embodiments, the fat replica is assembled to approximate the organization adipose tissue in meat. In some embodiments some or all of the components of the fat replica are suspended in a gel. In various embodiments the gel can be a proteinaceous gel, a hydrogel, an organogel, or a 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.

[00322] In particular embodiments, the fat replica is an emulsion comprising a solution of one or more proteins and one or more fats suspended therein as droplets. In some embodiments, the emulsion is stabilized by one or more cross-linking enzymes into a gel. In more particular embodiments, the one or more proteins in solution are isolated, purified proteins. In yet more particular embodiments, the isolated, purified proteins comprise a purified pea albumin enriched fraction. In other more particular embodiments, the isolated, purified proteins comprise a purified pea globulin enriched fraction. In other more particular embodiments, the isolated, purified proteins comprise a purified Moong bean 8S globulin

enriched fraction. In yet more particular embodiments, the isolated, purified proteins comprise a Rubisco enriched fraction. In other particular embodiments, the one or more fats are derived from plant-based oils. In more particular embodiments, the one or more fats are derived from one or more of: corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, 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 yet even more particular embodiments, the one or more fats is a rice bran oil. In another particular embodiment, the one or more fats is a canola oil. In other particular embodiments, the cross-linking enzyme is transglutaminase, lysyl oxidase, or other amine oxidase. In yet even more particular embodiments, the cross-linking enzyme is transglutaminase. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of purified pea albumin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% canola oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of purified pea albumin emulsified with 40-80% rice bran oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% rice bran oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially-purified moong bean 8S globulin emulsified with 40-80% canola oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel.

[00323] CONNECTIVE TISSUE REPLICA

[00324] Animal connective tissue provides key textural features that are an important component of the experience of eating meat. Accordingly, the present invention provides a composition derived from non-animal sources which recapitulates key features of animal connective tissue. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources which recapitulates important textural and visual features of animal connective tissue. Such a composition will be labeled herein as "connective tissue replica". In some embodiments, the connective tissue replica and/or meat substitute product comprising the connective tissue replica are partially derived from animal sources.

[00325] 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.

[00326] The protein content of most fascia-type connective tissue is comprised mainly of collagen. Collagen is characterized by a high fraction of proline and alanine, and also is assembled into characteristic elongated fibrils or rod-like, flexible structures. Prolamins are one family of proteins found in non-animal sources, such as plant sources. Prolamins are highly abundant in plants and are similar in amino acid composition to collagen. Among proteins we tested for this purpose, prolamins were particularly favorable because of their low cost and their ability to readily form fibers or sheets when spun or extruded. Non-limiting examples of prolamin family proteins include, e.g., zein (found in corn), these include hordein from barley, gliadin from wheat, secalin, extensins from rye, kafirin from sorghum, avenin from oats. In fascia-type connective tissue, the prolamin family of proteins, individually or combinations thereof, demonstrates suitability for the protein component because they are highly abundant, similar in global amino acid composition to collagen (high fraction of proline and alanine), and amenable to processing into films and fibers. In addition to zein (found in corn), these include hordein from barley, gliadin from wheat, secalin, extensins from rye, kafirin from sorghum, avenin from oats. Other proteins may be necessary to supplement prolamins in order to achieve targets specifications for physicochemical and nutritional properties. The list of potential candidates here is essentially open and may include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microorganisms, or synthesized in vitro. The proteins may be artificially designed to emulate physical properties of animal connective tissue. animal-derived or recombinant collagen, extensins (hydroxyproline-rich glycoproteins abundant in cell walls e.g. Arabidopsis thaliana, monomers of which are "collagen-like" rod-like flexible molecules). The proteins may be artificially designed to emulate physical properties of animal connective tissue. .

[00327] Methods for forming fascia-type connective tissue will be as those practiced in the art with a bias towards methods producing fibrous or fibrous-like structures by biological, chemical, or physical means, individually or in combination, serially or in parallel, before final forming. These methods may include extrusion or spinning.

[00328] 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).

[00329] 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.

[00330] Methods for forming cartilage-type connective tissue will be similar to those for fascia-type connective tissue, but with a bias towards methods producing isotropically homogenous structures.

[00331] The fat can be suspended in a gel. In some embodiments the present invention provides for methods for producing droplets of fat suspended in the proteinaceous gel. The fat can be isolated from plant tissues and emulsified. The emulsification can utilize high-speed blending, homogenization, agitation or temperature changes. The lipid suspension can be sonicated or extruded to further alter the properties of the lipid in the solution. At this point, in some embodiments other components of the consumable are added to the solution followed by a gelling agent. In some embodiments crosslinking agents (e.g. transglutaminase or lysyl oxidase) are added to bind the components of the consumable. In other embodiments the gelling agent is added and the lipid/gel suspension is later combined with additional components of the consumable. In fascia-type connective tissue, the prolamin family of proteins, individually or combinations thereof, demonstrates suitability for the protein component because they are highly abundant, similar in global amino acid composition to collagen (high fraction of proline and alanine), and amenable to processing into films. In addition to zein (found in corn), these include hordein from barley, gliadin from wheat, secalin, extensions from rye, kafirin from sorghum, avenin from oats. Other proteins may be necessary to supplement prolamins in order to achieve targets specifications for physicochemical and nutritional properties. The list of potential candidates here is essentially open and may include any major seed storage proteins, animal-derived or recombinant collagen, extensins (hydroxyproline-rich glycoproteins abundant in cell walls e.g. Arabidopsis thaliana, monomers of which are "collagen-like" rod-like flexible molecules).

[00332] In some embodiments some or all of the components of the consumable are suspended in a gel. In various embodiments the gel can be a hydrogel, an organogel, or a xerogel, The gel can be made thick using an agent based on polysaccharides or proteins. For example fecula, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, alginin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, cellulose, methylcellulose, hydroxymethylcellulose, acadia gum, konjac, starch, pectin, amylopectin or proteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, of fungi can be used alone or in combination to thicken the gel, forming an architecture or structure for the consumable. Enzymes that catalyze reactions leading to covalent crosslinks between proteins can also be used alone or in combination to form an architecture or structure

for the consumable. For example transclutaminase, lysyl oxidases, or other amine oxidases (e.g. Pichia pastoris lysyl oxidase (PPLO)) can be used alone or in combination to form an architecture or structure for the consumable. In some embodiments multiple gels with different components are combined to form the consumable. For example a gel containing a plant-based protein can be associated with a gel containing a plant-based fat. In some embodiments fibers or stings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

[00333] 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.

[00334] 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.

[00335] In some embodiments the meat replica contains no animal products, less than 1% wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[00336] In some embodiments the meat replica contains no animal products, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[00337] In some embodiments the meat replica contains no animal products, no soy protein isolate, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[00338] In some embodiments the meat replica contains no animal products, no soy protein concentrate, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[00339] In some embodiments the meat replica contains no animal products, no soy protein, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[00340] In some embodiments the meat replica contains no animal products, no tofu, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum.

[00341] In some embodiments the meat replica contains no animal products, no tofu, and no wheat gluten.

[00342] In some embodiments the meat replica contains no animal products, no soy protein, and no wheat gluten.

[00343] In some embodiments the meat replica contains no methylcellulose, no carrageenan, no caramel color, no Konjac flour, no gum Arabic, and no acacia gum.

[00344] In some embodiments the meat replica contains no animal products and less than 5% carbohydrates.

[00345] In some embodiments the meat replica contains no animal products, no soy protein, no wheat gluten, no methylcellulose, no carrageenan, no caramel color and no Konjac flour, no gum Arabic, and no acacia gum and less than 5% carbohydrates.

[00346] In some embodiments the meat replica contains no animal products, and less than 1% cellulose.

[00347] In some embodiments the meat replica contains no animal products, and less than 5% insoluble carbohydrates.

[00348] In some embodiments the meat replica contains no animal products, no soy protein, and less than 1% cellulose.

[00349] In some embodiments the meat replica contains no animal products, no soy protein, and less than 5% insoluble carbohydrates.

[00350] In some embodiments the meat replica contains no animal products, no wheat gluten, and less than 1% cellulose.

[00351] In some embodiments the meat replica contains no animal products, no wheat gluten, and less than 5% insoluble carbohydrates.

[00352] The percentage of different components may also be controlled. For example non-animal-based substitutes for muscle, fat tissue, connective tissue, and blood components can be combined in different ratios and physical organizations to best approximate the look and feel of meat. The various can also components can be arranged to insure consistency between bites of the consumable. The components can be arranged to insure that no waste is generated from the consumable. For example, while a traditional cut of meat may have portions that are not typically eaten, a meat replicate can improve upon meat by not including these inedible portions. Such an improvement allows for all of the product made or shipped to be consumed, which cuts down on waste and shipping costs. Alternatively, a meat replica may include inedible portions to mimic the experience of meat consumption. Such portions can include bone, cartilage, connective tissue, or other materials commonly referred to as gristle, or materials included simulating these components. In some embodiments the consumable may contain simulated inedible portions of meat products which are designed to serve secondary functions. For example a simulated bone can be designed to disperse heat during cooking, making the cooking of the consumable faster or more uniform than meat. In other embodiments a simulated bone may also serve to keep the consumable at a constant temperature during shipping. In other embodiments, the simulated inedible portions may be biodegradable.

[00353] In some embodiments the meat substitute compositions contains no animal protein, comprising between 10-30% protein, between 5-80% water, between 5-70% fat, comprising one or more isolated purified proteins. In particular embodiments, the meat substitute compositions comprise transglutaminase.

[00354] In some embodiments the consumable contains components to replicate the components of meat. The main component of meat is typically skeletal muscle. Skeletal muscle typically consists of roughly 75 percent water, 19 percent protein, 2.5 percent intramuscular fat, 1.2 percent carbohydrates and 2.3 percent other soluble non-protein substances. These include organic acids, sulfur compounds, nitrogenous

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

[00355] In some instances a meat replica is designed so that, when cooked, the percentages of components are similar to cooked meat. So, in some embodiments, the uncooked consumable has different percentages of components than uncooked meat, but when cooked the consumable is similar to cooked meat. For example, a meat replica may be made with a higher than typical water content for raw meat, but when cooked in a microwave the resulting product has percentages of components similar to meat cooked over a fire.

[00356] 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.

[00357] 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.

[00358] The protein in the consumable can come from a variety or combination of sources. Non-animal sources can provide some or all of the protein in the consumable. Non-animal sources can include

vegetables, fruits, nuts, grains, algae, bacteria, or fungi. The protein can be isolated or concentrated from one or more of these sources. In some embodiments the consumable is a meat replica comprising protein only obtained from non-animal sources.

[00359] 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.

[00360] INDICATORS OF COOKING MEAT

[00361] 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.

[00362] 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.

[00363] 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.

[00364] Evidence that the presence of leghemoglobin contributes favorably to aroma of meat replicas: A muscle replica comprising pea flour, sunflower oil, and glucose was heated for 10 minutes at 140C in the presence of either reduced leghemoglobin (LHb) or a mixture of iron (Fe3+), sodium and EDTA (EFS) in sealed containers carrying solid phase microextraction (SPME) fibers. These fibers contain polydimethylsiloxane (PDMS) which adsorbs volatile compounds for analysis by GC-MS. Analysis of GC-MS data from multiple replicas reveal consistent differences between the LHb and EFS samples. Non-limiting examples of compounds found exclusively or more abundantly in the LHb samples are: 2-octanone, 2-methyl furan, which are often associated with the aroma of cooked meat, and many other unidentified compounds. For example, Figure 1 shows a comparison of aligned GCMS profiles (in mirrored orientations) of otherwise identical muscle-replica samples cooked in the presence of leghemoglobin (LHb, Right side profile) or Ferric ion (Left side profile). In this illustration, the vertical axis represents retention time in the gas chromatography separation step, and the horizontal axis represents m/z ratios of ions produced by fragmentation of the corresponding separated volatile compounds. Selected compounds differentially represented in the two samples are labeled on the Right of the figure.

[00365] COLOR INDICATORS

[00366] The color of meat is an important part the experience of cooking and eating meat. For instance, cuts of beef are of a characteristic red color in a raw state and gradually transition to a brown color during

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

[00367] The main determinant of the nutritional definition of the color of meat is the concentration of iron carrying proteins in the meat. In the skeletal muscle component of meat products, one of the main iron-carrying proteins is myoglobin. It is estimated that the white meat of chicken has under 0.05%; pork and veal have 0.1-0.3%; young beef has 0.4-1.0%; and old beef has 1.5-2.0%. So, in some embodiments, the consumable is a meat replica which comprises an iron-carrying protein. In some embodiments, the meat replica comprises about 0.05%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, or more than about 2% of an iron-carrying protein by dry weight or total weight. In some cases, the iron carrying protein has been isolated and purified from a source. In other cases, the iron carrying protein has not been isolated and purified. In some cases, the source of the iron-carrying protein is an animal source, or a non-animal source such as a plant, fungus, or genetically modified organisms such as, e.g., bacteria or yeast. In some cases, the 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.

[00368] 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.

[00369] 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.

[00370] Examples of iron containing proteins include hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins. In various embodiments these iron containing proteins are added to the consumable to alter the visual characteristics or iron content of the consumable. In some embodiments the consumable comprises a hemoprotein (e.g. hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins,).

[00371] Leghemoglobin, similar in structure and physical properties to myoglobin, is readily available as an unused by-product of commodity legume crops (eg., soybean, pea). The leghemoglobin in the roots of these crops in the US exceeds the myoglobin content of all the red meat consumed in the US. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, including a muscle tissue replica, an adipose tissue replica, a connective tissue replica, and leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a heme protein. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a member of the globin protein family. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a high iron content from a heme protein. In some embodiments the iron content is similar to meat. In some embodiments the consumable has the distinctive red color of meat, such color provided by leghemoglobin.

[00372] Leghemoglobin is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.

[00373] 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.

[00374] A heme protein from the group of: Hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate

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

Food products comprising isolated, purified leghemoglobin

[00375] In some embodiments leghemoglobin is added to meat to enhance the properties of meat. For example, a leghemglobin containing solution can be injected into raw or cooked meat. In another example a leghemoglobin solution is dripped over meat or a consumable of the invention to enhance appearance. In one embodiment advertising, photography, or videography of food products such as meat or a meat substitute is enhanced with leghemoglobin.

Sources of leghemoglobin

[00376] 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, Figure 2). Figure 3 shows 100 mls of leghemoglobin solution isolated from 30 grams of pea root nodules. Leghemoglobins from different species are homologs and have similar color properties (Figure 4). In Figure 4, panel A shows an SDS_PAGE gels of lysed root-nodules of three legume plant species (1) Fava bean (2) English Pea (3) Soybean. Arrows mark respective leghemoglobins. Note that leghemoglobin is the most abundant soluble protein in each lysate. Panel B shows the similarity of UV-VIS spectral profile of leghemoglobins from two different plant species (Favabean and Soybean). We purified leghemoglobin from fava bean (green curve) and Soybean (red curve) root nodules using the protocol described elsewhere in the specification. UV-VIS spectra of both proteins shows that the heme iron is in the reduced (+2) state. Note that they are almost perfectly superimposed, consistent with their visually identical red color. The heme iron in the respective leghemoglobins was reduced to the +2 oxidation state by incubating Fava bean and Soybean leghemoglobin with 10mM sodium hydrosulfite in 20mM potassium-phosphate pH 7.4, 100mM sodium chloride buffer. Sodium hydrosulfite was then removed from the leghemoglobin solution using gel-exclusion chromatography. Inset shows a zoom-in of UV-VIS spectra in 450nm to 700nm region. Some plant species express several leghemoglobin isoforms (for example soybean has four leghemoglobin isoforms). Minor variations in precise amino acid sequence can modify overall charge of the protein at a particular pH and can modify precise structural conformation of iron containing heme group in leghemoglobin. Differences in structural conformation of heme group of different leghemoglobins can

influence oxidation and reduction rates of the heme iron. These differences may contribute to color and flavor generation properties of different leghemoglobins.

[00377] Leghemoglobin has a virtually identical absorbance spectrum and visual appearance to myoglobin from animal muscle. Figure 5 shows a comparison of reduced (heme iron 2+) and oxidized (heme iron 3+) soybean leghemoglobin (Figure 5 panel A) and equine heart muscle myoglobin (Figure 5 panel B) showing similarity of UV-VIS absorption profiles of two proteins. We purified soybean leghemoglobin from soybean root-nodules using here described protocol. Purified equine myoglobin was purchased from SigmaAldrich. Soybean leghemoglobin (Figure 5 panel A) and equine myoglobin (Figure 5 panel B) were reduced with 1mm sodium hydrosulfite. Shown are UV-VIS absorption spectra of heme Fe3+ (blue line) and heme Fe2+ (red line) of soybean leghemoglobin (Figure 5 panel A) and equine myoglobin (Figure 5 panel B). Insets show a zoom-in of UV-VIS spectra in 450nm to 700nm region. (Figure 5 panel C) Images of 10ul liquid droplet of a 40 mg/ml solution of soybean leghemoglobin in the heme-Fe3+ state (left droplet) showing characteristic rusty red color and a 40mg/ml solution of soybean leghemoglobin solution in the heme-Fe2+ state (right droplet) showing characteristic red color of and (right image) corresponding samples of equine myoglobin.

[00378] In other embodiments, leghemoglobin can be sourced from non-plant sources, such as from organisms such as bacteria or yeast which have been genetically modified to express high levels of leghemoglobin.

[00379] The oxidation state of the iron ion in leghemoglobin is important for its color. Leghemoglobin with the heme iron in the +2 oxidation state appears vivid red in color, while leghemoglobin with the heme iron in the +3 oxidation state appears brownish red. Thus, in using leghemoglobin as a source of red color in a meat replica, it is desireable to reduce the heme iron from the +3 state to the +2 state. Heme iron in leghemoglobin can be switched from oxidized (+3) state to reduced (+2) state with reducing reagents. Examples of successful reduction of leghemoglobin heme iron with sodium hydrosulfite and titanium citrate are illustrated in Figure 6. In Figure 6 the UV-VIS spectrogram of purified soybean leghemoglobin in which the heme iron is in the oxidized (+3) state is represented by the blue curves in each panel. The red curves in each panel represent the UV-VIS spectra of the same leghemoglobin species after reduction to the (+2) state (red lines) by addition of (Panel A) 1mM sodium hydrosulfite or (Panel B) 0.24 % (wt/v) titanium citrate in 20mM potassium phosphate pH 7.3, 100mM sodium chloride buffer. The Insets show a zoom-in of UV-VIS spectra in 450 -700 nm region. For this example, leghemoglobin was purified from soybean root nodules using 60/90% ammonium sulfate fractionation and exchanged into 20mM potassium phosphate pH 7.4, 100mM sodium chloride buffer. Sodium hydrosulfite stock solution was prepared by dissolving 100mM sodium hydrosulfite in1mM sodium hydroxide in water. Titanium citrate stock solution was prepared from 20 % (wt/v) Ti-chloride in hydrochloric acid by mixing it with 0.2M sodium citrate (1:10 v/v). pH was adjusted using sodium carbonate to pH 7.0.

[00380] Leghemoglobin can be purified from legume root nodules, such as the root nodules of peas or soybeans (Figure 1 shows pea root nodules, Figure 2 shows Leghemoglobin isolated from same). Root

nodules from soy beans were thoroughly cleaned to remove soil and extraneous root tissues prior to root nodule lysis in 20mM potassium phosphate pH 7.4, 100mM sodium chloride, 1mm EDTA and 1mM ascorbic acid. Root nodules were lysed by grinding root-nodules using a Vitamix blender. For some samples Polyvinylpyrrolidone polymer was added at 30% wt/v to aid in removal of plant phenolic small molecules that mediate oxidation of leghemoglobin heme-iron. Root nodule lysate was fractionated using ammonium sulfate in two steps, first ammonium sulfate was added to 60% wt/v. Pellet was discarded and supernatant brought to 90% wt/v. ammonium sulfate. Leghemoglobin was collected as a precipitated pellet in 90% ammonium sulfate fraction. Ammonium sulfate precipitated leghemoglobin was resuspended in 20mM potassium phosphate, 1mM EDTA, 50mM sodium chloride and ammonium sulfate was removed using dialysis or size-exclusion chromatography in the same buffer. In some instances this was the last purification step, while in other instances leghemoglobin was further purified using anion-exchange chromatography (FFQ GE Healthcare), which was sometimes followed by size-exclusion chromatography (Sephacryl S-100, GE Healthcare). Soybean leghemoglobin from 90% ammonium sulfate pellet was loaded on anion exchange columns (FFQ or DEAE, GE Healthcare) in different buffers (20mM potassium phosphate pH 7.4, containing 0 to 100mM sodium chloride, 20mM Tris pH 8 containing 0 to 100mM sodium chloride, 20mM sodium borax pH 9.8, 20mM sodium chloride, 20mM sodium carbonate pH 9, 20mM sodium chloride) and purified either in flow-through or using sodium chloride (0-1M salt gradient). An example of the leghemoglobin purification flow from soybean root nodules is represented in Figure 7. The figure shows SDS-PAGE fractionation of different soybean leghemoglobin purification steps (Lane 1) Soybean root-nodule lysate; (Lane 2) Soybean root-nodule lysate purified by 60/90 % (wt/v) ammonium sulfate fractionation. Shown is the protein content of 90% ammonium sulfate fractionated protein pellet resuspended in 20mm potassium phosphate pH 7.4, 100mM sodium chloride, 1mM EDTA buffer; Proteins from 90% ammonium sulfate pellet were further purified by anion-exchange chromatography (FFQ GE Healthcare) in 20mM potassium phosphate ph 7.4, 100mM sodium chloride. Leghemoglobin collected in the flowthrough is shown in Lane 3. Anion-exchange flowthrough was fractionated using size-exclusion chromatography (Sephacryl S-100 GE Healthcare) and resulting leghemoglobin fraction is shown in Lane 4. Leghemoglobin content at different purification steps was determined by determining the fraction of leghemoglobin band on SDS-PAGE gel in a respective sample using ImageDoc analysis software (BioRad). Purity (partial abundance) of leghemoglobin at respective steps in the purification steps was: lysate: 32.7% (lane 1), 60/90 % (wt/v) ammonium sulfate fractionation 78% (lane 2), anion-exchange chromatography ~ 83% (lane 3), and size-exclusion chromatography to ~ 95% (lane 4).

[00381] Leghemoglobin can also be produced by genetically engineering a bacterium or fungus to produce it. One illustrative example is shown in Figure 8. Figure 8 shows stained SDS-PAGE gel analysis of (A) soybean leghemoglobin expressed and purified using recombinant protein technology and (B) soybean leghemoglobin purified from soybean root nodules. (A) Recombinant Soybean leghemoglobin A carrying His-tag and TEV protease His-tag removal site was expressed in E.coli BL21 strain and purified using His-tag affinity chromatography (Talon resin, CloneTech). The left lane

contains molecular weight standards, the right lane contains purified recombinant soybean leghemoglobin A (arrow). Expected molecular weight of the recombinant soybean leghemoglobin A is 17.1kDa. (B) SDS-PAGE gel of purified Soybean leghemoglobin from root nodules. The left lane contains molecular weight standards, the right lane contains purified soybean leghemoglobin A (arrow). Mass spectrometry analysis of purified material determined that all four soybean leghemoglobin isoforms are present, and are full length (data not shown). Expected molecular weights (MW) of soybean leghemoglobin isoforms range from MW15.4 to 15.8kDa.

[00382] Leghemoglobin purified from soybean and fava root nodules, respectively was tasted by a panel of volunteers and in each case described as tasting like blood.

[00383] Leghemoglobin can be isolated from the roots nodules of legumes such as soy beans, fava beans, cow peas, lima beans, garbanzo beans, peas, lupine, lotus japonicum or other legumes. The root nodule (for example root nodules from a pea plant, Figure 1) is obtained and homogenized in an aqueous solution, soluble proteins including leghemoglobin are recovered after insoluble matter is removed by precipitation or filtration. Leghemoglobin can be purified by selective precipitation and/or chromatography and/or the use of molecules with specific affinity for leghemoglobin . (Figure 2, showing 100 mls of solution of leghemoglobin isolated from 30 grams of pea root nodules).

[00384] Heme proteins, for example leghemoglobin, can be combined with other plant based meat replica components. In some embodiments the heme proteins are captured in a gel which contains other components, for example lipids and or proteins. In some aspects a multiple gels are combined with non-gel based heme proteins. In some embodiments the combination of the heme proteins and the other compounds of the consumable are done to insure that the heme proteins are able to diffuse through the consumable. In some embodiments the consumable is ed in a heme-protein containing solution, for instance a leghemoglobin solution,. In some embodiments the consumable is soaked in a heme protein containing solution, for instance a leghemoglobin solution for 1, 5, 10, 15, 20 or 30 hours. In some embodiments the consumable is soaked in a heme containing solution, for instance a leghemoglobin solution for 1, 5, 10, 15, 30, or 45 minutes.

[00385] Figure 9 shows an example of 6 cubes of a commercial meat analog (Quorn chicken analog), about 1cm on a side, 4 of which (Left and lower right) have been soaked in a solution of about 10 mg/ml soybean leghemoglobin in 20 mM Potassium phosphate pH 7.4 and 100 mM NaCl; the remaining two (Upper right) were soaked in the same buffer without leghemoglobin. Note the deep pink color of the leghemoglobin-infused cubes in contrast to the pale tan color of the un-infused cubes.

[00386] Figure 10 shows the 4 leghemoglobin-infused cubes of Quorn chicken analog in the process of cooking in a pan at 350°C. The two lower cubes have been turned over to expose the grilled surface, which has turned brown. Note in the upper two cubes that the heated portion has turned grey-brown, while the cooler top surface retains its pink color. In some embodiments the consumable is injected with a heme containing solution, for instance a leghemoglobin solution, until the consumable is the color of uncooked meat.

[00387] Given the usefulness of heme proteins for coloring consumables it will be useful to detect whether a product contains a particular heme protein. Accordingly the present invention includes in some embodiments methods to determine whether a product contains a heme protein. Methods for detecting proteins are well known in the art. For example an ELISA or proximity-ligation assoacy or luninex assay or western blot analysis can be performed to determine whether leghemoglobin is present in a food product such as meat or a meat replica. In one embodiment the detection methods are performed to determine whether meat has been altered with leghemoglobin.

Examples

[00388] An exemplary muscle replica composition comprising one or more isolated, purified plant proteins is described herein.

[00389] Protein purification for components of the replica

[00390] Moong bean seeds, Green Pea dry seed were purchased as milled flour and used for purification of respective seed storage proteins. Rubsico was purified from fresh alfalfa plant. Protein composition at individual fractionation steps was monitored by SDS-PAGE and protein concentrations were measured by standard UV-VIS and Pierce assay methods.

[00391] Moong bean 8S globulins: Moong bean flour was resuspended in in 50mM potassium phosphate buffer pH 7 and 0.5M NaCl at 1:4 (wt/v) ratio, and mixture was incubated for 1hr. Unsoluble material was separated by centrifugation and proteins in the supernatant were fractionated by addition of ammonium sulfate in 2 steps: 50% (wt/v) followed by 90% (wt/v). Protein precipitated in 90% fraction contained the moong bean 8S globulins and was stored at -20C until further use.

[00392] Pea-albumins: Green pea dry seed flour was resuspended at 1: 10 (wt/v) ratio in 50mM sodium acetate buffer pH 5 and incubated for 1hr. Unsoluble material was separated by centrifugation and proteins in the supernatant were fractionated by ammonium sulfate precipitation in two steps: 50% (wt/v) followed by 90% (wt/v). Ammonium sulfate solutions were stirred for 1 hour and ammonium sulfate precipitated proteins removed by centrifugation. Proteins of interest precipitated in 90% (wt/v) ammonium sulfate. Pellet was stored at -20C until further use.

[00393] Pea-globulins: Green pea dry seed flour was resuspended at 1: 10 (wt/v) ratio in 20mM potassium phosphate buffer pH 8, 0.4M sodium chloride and stirred for 1hr. After centrifugation, the supernatant was subjected to ammonium sulfate fractionation. First, supernatant was brought to 50% (wt/v) ammonium sulfate, and precipitated proteins removed. Second, 50% (wt/v) ammonium sulfate supernatant was brought to 80% (wt/v) ammonium sulfate saturation. The 80% (wt/v) ammonium sulfate pelleted protein contained globulins of interest. Pellet was stored at -20'C until further use.

[00394] RuBisCO: RuBisCO was fractionated from alfalfa greens (or other green plants eg soybean plants, spinach etc) by first grinding leaves with 4 volumes of cold 50mM KPhosphate buffer pH 7.4 buffer (with (in lab) or without (in field) 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 -20C until used.

[00395] Obtaining plant proteins.

Moong bean seed 8S protein was purified by ammonium sulfate fractionation as described. Pellet was resuspended in 20mM potassium phosphate pH pH 7.4 and 0.5M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16 000g, 10min and protein concentrated to desired concentration. Pea globulins purified by ammonium sulfate fractionation as described. Protein pellet was resuspended in 20mM potassium phosphate pH pH 7.4 and 0.4M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16 000g, 10min and protein concentrated to desired concentration. Pea albumin purified by ammonium sulfate fractionation as described. Protein pellet was resuspended in 20mM potassium phosphate pH pH 7.4 and 0.1M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16 000g, 10min and protein concentrated to desired concentration.

[00396] Constructing a Muscle Tissue Analog

[00397] Moong bean seed 8S protein was purified by ammonium sulfate fractionation as described above, For preparation of gels, 200 g of pellet was dissolved in 400 ml of dialysis buffer (20 mM potassium phosphate, 400 mM NaCl, pH 7.3) and the resulting solution dialyzed for 6 hours against 5 l of dialysis buffer, replaced twice with fresh buffer. Protein solution was centrifuged at 12,000 g for 15 min to remove debris. Protein was concentrated by dialyzing for 36 hours against 5 l of 30% w/w solution of PEG 8000 (polyethylene glycol, molecular weight 8000) in dialysis buffer. Final protein concentration was 150 mg/ml.

[00398] Leghemoglobin was purified from soybean root nodules. Legume root nodules were cleaned to remove soil and extraneous root tissues prior to root nodule lysis in 20mM potassium phosphate pH 7.4, 100mM sodium chloride, 1mm EDTA and 1mM ascorbic acid. Root nodules were lysed by grinding root-nodules using juicer blender. Unsoluble material was separated by centrifugation. Root nodule lysate was fractionated using ammonium sulfate in two steps, first ammonium sulfate was added to 60% wt/v and solution incubated for 1hr, 4'C. Pellet was discarded and supernatant brought to 90% wt/v ammonium sulfate and incubated for 12hr, 4'C. Leghemoglobin was collected as a precipitated pellet in 90% ammonium sulfate fraction and resuspended in 20mM potassium phosphate, 1mM EDTA, 100mM sodium chloride. SDS-PAGE gel analysis determined that protein solution contains 70% leghemoglobin and 30% other root nodule proteins. Ammonium sulfate was removed using size-exclusion chromatography in the same buffer. Leghemoglobin was concentrated by dialyzing for 48hr against 30% PEG 8000 (polyethylene glycol, molecular weight 8000) in 20mM potassium phosphate pH 7.3, 100mM

sodium chloride. Total protein concentration was 57mg/ml. UV-VIS spectra suggested that leghemoglobin was in heme-iron oxidized state. Thus, leghemoglobin was incubated with 5mM sodium hydrosulfite for 5min and sodium hydrosulfite was removed using size-exclusion chromatography in 20mM potassium phosphate, 100mM sodium chloride buffer. Leghemoglobin was further concentrated to 35.4 mg/ml. UV-VIS spectra analysis confirmed that leghemoglobin is in heme-iron reduced state.

[00399] Transglutaminase was obtained commercially from (Activa TI, Ajimoto). Stock solution (20% wt/v) was made in 20mM potassium phosphate pH 7.3, 100mM sodium chloride buffer.

[00400] To prepare "dark" muscle tissue analog (Figure 11), 43 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 37 ml of leghemoglobin solution (46.5 mg/ml leghemoglobin and 20mg/ml of other soybean root nodule protein) in 20 mM potassium phosphate, 100 mM NaCl, pH 7.3). 20 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly mixed, divided into two 50 ml Falcon tubes and incubated overnight at room temperature. Final protein concentrations were 65 mg/ml for moong bean protein, 18 mg/ml of leghemoglobin, 91 mg/ml total protein.

[00401] "White" muscle analog (Figure 12) was prepared by mixing 43ml moong bean protein solution (150mg/ml) with 45ml of 11.7 mg/ml solution of leghemoglobin and 0.8 % (wt/v) of transglutaminase solution. Final protein concentrations were 63 mg/ml for moong bean protein, 5.2 mg/ml of leghemoglobin, 68 mg/ml total protein.

[00402] The "dark" muscle tissue analog formed an opaque gel of dark chocolate color, smooth uniform texture, with glistening surface, and a small amount (< 1 ml) of dark red, venous blood colored liquid on top. The gel was freely standing, elastic but fragile, similar in appearance to thin Jell-O. The gel has a medium aroma with notes of beans and blood clearly discernible. The flavor is dominated by notes of beans and iron/blood, with weaker grassy and medicinal/chemical flavors. The taste is salty, with a long aftertaste of blood.

[00403] The "white" muscle tissue analog was very similar, but with much lighter, cappuccino-like, color. It was also more fragile, 2-3-fold less strong against compression.

[00404] Fat Tissue Analog

[00405] Fat tissue analog using moong bean 8S globulin fraction was prepared as follows: 15 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 15 ml of rice bran oil. 6 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly emulsified using a homogenizer (VWR) at speed #2. Emulsion was aliquoted into 1.6 eppendorf tubes and incubated overnight at room temperature. After that, tubes were heated at 95 °C for 5 min in a heat block, and allowed to cool down to room temperature on a bench. Final concentrations were 75 mg/ml for moong bean protein, 50% w/w oil.

[00406] Fat tissue analog using pea globulin (100 mg/ml) was prepared by the same method. Additionally, fat tissue analog was prepared from pea globulin, and either rice bran or canola oil, in bulk by the same method, but without aliquoting emulsions into eppendorf tubes. Instead, emulsions in 50 ml Falcon tubes were rotated overnight on a nutator, and were subsequently incubated at 90 °C for 30 min.

[00407] Fat tissue analog based on moong beans (Figure 13) and prepared in eppendorf tubes formed an opaque gel of off-white color, smooth uniform texture, with no visible discernible liquid that was not incorporated into the gel. The gel was freely standing, elastic and springy. The gel has a slight, pleasant aroma and a mild and pleasant flavor. The taste is mildly salty.

[00408] Fat tissue analog based on pea globulin (Figure 14) and prepared in eppendorf tubes was very similar to moong bean-based fat analog, except that it gave up a little of oil upon compression.
Fat tissue analog prepared in 50 ml Falcon tubes were similar in appearance, texture and aromas, but substantially softer (2-fold softer for canola oil, and 3-fold softer for rice bran oil, according to compressibility measurements).

[00409] Connective Tissue Analog

Connective tissue analog prototypes were developed using zein protein sourced from 100% yellow corn gluten meal, or from commercial sources, such as Amazein (Prairie Gold, Bloomington, IL). Zein proteins were solubilized in 70 - 90% ethanol with desired ratios at 1:3 to 1:5 (solids:solution). By precipitating zein proteins, for example by a change in pH, in a controlled manner, large zein structures result with physicochemical properties that can be manipulated as desired. For example, Figure 15 shows connective-tissue analog strands that were created using a 1:3 ratio in 70% ethanol, loaded into a syringe with a 23 gauge needle (ID 0.337 mm). The solution was slowly extruded from the bottom of a 5 inch-high vessel into an excess of 5 M NaCl solution. The ethanol-zein solution being less dense than the NaCl solution, floated upward, drawing out a fibrous stand of solidifying zein. The NaCl was constantly stirred as the strands began to develop to assist in the strand lengthening. The strands bunch together and become a hard, dense mass.

[00410] Ground beef replica prototypes made from gels of plant proteins and plant oils.

[00411] A ground beef prototype patty was made by combining 62% (wt/wt) muscle analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "white muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (Figure 16 panel A). A ground beef prototype patty was made by combining 62% muscle analog (62% "dark muscle analog" and 38% "white muscle analog), 29% fat tissue analog (from moong bean seed 8S protein and rice bran oil) , 5% connective tissue analog (Figure 16 panel B). A ground beef prototype patty was made by combining 71 % (wt/wt) muscle tissue analog (composed of 60% "white" muscle analog, 40 % "dark" muscle analog) , 23% fat tissue (from pea seed globulin proteins and canola oil) (Figure 16 panel C). A ground beef prototype patty was made by combining 67% "White" muscle analog, with 28% fat tissue analog (from pea globulins and rice bran oil), (Figure 16, panel D)

[00412] In a further test, the effect of cooking the ground beef replica patties was evaluated by grilling on a 350°F pan. A ground beef patty analog was made by combining 62% (wt/wt) muscle tissue analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (Figure 17). The panel on the

left shows the patty before cooking and the panel on the right shows the same patty after cooking for about 2 minutes. Observers described the aroma of the cooking ground beef replica as distinctly "beefy".

CLAIMS

WHAT IS CLAIMED IS:

1. A meat substitute composition comprising a protein content, wherein one or more isolated and purified proteins accounts for 10% or more of said protein content by weight, wherein said meat substitute composition accurately mimics the taste, texture, or color of a meat product derived from animal sources.
2. The meat substitute composition of claim 1, wherein said composition accurately mimics the color of said meat product in its raw state and in a cooked state after cooking.
3. The meat substitute composition of claim 1, wherein said one or more isolated and purified proteins accounts for 25% or more of said protein content by weight.
4. The meat substitute composition of claim 3, wherein said one or more isolated and purified proteins accounts for 50% or more of said protein content by weight.
5. The meat substitute composition of claim 4, wherein said one or more isolated and purified proteins accounts for 75% or more of said protein content by weight.
6. The meat substitute composition of claim 5, wherein said one or more isolated and purified proteins accounts for 90% or more of said protein content by weight.
7. The meat substitute composition of claim 1, wherein gluten does not account for 10% or more of said protein content by weight.
8. The meat substitute composition of claim 1, wherein each of said isolated, purified proteins is separately isolated and purified.
9. The meat substitute composition of claim 1, comprising 1-7 isolated and purified proteins.
10. The meat substitute composition of claim 9, wherein said 1-7 isolated and purified proteins are each isolated from different plant species.
11. The meat substitute composition of claim 10, wherein said protein content comprises no more than trace amounts of any other proteins derived from the different plant species.
12. The meat substitute composition of any claim 1, wherein said one or more isolated and purified proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or

other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

13. The meat substitute composition of claim 1, wherein said one or more isolated and purified proteins are not isolated from an animal.
14. The meat substitute composition of claim 13, wherein said one or more isolated and purified proteins are isolated from a single plant source.
15. The meat substitute composition of claim 14, wherein said one or more isolated and purified proteins are isolated from multiple plant sources.
16. The meat substitute composition of claim 13, wherein said one or more isolated, purified proteins are isolated from a genetically modified organism.
17. The meat substitute composition of claim 16, wherein said genetically modified organism is a genetically modified bacteria or yeast organism.
18. The meat substitute composition of any of the preceding claims, wherein said isolated, purified protein has been formed into fibers.
19. The meat substitute composition of claim 18, wherein said fibers resemble skeletal muscle fibers.
20. The meat substitute composition of claim 19, wherein said fibers are asymmetric fibers.
21. The meat substitute composition of any of the preceding claims, further comprising one or more isolated and purified iron-containing proteins.
22. The meat substitute composition of claim 21, wherein said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, and flavohemoglobins.
23. The meat substitute composition of claim 22, wherein said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1.
24. The meat substitute composition of any of the preceding claims, wherein said isolated and purified proteins are assembled into one or more gels.
25. The meat substitute composition of any of the preceding claims, further comprising one or more fats.
26. The meat substitute composition of claim 25, wherein said one or more fats are derived from a plant source.
27. A meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources.
28. The meat substitute product of claim 27, wherein said indicator is a visual indicator that accurately mimics the color transition of a meat product during said cooking progression.
29. The meat substitute product of claim 28, wherein said color transition is from red to brown.

30. The meat substitute product of claim 28, wherein said color transition is from pink to white or tan.
31. The meat substitute product of claim 28, wherein said visual indicator transitions from a translucent to opaque color during said cooking progression.
32. A meat substitute product of claim 27 wherein the indicator is an olfactory indicator that indicates cooking progression.
33. The meat substitute product of claim 32, wherein said olfactory indicator is one or more volatile odorants released during cooking.
34. The meat substitute product of any of claims 32, wherein said indicator comprises one or more isolated, purified iron-containing proteins.
35. The meat substitute product of claim 34, wherein said one or more isolated, purified iron-containing proteins is in a reduced state before cooking.
36. The meat substitute product of 34, wherein said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, and flavohemoglobins.
37. The meat substitute product of 36, wherein said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1.
38. The meat substitute product of claim 34, wherein said one or more isolated and purified iron-containing proteins are not isolated from an animal.
39. The meat substitute product of claim 34, wherein said one or more isolated and purified iron-containing proteins are isolated from one or more plant sources.
40. The meat substitute product of claim 39, wherein said one or more isolated, purified proteins are isolated from the root nodules, roots, seeds, leaves, or stems of said one or more plant sources.
41. The meat substitute product of claim 40, wherein said one or more plant sources are soy or pea plants.
42. The meat substitute composition of claim 39, wherein said one or more plant sources comprises one or more plants of the legume family.
43. The meat substitute product of claim 38, wherein said one or more isolated and purified iron carrying proteins in a reduced or oxidized state has a similar UV-VIS profile to a myoglobin protein derived from an animal source when in an equivalent reduced or oxidized state.
44. The meat substitute product of claim 43, wherein the difference between the peak absorbance wavelength of said one or more isolated and purified iron-containing proteins and the peak absorbance wavelength of myoglobin derived from an animal source is less than 5%.
45. The meat substitute product of claim 38, wherein said one or more isolated, purified proteins are isolated from a genetically modified organism.

46. The meat substitute product of claim 45, wherein said genetically modified organism is a genetically modified bacteria or yeast organism.
47. The meat substitute product of any of claims 1-46 wherein said meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.
48. The meat substitute product of claim 47, wherein said meat substitute product contains less than 1% wheat gluten.
49. The meat substitute product of claim 48, wherein said meat substitute product contains no wheat gluten.
50. The meat substitute product of claim 49, wherein said meat substitute product contains no soy protein isolate.
51. The meat substitute product of claim 49, wherein said meat substitute product contains no soy protein concentrate.
52. The meat substitute product of claim 49, wherein said meat substitute product contains no soy protein.
53. The meat substitute product of claim 49, wherein said meat substitute product contains less than 5% carbohydrates.
54. The meat substitute product of claim 49, wherein said meat substitute product contains no tofu.
55. The meat substitute product of any of claims 1-46, wherein said meat substitute product contains no tofu, and no wheat gluten.
56. The meat substitute product of any of claims 1-46, wherein said meat substitute product contains no soy protein, and no wheat gluten.
57. The meat substitute product of any of claims 1-46, wherein said meat substitute product contains no animal products and less than 5% carbohydrates.
58. The meat substitute product of any of claims 1-46, wherein said meat substitute product contains less than 1% cellulose.
59. The meat substitute product of any of claims 1-46, wherein said meat substitute product contains less than 5% insoluble carbohydrates.
60. The meat substitute product of any of claims 1-46, wherein said meat substitute product contains no soy protein, and less than 1% cellulose.
61. The meat substitute product of any of claims 1-46, wherein said meat substitute product contains no soy protein, and less than 5% insoluble carbohydrates.
62. The meat substitute product of any of claims 1-46, wherein said meat substitute product contains no wheat gluten, and less than 1% cellulose.
63. The meat substitute product of any of claims 1-46, wherein said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.

64. A muscle tissue replica comprising a protein content, wherein said protein content comprises one or more isolated and purified proteins, wherein said muscle tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source.
65. The muscle tissue replica of claim 64, wherein said one or more isolated and purified proteins accounts for at least 50% of said protein content by weight.
66. The muscle tissue replica of claim 64, wherein said protein content is derived from one or more non-animal sources.
67. The muscle tissue replica of claim 66, wherein said one or more non-animal sources is a plant source.
68. The muscle tissue replica of claim 66, wherein said one or more non-animal sources is a genetically modified yeast or bacteria.
69. The muscle tissue replica of claim 64, wherein each of said one or more isolated proteins is isolated and purified separately.
70. The muscle tissue replica of any of the preceding claims, wherein said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, and moong seed storage 8S globulin.
71. The muscle tissue replica of claim 70, wherein said seed storage protein is moong bean 8S protein.
72. The muscle tissue replica of claim 64, wherein said protein content is suspended in a gel.
73. The muscle tissue replica of claim 64, wherein said protein content is in the form of a gel.
74. The muscle tissue replica of claim 72 or 73, wherein said gel comprises an isolated, purified cross-linking enzyme.
75. The muscle tissue replica of claim 74, wherein said isolated, purified cross-linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.
76. The muscle tissue replica of claim 75, wherein said isolated, purified cross-linking enzyme is transglutaminase.

77. The muscle tissue replica of claim 64, wherein said protein content has been assembled into fibers.
78. The muscle tissue replica of claim 77, wherein said fibers are arranged isotropically.
79. The muscle tissue replica of claim 78, wherein said fibers are asymmetric fibers.
80. The muscle tissue replica of claim 64, further comprising one or more isolated and purified iron-containing proteins.
81. The muscle tissue replica of claim 80, wherein said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.
82. The muscle tissue replica of claim 80, wherein said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1.
83. The muscle tissue replica of claim 64, comprising a protein content, wherein (i) one isolated and purified protein that is not an iron-containing protein accounts for 40-95% of said protein content, (ii) one or more isolated and purified iron-containing proteins accounts for 1-20% of said protein content, and (iii) one or more cross-linking reagents accounts for 0.1-35% of said protein content.
84. The muscle tissue replica of claim 64, wherein said protein content accounts for 5-50% of said replica by weight or by weight/volume.
85. The muscle tissue replica of claim 83, wherein said one isolated and purified protein is moong bean 8S protein.
86. The muscle tissue replica of claim 83, wherein said one or more isolated and purified iron-containing proteins is leghemoglobin.
87. The muscle tissue replica of claim 83, wherein said one or more cross-linking reagents is transglutaminase.
88. The muscle tissue replica of any of claims 64-87, wherein said muscle tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.
89. The muscle tissue replica of claim 88, wherein said muscle tissue replica contains less than 1% wheat gluten.
90. The muscle tissue replica of claim 89, wherein said muscle tissue replica contains no wheat gluten.
91. The muscle tissue replica of claim 90, wherein said muscle tissue replica contains no soy protein isolate.
92. The muscle tissue replica of claim 90, wherein said muscle tissue replica contains no soy protein concentrate.
93. The muscle tissue replica of claim 90, wherein said muscle tissue replica contains no soy protein.

94. The muscle tissue replica of claim 93, wherein said muscle tissue replica contains less than 5% carbohydrates.
95. The muscle tissue replica of claim 90, wherein said muscle tissue replica contains no tofu.
96. The muscle tissue replica of any of claims 64-87, wherein said muscle tissue replica contains no tofu, and no wheat gluten.
97. The muscle tissue replica of any of claims 64-87, wherein said muscle tissue replica contains no soy protein, and no wheat gluten.
98. The muscle tissue replica of any of claims 64-87, wherein said muscle tissue replica contains no animal products and less than 5% carbohydrates.
99. The muscle tissue replica of any of claims 64-87, wherein said muscle tissue replica contains less than 1% cellulose.
100. The muscle tissue replica of any of claims 64-87, wherein said muscle tissue replica contains less than 5% insoluble carbohydrates.
101. The muscle tissue replica of any of claims 64-87, wherein said muscle tissue replica contains no soy protein, and less than 1% cellulose.
102. The muscle tissue replica of any of claims 64-87, wherein said muscle tissue replica contains no soy protein, and less than 5% insoluble carbohydrates.
103. The muscle tissue replica of any of claims 64-87, wherein said muscle tissue replica contains no wheat gluten, and less than 1% cellulose.
104. The muscle tissue replica of any of claims 64-87, wherein said muscle tissue replica contains no wheat gluten, and less than 5% insoluble carbohydrates.
105. A fat tissue replica comprising a gelled emulsion, said gelled emulsion comprising a protein solution with fat droplets suspended therein.
106. The fat tissue replica of claim 105, wherein said fat droplets are derived from a non-animal source.
107. The fat tissue replica of claim 105, wherein said fat droplets are comprised of one or more plant oils.
108. The fat tissue replica of claim 107, wherein said one or more plant oils is selected from the group consisting of corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, 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.

109. The fat tissue replica of claim 108, wherein said one or more plant oils is rice bran oil or canola oil.
110. The fat tissue replica of claim 105, wherein said protein solution comprises one or more isolated, purified proteins.
111. The fat tissue replica of claim 110, wherein said one or more isolated, purified proteins accounts for 75% or more of the protein in said protein solution.
112. The fat tissue replica of claim 112, wherein said one or more isolated, purified proteins are derived from a non-animal source.
113. The fat tissue replica of claim 112 wherein said non-animal source is a plant source.
114. The fat tissue replica of claim 110, wherein said non-animal source is a genetically modified yeast or bacteria.
115. The fat tissue replica of claim 110, wherein each of said one or more isolated proteins is isolated and purified separately.
116. The fat tissue replica of claim 110 wherein said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

117. The fat tissue replica of claim 116 wherein said one or more isolated, purified proteins is an albumin protein, a seed storage protein, or pea globulin protein.
118. The fat tissue replica of claim 116 wherein said albumin protein is isolated pea albumin protein.
119. The fat tissue replica of claim 116, wherein said seed storage protein is moong bean 8S protein.
120. The fat tissue replica of claim 105, wherein said gelled emulsion comprises
a) a protein solution comprising 1-3 isolated and purified proteins, wherein said solution accounts for 30-70% of the volume of said emulsion;
b) a plant oil, wherein said plant oil accounts for 30-70% of the volume of said emulsion; and
c) an isolated, purified cross-linking enzyme, wherein said cross-linking enzyme accounts for 0.0001-1% of said emulsion by wt/volume;
wherein said plant oil is emulsified in said protein solution, wherein said emulsion is formed into a gel by said cross-linking enzyme.
121. The fat tissue replica of claim 105, wherein said gelled emulsion comprises
a) a protein solution comprising 1-3 isolated and purified proteins, wherein said solution accounts for 1-30% of the volume of said emulsion;
b) a plant oil, wherein said plant oil accounts for 70-99% of the volume of said emulsion; and
c) an isolated, purified cross-linking enzyme, wherein said cross-linking enzyme accounts for 0.5-0.0001-1% of said emulsion by wt/volume;
wherein said plant oil is emulsified in said protein solution, wherein said emulsion is formed into a gel by said cross-linking enzyme.
122. The fat tissue replica of claim 105, further comprising a cross-linking enzyme.
123. The fat tissue replica of claim 122, wherein said cross-linking enzyme is transglutaminase.
124. The fat tissue replica of claim 120 or 121, wherein one of said 1-3 isolated and purified proteins is moong bean 8S protein, pea albumin protein, or pea globulin protein.
125. The fat tissue replica of claim 120 or 121, wherein said plant oil is rice bran oil or canola oil.
126. The fat tissue replica of any of claims 105-123, wherein said fat tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.
127. The fat tissue replica of claim 126, wherein said fat tissue replica contains less than 1% wheat gluten.
128. The fat tissue replica of claim 127, wherein said fat tissue replica contains no wheat gluten.
129. The fat tissue replica of claim 128, wherein said fat tissue replica contains no soy protein isolate.
130. The fat tissue replica of claim 128, wherein said fat tissue replica contains no soy protein concentrate.
131. The fat tissue replica of claim 128, wherein said fat tissue replica contains no soy protein.
132. The fat tissue replica of claim 131, wherein said fat tissue replica contains less than 5% carbohydrates.
133. The fat tissue replica of claim 128, wherein said fat tissue replica contains no tofu.

134. The fat tissue replica of any of claims 105-123, wherein said fat tissue replica contains no tofu, and no wheat gluten.
135. The fat tissue replica of any of claims 105-123, wherein said fat tissue replica contains no soy protein, and no wheat gluten.
136. The fat tissue replica of any of claims 105-123, wherein said fat tissue replica contains no animal products and less than 5% carbohydrates.
137. The fat tissue replica of any of claims 105-123, wherein said fat tissue replica contains less than 1% cellulose.
138. The fat tissue replica of any of claims 105-123, wherein said fat tissue replica contains less than 5% insoluble carbohydrates.
139. The fat tissue replica of any of claims 105-123, wherein said fat tissue replica contains no soy protein, and less than 1% cellulose.
140. The fat tissue replica of any of claims 105-123, wherein said fat tissue replica contains no soy protein, and less than 5% insoluble carbohydrates.
141. The fat tissue replica of any of claims 105-123, wherein said fat tissue replica contains no wheat gluten, and less than 1% cellulose.
142. The fat tissue replica of any of claims 105-123, wherein said fat tissue replica contains no wheat gluten, and less than 5% insoluble carbohydrates.
143. A connective tissue replica, comprising a protein content comprising one or more isolated, purified proteins, wherein said protein content has been assembled into structures approximating the texture and visual appearance of connective tissue or skin.
144. The connective tissue replica of claim 143, wherein said protein content is derived from non-animal source.
145. The connective tissue replica of claim 143, wherein said non-animal source is a plant source.
146. The connective tissue replica of claim 143, wherein said non-animal source is a genetically modified yeast or bacteria.
147. The connective tissue replica of claim 143, wherein said one or more isolated proteins account for 50% or more of said protein content by weight.
148. The connective tissue replica of claim 143, wherein said one isolated and purified protein accounts for 90% or more of said protein content by weight.
149. The connective tissue replica of claim 143, wherein each of said one or more isolated proteins is isolated and purified separately.
150. The connective tissue replica of claim 143, wherein said one or more isolated proteins are selected from the group consisting of hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate

mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phascolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

151. The connective tissue replica of claim 150, wherein said one or more isolated and purified proteins is a prolamin family protein.
152. The connective tissue replica of claim 150, wherein said one or more isolated and purified proteins is zein.
153. The connective tissue replica of claim 143, wherein said protein content is suspended in a gel.
154. The connective tissue replica of claim 153, wherein said gel comprises an isolated, purified cross-linking enzyme.
155. The connective tissue replica of claim 154, wherein said isolated, purified cross-linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.
156. The connective tissue replica of claim 155, wherein said isolated, purified cross-linking enzyme is transglutaminase.
157. The connective tissue replica of claim 143, wherein said protein content is formed into a fiber.
158. The connective tissue replica of claim 157, wherein said fiber is produced by an extrusion process.
159. The connective tissue replica of claim 157, wherein said fiber is stabilized by protein crosslinks.
160. The connective tissue replica of claim 157, wherein said fiber contains an isolated, purified cross-linking enzyme.
161. The connective tissue replica of claim 160, wherein said isolated, purified cross-linking enzyme is selected from the group consisting of transglutaminase, lysyl oxidases, and amine oxidases.
162. The connective tissue replica of claim 161, wherein said isolated, purified cross-linking enzyme is transglutaminase.
163. A meat substitute product, comprising:

a) a muscle replica;
b) a fat tissue replica; and
c) a connective tissue replica;

wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat.

164. The meat substitute product of claim 163, comprising two or more of said muscle replica, fat tissue replica, and connective tissue replica.

165. The meat substitute product of claim 163, wherein (i) said muscle replica accounts for 40-90% of said product by weight, (ii) said fat tissue replica accounts for 1-60% of said product by weight, and (iii) said connective tissue replica accounts for 1-30% of said product by weight.

166. The meat substitute product of claim 163, comprising:

a) 60-90% water;

b) 5-30% protein content;

c) 1-20% of a fat or fat replica;

wherein said protein content comprises one or more isolated, purified plant proteins.

167. The meat substitute product of claim 166, wherein said protein content is derived from non-animal source.

168. The meat substitute product of claim 167, wherein said non-animal source is a plant source.

169. The meat substitute product of claim 167, wherein said non-animal source is a genetically modified yeast or bacteria.

170. The meat substitute product of claim 163, wherein 50% or more of said protein content by weight are one or more isolated purified proteins.

171. The meat substitute product of claim 170, wherein each of said one or more isolated proteins is isolated and purified separately from different plant species.

172. The meat substitute product of claim 170, wherein one or more of said isolated proteins is selected from the group consisting of: hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, ribulose-1,5-bisphosphate carboxylase oxygenase (rubisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (rubisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

173. The meat substitute product of claim 163, further comprising one or more isolated and purified iron-containing proteins.

174. The meat substitute product of claim 173, wherein said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, and flavohemoglobins.

175. The meat substitute composition of claim 174 wherein said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1.
176. The meat substitute product of any of claims 163-175, wherein said meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.
177. The meat substitute product of claim 176, wherein said meat substitute product contains less than 1% wheat gluten.
178. The meat substitute product of claim 177, wherein said meat substitute product contains no wheat gluten.
179. The meat substitute product of claim 178, wherein said meat substitute product contains no soy protein isolate.
180. The meat substitute product of claim 178, wherein said meat substitute product contains no soy protein concentrate.
181. The meat substitute product of claim 178, wherein said meat substitute product contains no soy protein.
182. The meat substitute product of claim 181, wherein said meat substitute product contains less than 5% carbohydrates.
183. The meat substitute product of claim 178, wherein said meat substitute product contains no tofu.
184. The meat substitute product of any of claims 163-175, wherein said meat substitute product contains no tofu, and no wheat gluten.
185. The meat substitute product of any of claims 163-175, wherein said meat substitute product contains no soy protein, and no wheat gluten.
186. The meat substitute product of any of claims 163-175, wherein said meat substitute product contains no animal products and less than 5% carbohydrates.
187. The meat substitute product of any of claims 163-175, wherein said meat substitute product contains less than 1% cellulose.
188. The meat substitute product of any of claims 163-175, wherein said meat substitute product contains less than 5% insoluble carbohydrates.
189. The meat substitute product of any of claims 163-175, wherein said meat substitute product contains no soy protein, and less than 1% cellulose.
190. The meat substitute product of any of claims 163-175, wherein said meat substitute product contains no soy protein, and less than 5% insoluble carbohydrates.
191. The meat substitute product of any of claims 163-175, wherein said meat substitute product contains no wheat gluten, and less than 1% cellulose.
192. The meat substitute product of any of claims 163-175, wherein said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.

193. A food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by an animal.

194. The food product of claim 193, wherein said one or more isolated, purified iron-containing proteins is derived from a non-animal source.
195. The food product of claim 194, wherein said non-animal source is a plant source.
196. The food product of claim 195, wherein said plant source comprises one or more plants of the legume family.
197. The meat substitute product of claim 196, wherein said one or more plants of the legume family is a soy or pea plant.
198. The food product of claim 194, wherein said non-animal source is a genetically modified yeast or bacteria.
199. The food product of claim 193, wherein said iron-containing protein is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.
200. The food product of claim 193, wherein said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1.
201. The food product of any of claims 193-200, wherein said product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum
202. The food product of claim 201, wherein said food product contains less than 1% wheat gluten.
203. The food product of claim 202, wherein said food product contains no wheat gluten.
204. The food product of claim 203, wherein said food product contains no soy protein isolate.
205. The food product of claim 203, wherein said food product contains no soy protein concentrate.
206. The food product of claim 203, wherein said food product contains no soy protein.
207. The food product of claim 206, wherein said food product contains less than 5% carbohydrates.
208. The food product of claim 203, wherein said food product contains no tofu.
209. The food product of any of claims 193-200, wherein said food product contains no tofu, and no wheat gluten.
210. The food product of any of claims 193-200, wherein said food product contains no soy protein, and no wheat gluten.
211. The food product of any of claims 193-200, wherein said food product contains no animal products and less than 5% carbohydrates.
212. The food product of any of claims 193-200, wherein said food product contains less than 1% cellulose.
213. The food product of any of claims 193-200, wherein said food product contains less than 5% insoluble carbohydrates.
214. The food product of any of claims 193-200, wherein said food product contains no soy protein, and less than 1% cellulose.

215. The food product of any of claims 193-200, wherein said food product contains no soy protein, and less than 5% insoluble carbohydrates.

216. The food product of any of claims 193-200, wherein said food product contains no wheat gluten, and less than 1% cellulose.

217. The food product of any of claims 193-200, wherein said food product contains no wheat gluten, and less than 5% insoluble carbohydrates.

218. A method of making a meat substitute composition, comprising:

a) isolating and purifying one or more proteins; and

b) assembling said one or more proteins into a physical organization that approximates the physical organization of meat.

219. A method of making a muscle tissue replica, comprising:

a) isolating and purifying one or more proteins; and

b) assembling said one or more proteins into a physical organization that approximates the physical organization of skeletal muscle.

220. A method of making a fat tissue replica, comprising:

a) isolating and purifying one or more proteins;

b) preparing a solution comprising one or more proteins;

c) emulsifying one or more fats in said solution; and

d) stabilizing said solution into a gelled emulsification with one or more cross-linking reagents.

221. A method of making a connective tissue replica comprising:

a) isolating and purifying one or more proteins; and

b) precipitating said one or more proteins,

wherein said precipitating results in said one or more proteins forming physical structures approximating the physical organization of connective tissue.

222. The method of claim 221, wherein said precipitating comprises

a) solubilizing said one or more proteins in a first solution; and

b) extruding said first solution into a second solution,

c) wherein said one or more proteins is insoluble in said second solution, wherein said extruding induces precipitation of said one or more proteins.

Figure 1

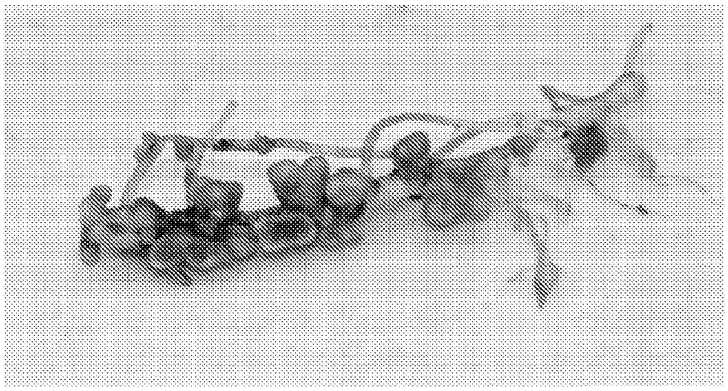


Figure 2.

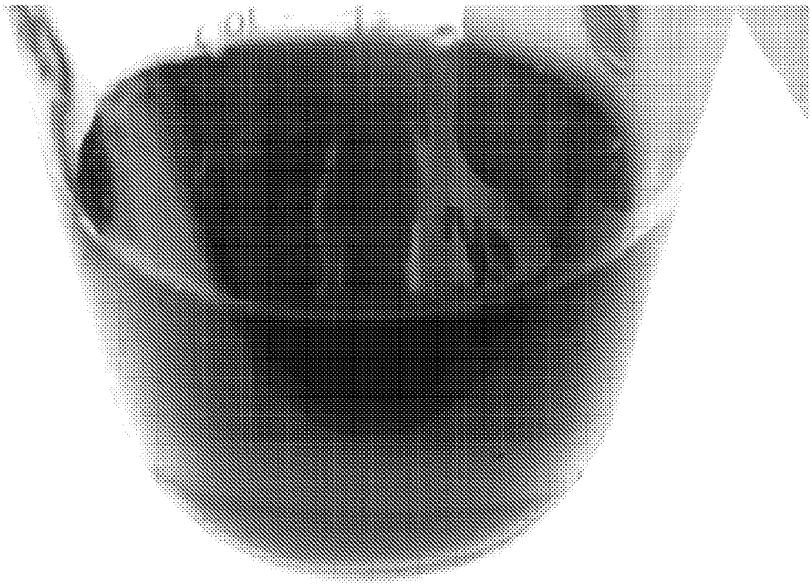


Figure 3.

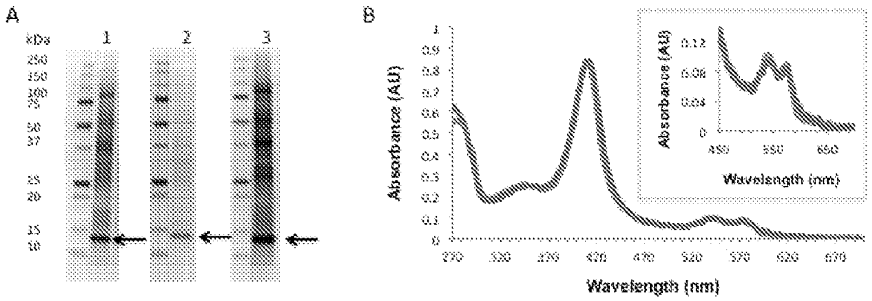


Figure 4

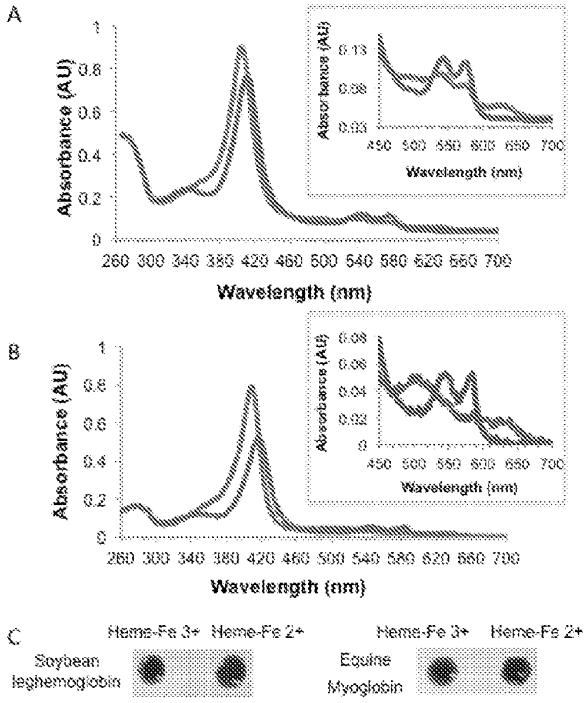


Figure 5

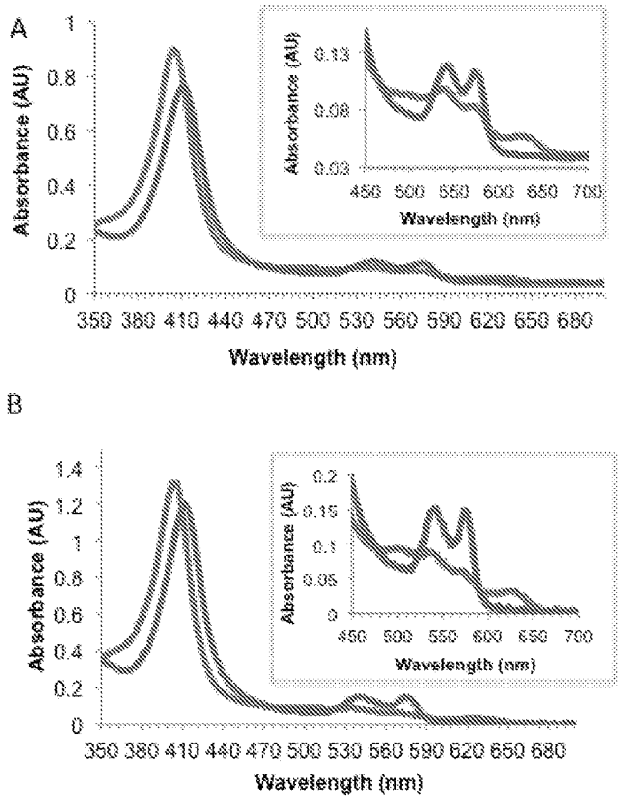
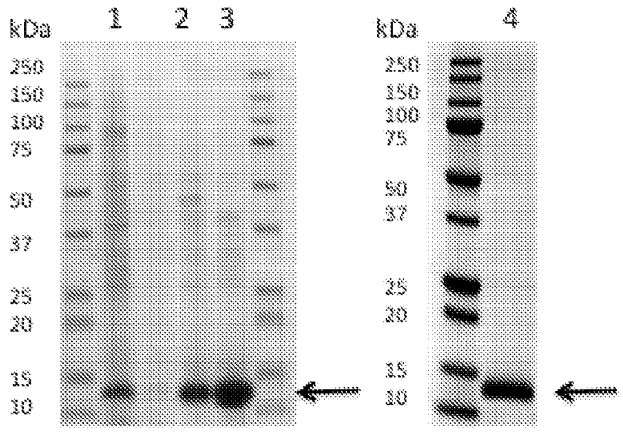


Figure 6



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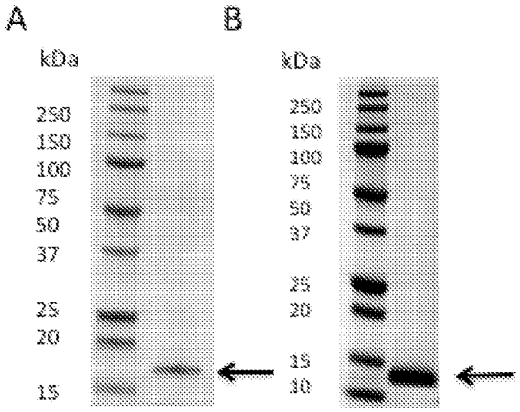
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Figure 8

Figure 9

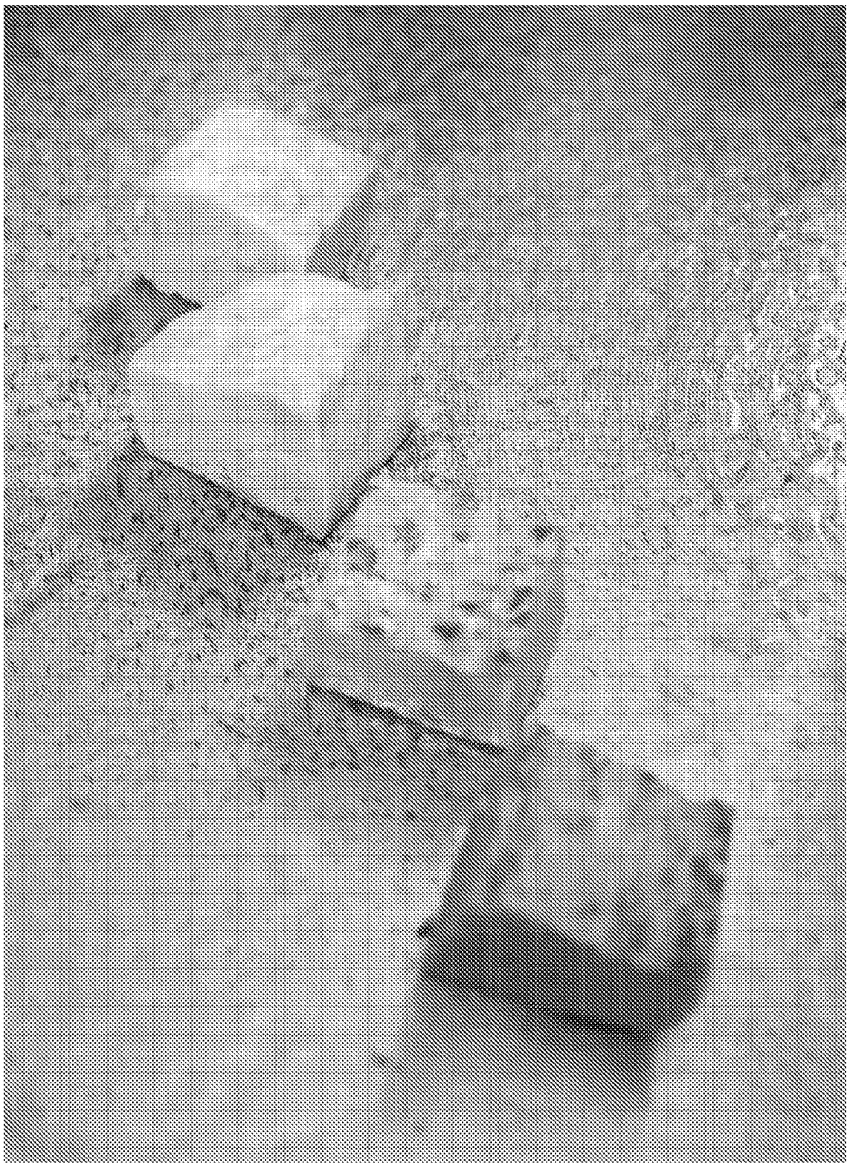

Figure 10

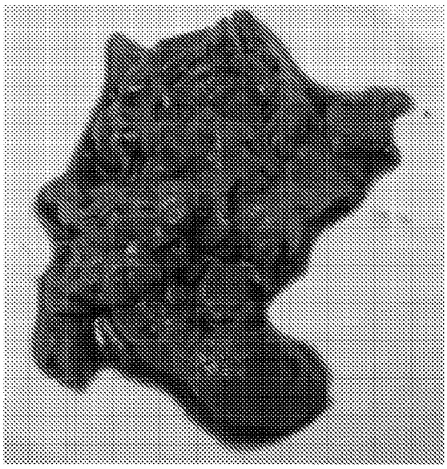

Figure 11

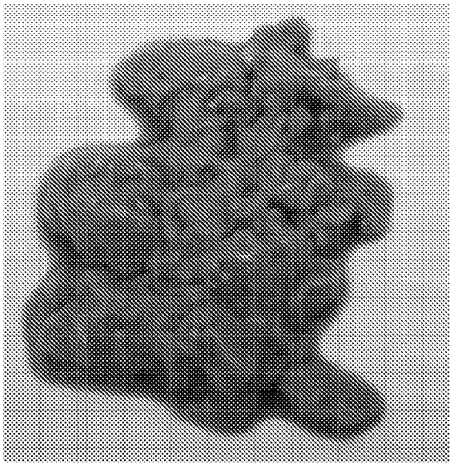

Figure 12

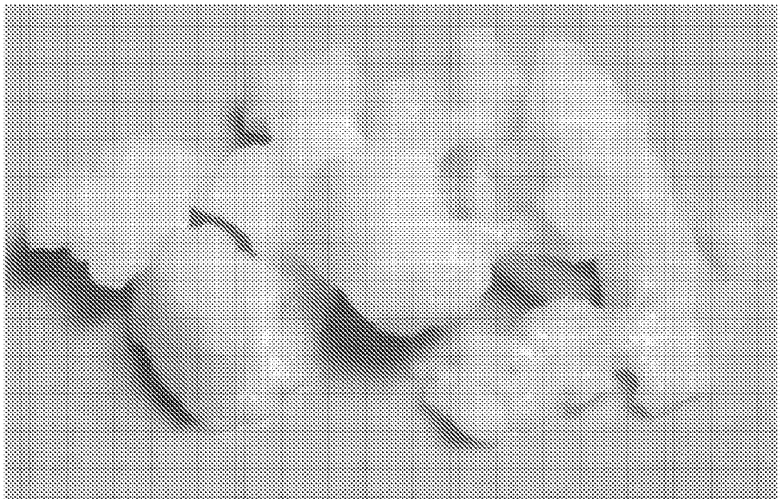

Figure 13

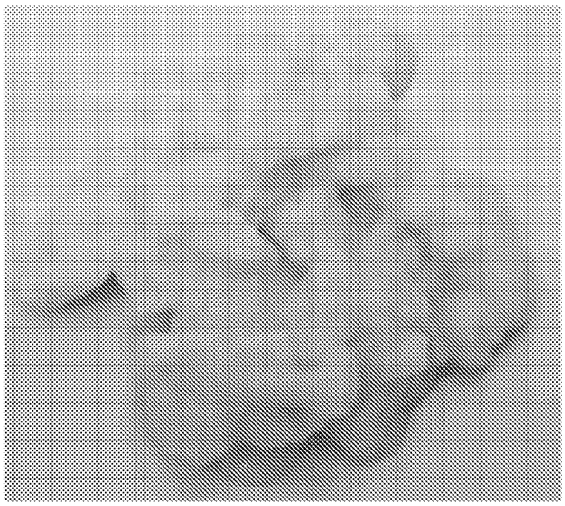

Figure 14

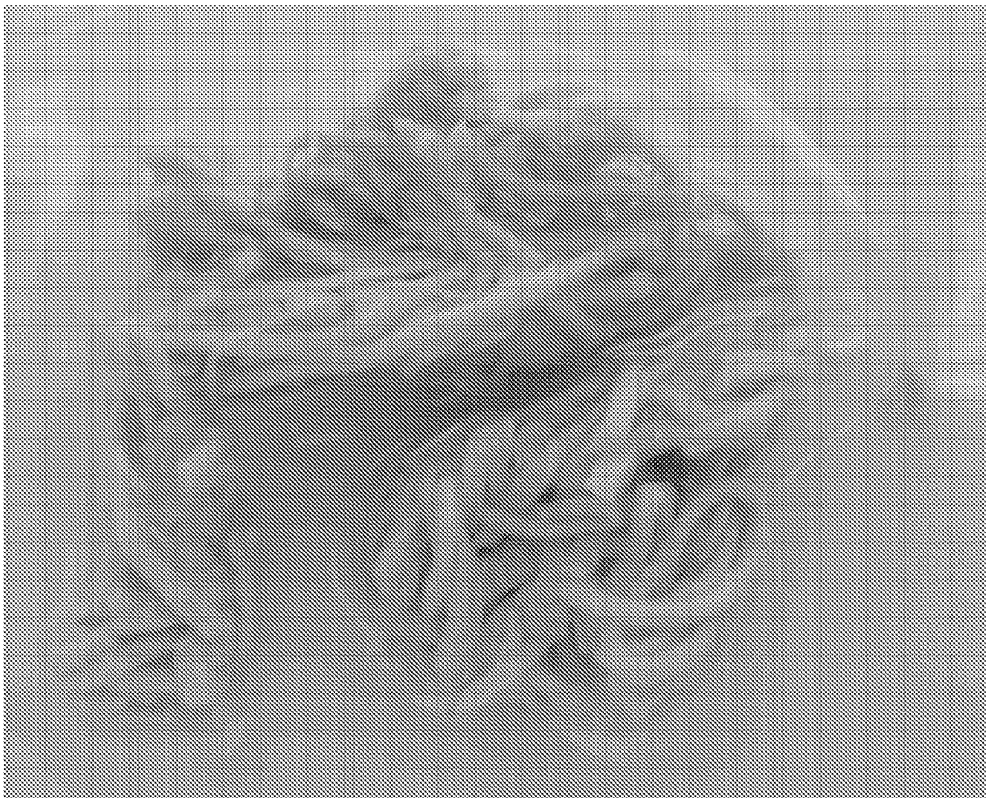

Figure 15

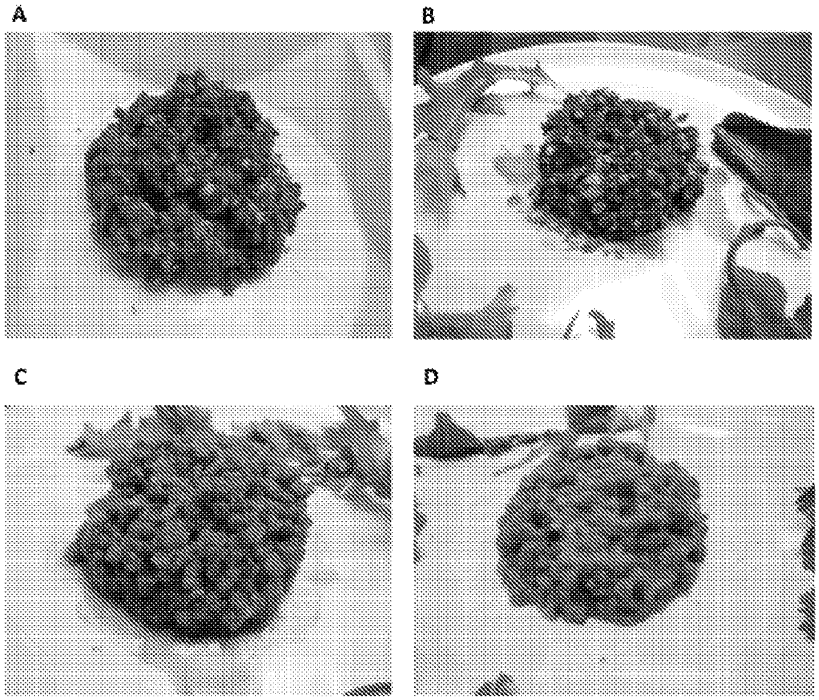

Figure 16

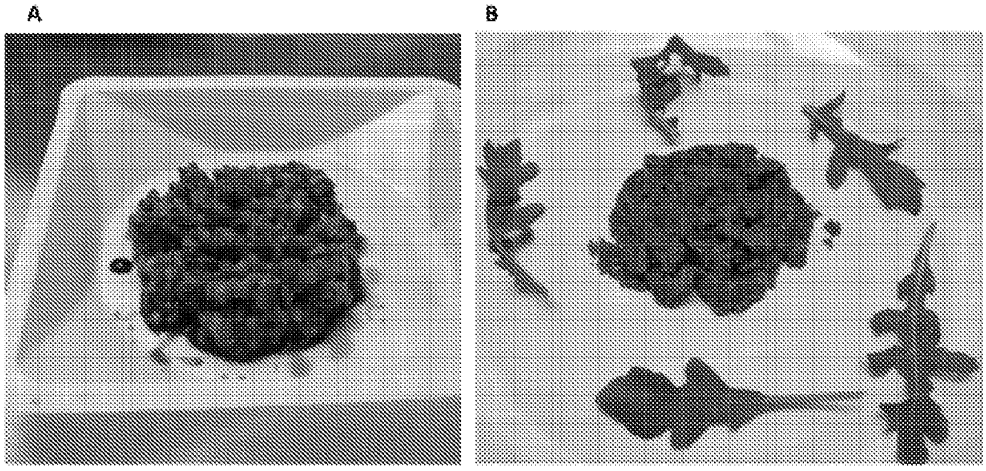

Figure 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/46560

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A23J 1/00 (2012.01)
USPC - 426/656
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 426/656

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 426/656, 574, 573, 531, 615
(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase; Google; PubMed
Search terms: meat, substitute, isolated, purified, protein, gluten, raw, cook, recombinant, yeast

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/0035003 A1 (MCMINDES et al.) 16 February 2006 (16.02.2006) para [0004], [0033], [0039], [0047], [0050], [0052]-[0054], [0058], [0065], [0110], [0118]	1-20
Y	US 2007/0269571 A1 (AKITA et al.) 22 November 2007 (22.11.2007) entire document.	1-20
Y	US 2010/0074998 A1 (ESPELETA VEGA et al.) 25 March 2010 (25.03.2010) entire document.	1-20
Y	US 2004/0161513 A1 (AKASHE et al.) 19 August 2004 (19.08.2004) entire document.	1-20
Y	US 2011/0008502 A1 (HOSOMI et al.) 13 January 2011 (13.01.2011) entire document.	1-20

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 December 2012 (05.12.2012)	14 DEC 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/46560

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 21-26, 47-63, 70-71, and 88-104
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I claims 1-20 directed to a meat substitute composition comprising a protein content.

Group II claims 27-46 directed to a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources.

Group III claims 64-69 and 72-87 directed to a muscle tissue replica.

Group IV claims 105-142 directed to a fat tissue replica.
----------(Continued on extra sheet)----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-20

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/46560

Continuation of Box III - observations where unity of invention is lacking:

Group V claims 143-162 directed to a connective tissue replica.

Group VI claims 163-192 directed to a meat substitute product.

Group VII claims 193-217 directed to a food product comprising one or more isolated, purified iron-containing proteins.

Group VIII claim 218 directed to a method of making a meat substitute composition.

Group IX claim 219 directed to a method of making a muscle tissue replica.

Group X claim 220 directed to a method of making a fat tissue replica.

Group XI claims 221 and 222 directed to a method of making a connective tissue replica.

The inventions listed as Groups I-XI do not relate to a single inventive concept under Rule 13.1 because under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of the Groups are delineated as above. Groups I, II, VI, and VIII share the special technical feature of a meat substitute product. Groups I, III, V, and VII-XI share the special technical feature of an isolated and purified protein or isolating and purifying a protein. However, this is not an improvement over the prior art of US 2010/0074998 A1 to ESPELETA VEGA et al. (hereinafter Espeleta Vega) that teaches a meat substitute composition comprising a protein content comprising one or more isolated and purified proteins (abstract; para [0037] - vegetable protein isolate), wherein said meat substitute composition accurately mimics the taste or color of a meat product derived from animal sources (para [0047]). Groups III, VI, and IX share the special technical feature of a muscle tissue replica. Likewise, this is not an improvement over the prior art of US 2004/0161513 A1 to AKASHE et al. (hereinafter Akashe) that teaches a muscle replica (para [0065] - "the deflavored soy materials are texturized prior to incorporation into the meat or meat analog product in order to develop meat-like texture (i.e., similar to fibers found in meat)"). Groups IV, VI and X share the special technical feature of a fat tissue replica. Likewise this is not an improvement over the prior art of US 2011/0008502 A1 to HOSOMI et al. (hereinafter Hosomi) that teaches a fat tissue replica for use in processed meat products in order to impart a fatty tissue-specific mouthfeel and juiciness (para [0277]). Groups V, VI and XI share the special technical feature of a connective tissue replica. Likewise, this is not an improvement over the prior art of Akashe that teaches a connective tissue replica (para [0065] - "Preferred processing includes adding water and carrying out further size reduction by grinding, for example, so as to provide thin protein filaments. In the meat and meat analog products of this invention, these filaments provide texture properties similar to those of connective tissue proteins such as collagen normally present in meat products."); wherein said muscle replica and connective tissue replica are assembled in a manner that approximates the physical organization of meat (" In the meat and meat analog products of this invention, these filaments provide texture properties similar to those of connective tissue proteins such as collagen normally present in meat products."). Further, Group II requires a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from non-animal sources which is not required by any of the other Groups. Group III further requires the muscle tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source which is not required by any of the other Groups. Group IV further requires the fat tissue replica comprising a gelled emulsion, said gelled emulsion comprising a protein solution with fat droplets suspended therein which is not required by any of the other Groups. Group V further requires the connective tissue replica has been assembled into structures approximating the texture and visual appearance of connective tissue or skin which is not required by any of the other Groups. Group VI further requires the muscle replica, fat tissue replica, and/or connective tissue replica be assembled in a manner that approximates the physical organization of meat which is not required by any of the other Groups. Group VII further requires purified iron-containing proteins, wherein configured for consumption by an animal which is not required by any of the other Groups. Group VIII further requires assembling said one or more proteins into a physical organization that approximates the physical organization of meat which is not required by any of the other Groups. Group IX further requires assembling said one or more proteins into a physical organization that approximates the physical organization of skeletal muscle which is not required by any of the other groups. Group X further requires preparing a solution comprising one or more proteins, emulsifying one or more fats in said solution, and stabilizing said solution into a gelled emulsification with one or more cross-linking reagents which is not required by any of the other Groups. Group XI further requires solubilizing one or more proteins in a first solution, extruding said first solution into a second solution, wherein said one or more proteins is insoluble in said second solution, wherein said extruding induces precipitation of said one or more proteins which is not required by any of the other Groups.

Therefore the inventions listed as Groups I-XI do not relate to a single general inventive concept under PCT Rule 13.1 because they do not share a same or corresponding special technical feature. This ISA will establish the ISR for the first Group mentioned, specifically, Group I claims 1-20 without additional fees. In order for all inventions to be examined, the appropriate examination fees must be paid.

+++++++

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(74) Agents: **EASTERDAY, Mathew** et al.; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, CA 94304-1050 (US).

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Published:

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WO 2013/138793 A2

(54) Title: AFFINITY REAGANTS FOR PROTEIN PURIFICATION

(57) Abstract: Disclosed herein are methods and compositions for purifying proteins from crude solutions.

AFFINITY REAGANTS FOR PROTEIN PURIFICATION

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 61/611,999 filed 03-16-2012, which application is incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] Proteins and other molecules with potential commercial value — for use as food ingredients (*e.g.*, rubisco, seed-storage proteins, leghemoglobin), industrial enzymes (*e.g.*, papain, bromelain, etc.), structural materials or pharmaceuticals are present in commercially useful quantities in natural sources. Purity of the protein or other molecule can be important for optimal utility.

[0003] The rarity of commercial sources of pure plant, animal or microbial proteins highlights an existing need in the art for general, scalable methods of efficiently and economically purifying proteins or other molecules from a crude solution.

SUMMARY OF THE INVENTION

[0004] Disclosed herein are methods for isolating one or more target proteins comprising: (a) applying a composition comprising the one or more target proteins to a substrate comprising one or more switchable affinity reagents at a binding pH such that the target proteins bind to the switchable affinity reagents; and (b) applying a solution at a release pH to the substrate comprising the one or more switchable affinity reagents such that the one or more target proteins are released from the one or more switchable affinity reagents, thereby isolating the one or more target proteins. Some embodiments further comprise (c) applying a wash solution at the binding pH between step (a) and step (b), wherein the wash solution removes one or more contaminants. In one embodiment, the one or more switchable affinity reagents do not comprise an antibody. In one embodiment, the one or more target proteins are antibodies. In one embodiment, the one or more target proteins can be used to treat a disease or disorder. In one embodiment, the disease or disorder is a cancer.

[0005] In some embodiments, the one or more target proteins comprise plant proteins. In one embodiment, said one or more target proteins are selected from the group consisting of leghemoglobin, non-symbiotic hemoglobin, hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose

bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, 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, pea globulins, pea albumins, or combinations thereof.

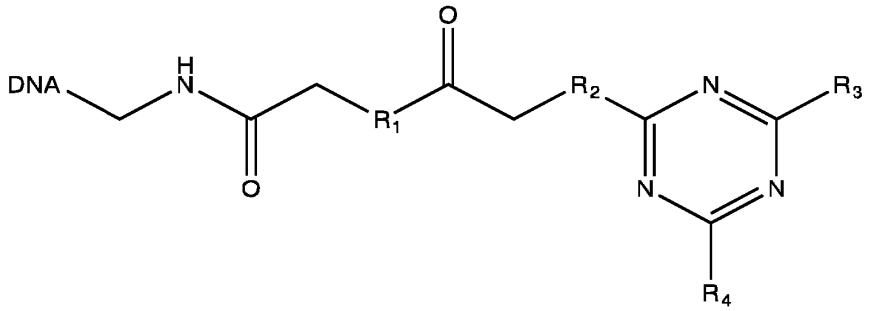
[0006] In some embodiments, the one or more switchable affinity reagents comprise a small molecule compound. In some embodiments, the small molecule compound comprises peptide, peptoid, 3-peptide, or D-peptide subunits. In some embodiments, the binding pH is between about 7 and about 11. In some embodiments, the binding pH is about 9. In some embodiments, the release pH is between about 3 and about 7. In some embodiments, the release pH is about 6. In some embodiments, the one or more target proteins are used to produce a consumable. In some embodiments, the consumable is a meat-substitute. In some embodiments, the consumable is a cheese-substitute. In some embodiments, the consumable is a milk-substitute. In some embodiments, the consumable is an egg-substitute.

[0007] In some embodiments, the invention provides a composition comprising a non-polypeptide pH switchable reagent which binds a target at a first pH and does not bind the target at a second pH.

[0008] In some embodiments, the pH switchable reagent is a peptoid. In some embodiments, the peptoid is comprised of N subunits. In some embodiments, the N subunits are 1, 2, 3, 4, or 5 subunits. In some embodiments, N subunits is 2 subunits. In some embodiments, the peptoid is modified with multiple diversity points. In some embodiments, the multiple diversity points are at least 1, 2, 3, 4, 5, 6 diversity points. In some embodiments, the multiple diversity points are 4 diversity points.

[0009] In some embodiments, the peptoid comprises a triazine. In some embodiments, the triazine is tri-substituted.

[0010] In some embodiments, wherein the pH switchable reagent has a backbone structure comprising:



[0011] In some embodiments, the composition comprises a mixture of diastereomers. The composition can comprise a mixture of enantiomers. The composition can comprise an essentially pure diastereomer, wherein the essentially pure diastereomer refers to a diastereomer that is greater than 70% purified from other diastereomers. The composition can comprise an essentially pure enantiomer, wherein the essentially pure enantiomer refers to a enantiomer that is greater than 70% purified from other enantiomers.

[0012] In some embodiments, the peptoid is non-degradable by proteases.

[0013] In some embodiments, the first pH is greater than 7. In some embodiments, the first pH is less than 7. In some embodiments, the second pH is greater than 7. In some embodiments, the second pH is less than 7.

[0014] In some embodiments, the pH switchable reagent is conjugated to a substrate. In some embodiments, the substrate is a bead that is selected from a group comprising agarose, sepharose, polystyrene, styrene, iron oxide, magnetic, or paramagnetic. In some embodiments, the substrate is coated with a coat selected from a group comprising silica, carboxyl functional groups, aldehyde functional groups, or N-hydroxysuccinimide functional groups. In some embodiments, the substrate is coated with a functional group able to conjugate primary amines and thiols. In some embodiments, the substrate is a component of a resin used for chromatography. In some embodiments, the resin is used for protein purification chromatography. In some embodiments, the resin is used for affinity protein purification chromatography.

[0015] In some embodiments, the invention is a consumable product comprising a pH switchable reagent. In some embodiments, switchable reagent is present at a concentration of less than 1,000 parts per million target protein. In some embodiments, at least 90% of the dry weight of the composition comprises plant based protein. In some embodiments, the consumable is substantially free of chlorophylls, chlorins, metalloids, transition metals, celluloses, or complex polysaccharides. In some embodiments, the consumable product is food-safe.

[0016] In some embodiments, the composition comprises a protein content, wherein the protein content is obtained from a plant; wherein the composition is substantially free of odorants found

in the plant. In some embodiments, the composition comprises the pH switchable reagent is present at a concentration of less than 1,000 parts per million target protein. In some embodiments, least 90% of the composition comprises plant based protein. In some embodiments, the composition is substantially free of chlorophylls, chlorins, metalloids, transition metals, celluloses, or complex polysaccharides. In some embodiments, the consumable product is food-safe.

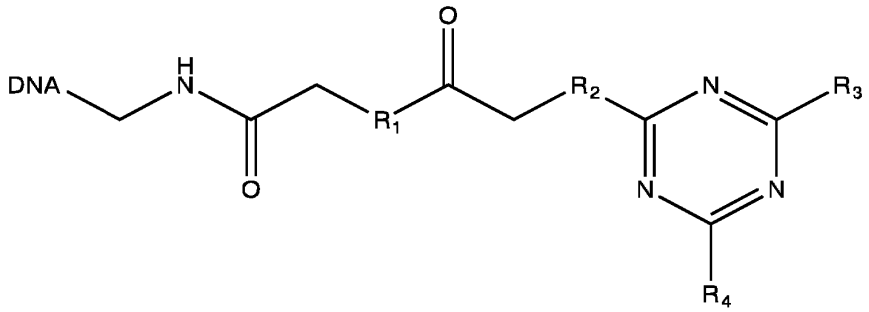
[0017] In some embodiments, the composition comprises a protein content, wherein the protein content is obtained from a plant; wherein the composition is substantially free of colorants found in the plant. In some embodiments, the composition comprises the pH switchable reagent is present at a concentration of less than 1,000 parts per million target protein. In some embodiments, at least 90% of the composition comprises plant based protein. In some embodiments, the composition is substantially free of chlorophylls, chlorins, metalloids, transition metals, celluloses, or complex polysaccharides. In some embodiments, the consumable product is food-safe.

[0018] In some embodiments, the invention provides a method for identifying a non-polypeptide pH switchable reagent, the method comprising: a) generating a library of non-polypeptides by tag-directed synthesis; b) exposing at least a subset of the library to a protein in a solution at a first pH; c) altering the pH of the solution to a second pH; and d) identifying a non-polypeptide pH switchable reagent which binds a target at a first pH and does not bind the target at a second pH.

[0019] In some embodiments, the invention provides a method for isolating one or more target proteins comprising: a) applying a composition comprising the one or more target proteins to a substrate; wherein the substrate comprises one or more non-polypeptide pH switchable reagents; wherein at least part of the applying step occurs at a first pH; wherein at the first pH at least one of the one or more target proteins binds to the one or more non-polypeptide pH switchable reagents; b) releasing the target proteins by adjusting the pH of the solution to a second pH; wherein at the second pH at least one or more of the target proteins does not bind the non-polypeptide pH switchable reagent and; c) collecting the target proteins that eluted from the one or more non-polypeptide pH switchable reagents.

[0020] In some embodiments of the method, the pH switchable reagent is a peptoid. In some embodiments of the method, the peptoid is comprised of N subunits. In some embodiments of the method, N subunits is 1, 2, 3, 4, 5 subunits. In some embodiments of the method, N subunits is 2 subunits. In some embodiments of the method, the peptoid is modified with multiple diversity points. In some embodiments of the method, the multiple diversity points are at least 1,

2, 3, 4, 5, 6 diversity points. In some embodiments of the method, the multiple diversity points are 4 diversity points. In some embodiments of the method, the peptoid comprises a triazine. In some embodiments of the method, the triazine is tri-substituted. In some embodiments of the method, the pH switchable reagent has a backbone structure comprising:



[0021] In some embodiments of the method, the pH switchable reagent comprises a mixture of diastereomers. In some embodiments of the method, the pH switchable reagent comprises a mixture of enantiomers. In some embodiments of the method, wherein the pH switchable reagent comprises an essentially pure diastereomer, wherein the essentially pure diastereomer refers to a diastereomer that is greater than 70% purified from other diastereomers. In some embodiments of the method, the pH switchable reagent comprises an essentially pure enantiomer, wherein the essentially pure enantiomer refers to an enantiomer that is greater than 70% purified from other enantiomers.

[0022] In some embodiments of the method, the peptoid is non-degradable by proteases. In some embodiments of the method, the first pH is greater than 7. In some embodiments of the method, the first pH is less than 7. In some embodiments of the method, the second pH is greater than 7. In some embodiments of the method, the second pH is less than 7.

[0023] In some embodiments of the method, the pH switchable reagent is conjugated to a substrate. In some embodiments of the method, the substrate is a bead that is selected from a group comprising agarose, sepharose, polystyrene, styrene, iron oxide, magnetic, or paramagnetic. In some embodiments of the method, the substrate is coated with a coat selected from a group comprising silica, carboxyl functional groups, aldehyde functional groups, amine functional groups, alkyne functional groups, azide functional groups or N-hydroxysuccinimide functional groups. In some embodiments of the method, the substrate is coated with a functional group able to conjugate primary amines and thiols. In some embodiments of the method, the substrate is a component of a resin used for chromatography. In some embodiments of the method, the resin is used for protein purification chromatography. In some embodiments of the method, the resin is used for affinity protein purification chromatography.

[0024] In some embodiments rubsco binds at pH 9 and releases at pH 6. In some embodiments rubsco binds at pH 6 and releases at pH 9. In some embodiments leghemoglobin binds at pH 9 and releases at pH 6. In some embodiments leghemoglobin binds at pH 6 and releases at pH 9.

INCORPORATION BY REFERENCE

[0025] 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

[0026] Disclosed herein are methods and compositions for purifying one or more target proteins or compounds, or molecules from a crude solution. The methods and compositions disclosed herein can be used to purify quantities of the target proteins without the need to produce recombinant, tagged versions of the proteins. The methods and compositions can comprise switchable affinity reagents. The switchable affinity reagent can be a molecule that has controllable or regulatable affinity for a target protein, compound, or molecule. For example, a switchable affinity reagent can be a reagent that binds to the target protein, compound, or molecule with one affinity under a first set of conditions and does not bind, or binds with a lower affinity to, the target protein, compound, or molecule under a second set of conditions. The switchable affinity reagent can be a small molecule. The switchable affinity reagent can be designed. The switchable affinity reagent can be selected from a library of candidate affinity reagents or small molecules. The switchable affinity reagent can be selected from a library of candidate affinity reagents produced using DNA programmable combinatorial chemistry. The library of candidate affinity reagents, optionally produced using DNA programmable combinatorial chemistry, can be biased towards molecules that are likely to have a functional group or moiety that can be altered by environmental conditions. The switchable affinity reagent can be immobilized on a solid support structure, such as a bead or resin. The conditions to control or regulate the affinity of the switchable affinity reagent can include changes in pH, temperature, salinity, or concentration of a metal ion or other reagent.

DEFINITIONS

[0027] As used herein, “about” can refer to ± 10% and includes ± 1% and ± 0.1%.

[0028] As used herein, in addition to definitions known in the art, “resin” can refer to a solid support with a large surface area (e.g. a membrane, a fine powder or gel.)

[0029] As used herein, the term "switchable affinity reagent" can mean a reagent that binds to a target (e.g. a protein) under one set of conditions but does not bind, or binds with a lower affinity to, the target under a second set of conditions.

Switchable Affinity Reagent

[0030] Methods to purify one or more targets (*e.g.*, proteins from *e.g.* a crude solution) can utilize a switchable affinity reagent. The switchable reagent can be immobilized on a solid or semi-solid support. Conditions that can be used to regulate the affinity of a switchable affinity reagent for a target protein can include pH, temperature, salinity or ionic strength, or the concentration a metal ion or other reagent. In one example, the affinity of a switchable affinity reagent can be controlled by relying on pH dependent changes in charge of residues or chemical moieties on the target, the switchable affinity reagent, or both. The effected residues or chemical moieties can be located at the interface between the affinity reagent and the target. The effected residues or chemical moieties can be residues or chemical moieties whose charge affects the conformation of the affinity reagent, the target, or both. The switchable affinity reagent can comprise a small molecule compound. The switchable affinity reagent can optionally comprise a nucleic acid sequence that encodes a program for the synthesis of the small molecule compound. The switchable affinity reagent can be conjugated to a solid support substrate such as a bead. The switchable affinity reagent can be conjugated to a semi-solid support substrate.

[0031] A switchable affinity reagent can comprise a molecule or small molecule compound that is synthesized from smaller subunits, building blocks, or reagents, precursors, monomers or residues (collectively "subunits"). The subunits of a switchable affinity reagent can optionally comprise a common parent structure. The common parent structure can be a peptide or peptidomimetic backbone (*e.g.*, an L-peptide backbone, a D-peptide backbone, a (3-peptide backbone, a peptoid backbone). The common parent structure can be a ring structure (*e.g.*, a benzene ring, a phenol ring, a toluene, a napthalene, a cyclohexyl, a sugar, an aniline, a biphenyl structure, a pyridine, a triazine, *etc.*).

[0032] The common parent structure can comprise one or more (*e.g.*, two) functional moieties that can be used to link subunits together. The subunits of the switchable affinity reagent can comprise, but are not limited to, L-amino acids or L-peptide residues, D-amino acids or D-peptide residues, (3-amino acids or 3-peptide residues, or peptoid residues).

[0033] The subunits can be comprised of one or more side chains or ring-structure modifications (collectively called "R groups") each of which can take a variety of chemical forms. The R groups of the subunits can vary in terms of chain length, ring size or number, and/or patterns of substitution. The R groups of the subunit of a switchable affinity reagent can comprise naturally occurring side chains such as the side chains found in L-peptides, proteins, or amino acids. The R groups of the subunits of a switchable affinity reagent can comprise side chains or groups not found in biology.

[0034] A switchable affinity reagent can comprise a molecule or small molecule compound that is synthesized from smaller subunits, building blocks, reagents, precursors, monomers, or residues (collectively "subunits"). The switchable affinity reagent can comprise between about 2 and about 1000 subunits; for example, the switchable affinity reagent can comprise about 2-1000, 2-750, 2-500, 2-250, 2-100, 2-75, 2-50, 2-40, 2-30, 2-25, 2-20, 2-15, 2-10, 2-5, 5-1000, 5-750, 5-500, 5-250, 5-100, 5-75, 5-50, 5-40, 5-30, 5-25, 5-20, 5-15, 5-10, 10-1000, 10-750, 10-500, 10-250, 10-100, 10-75, 10¬50, 10-40, 10-30, 10-25, 10-20, 10-15, 15-1000, 15-750, 15-500, 15-250, 15-100, 15-75, 15-50, 15¬40, 15-30, 15-25, 15-20, 20-1000, 20-750, 20-500, 20-250, 20-100, 20-75, 20-50, 20-40, 20-30, 30¬1000, 30-750, 30-500, 30-250, 30-100, 30-75, 30-50, 30-40, 40-1000, 40-750, 40-500, 40-250, 40¬100, 40-75, 40-50, 50-1000, 50-750, 50-500, 50-250, 50-100, 50-75, 75-1000, 75-750, 75-500, 75¬250, 75-100, 100-1000, 100-750, 100-500, 100-250, 250-1000, 250-750, 250-500, 500-1000, 500¬750, 750-1000, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850,900, 950, 1000. In one example, a switchable affinity reagent comprises between about 2 and about 10 subunits. In another example, the switchable affinity reagent comprises between about 2 and about 6 subunits. The switchable affinity reagent can optionally further comprise a nucleotide sequence (*e.g.,* DNA, RNA) that encodes a program for the synthesis of the small molecule compound.

[0035] A switchable affinity reagent can be characterized by having a molecular weight of between about 10 Da and 1000000 Da; for example, the molecular weight of the switchable affinity reagent can be about 10-1000000 Da, 10-75000 Da, 10-50000 Da, 10-25000 Da, 10-10000 Da, 10-5000 Da, 10-2500 Da, 10-1000 Da, 10-750 Da, 10-500 Da, 10-250 Da, 10-100 Da, 100-1000000 Da, 100-75000 Da, 100-50000 Da, 100-25000 Da, 100-10000 Da, 100-5000 Da, 100-2500 Da, 100-1000 Da, 100-750 Da, 100-500 Da, 100-250 Da, 250-1000000 Da, 250-75000 Da, 250-50000 Da, 250-25000 Da, 250-10000 Da, 250-5000 Da, 250-2500 Da, 250-1000 Da, 250-750 Da, 250-500 Da, 500-1000000 Da, 500-75000 Da, 500-50000 Da, 500-25000 Da, 500-

10000 Da, 500-5000 Da, 500-2500 Da, 500-1000 Da, 500-750 Da, 750-1000000 Da, 750-75000 Da, 750-50000 Da, 750-25000 Da, 750-10000 Da, 750-5000 Da, 750-2500 Da, 750-1000 Da, 1000-1000000 Da, 1000-75000 Da, 1000-50000 Da, 1000-25000 Da, 1000-10000 Da, 1000-5000 Da, 1000-2500 Da, 2500¬1000000 Da, 2500-75000 Da, 2500-50000 Da, 2500-25000 Da, 2500-10000 Da, 2500-5000 Da, 5000¬1000000 Da, 5000-75000 Da, 5000-50000 Da, 5000-25000 Da, 5000-10000 Da, 10000-1000000 Da, 10000¬75000 Da, 10000-50000 Da, 10000-25000 Da, 25000-1000000 Da, 25000-75000 Da, 25000-50000 Da, 50000-1000000 Da, 50000-75000 Da, 75000-1000000 Da, 10 Da, 20 Da, 30 Da, 40 Da, 50 Da, 60 Da, 70 Da, 80 Da, 90 Da, 100 Da, 125 Da, 150 Da, 175 Da, 200 Da, 225 Da, 250 Da, 300 Da, 350 Da, 400 Da, 450 Da, 500 Da, 550 Da, 600 Da, 650 Da, 700 Da, 750 Da, 800 Da, 850 Da, 900 Da, 950 Da, 1000 Da, 1100 Da, 1200 Da, 1300 Da, 1400 Da, 1500 Da, 1600 Da, 1700 Da, 1800 Da, 1900 Da, 2000 Da, 2100 Da, 2200 Da, 2300 Da, 2400 Da, 2500 Da, 2750 Da, 3000 Da, 3250 Da, 3500 Da, 3750 Da, 4000 Da, 4250 Da, 4500 Da, 4750 Da, 5000 Da, 6000 Da, 7000 Da, 8000 Da, 9000 Da, 10000 Da, 12500 Da, 15000 Da, 17500 Da, 20000 Da, 22500 Da, 25000 Da, 30000 Da, 35000 Da, 40000 Da, 45000 Da, 50000 Da, 55000 Da, 60000 Da, 65000 Da, 70000 Da, 75000 Da, 80000 Da, 85000 Da, 90000 Da, 95000 Da, or 100000 Da, or more, or any intervening amount. In one example, the molecular weight of a switchable affinity reagent can be between about 100 Da and 1000 Da. In another example, the molecular weight of a switchable affinity reagent can be less than about 1000 Da.

[0036] In another example, the molecular weight of a switchable affinity reagent can be between about 600 and about 900 Da.

[0037] A switchable affinity reagent can be characterized as having a first binding affinity for a target protein, compound, or molecule under a first set of conditions (*e.g.*, binding conditions) and a second binding affinity for the target protein under a second set of conditions (*e.g.*, release conditions). The first binding affinity can be stronger than the second binding affinity. For example, the first binding affinity can be at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 460, 480, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2200, 2400, 2600, 2800, 3000, 3200, 3400, 3600, 3800, 4000, 4200, 4400, 4600, 4800, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, or 10000 times stronger than the second binding affinity.

[0038] A switchable affinity reagent can be characterized as having a first binding affinity for a target protein, compound, or molecule under a first set of conditions (*e.g.*, binding conditions)

and a second binding affinity for the target protein under a second set of conditions (*e.g.*, release conditions), wherein the first binding affinity and the second binding affinity are expressed as dissociation constants (K_d). The dissociation constant can have molar units and a lower dissociation constant can mean that the switchable affinity reagent and the target protein bind with a higher affinity. Similarly, a higher dissociation constant can be associated with lower binding affinity between the switchable affinity reagent and the target protein. The first binding affinity between the switchable affinity reagent and the target protein can be a K_d of less than about 2 mM; for example, the first binding affinity can be a K_d of less than about 2 mM, 1000 uM, 900 uM, 800 uM, 700 uM, 600 uM, 500 uM, 400 uM, 300 uM, 200 uM, 100 uM, 75 uM, 50 uM, 25 uM, 20 uM, 15 uM, 10 uM, 9 uM, 8 uM, 7 uM, 6 uM, 5 uM, 4 uM, 3 uM, 2 uM, 1 uM, 900 nM, 800 nM, 700 nM, 600 nM, 500 nM, 400 nM, 300 nM, 200 nM, 100 nM, 75 nM, 50 nM, 25 nM, 20 nM, 15 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, or lower. The second binding affinity can be a Kd that is at least about 2 times higher than the first binding affinity; for example, the second binding affinity can be a Kd that is at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 460, 480, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2200, 2400, 2600, 2800, 3000, 3200, 3400, 3600, 3800, 4000, 4200, 4400, 4600, 4800, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, or 10000 times higher than the first binding affinity.

pH sensitive Switchable Affinity Reagents

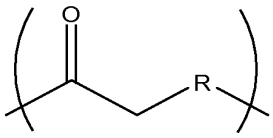
[0039] A switchable affinity reagent can be a pH sensitive switchable reagent, wherein the reagent binds a target protein at a binding pH and does not bind the target protein at a release pH. The binding pH can be a pH between about 1 and 14; for example, about, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or any included decimal. The release pH can be a pH between about 1 and 14; for example, about, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or any included decimal. The binding and release pH can be separated by between about 1 and about 13 pH units; for example, the binding and release pH can be separated by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 pH units. In some instances binding of a target protein occurs in acidic conditions and release of the target protein occurs in basic conditions. In some instances binding of a target protein occurs in basic conditions and release of the target protein occurs in acidic conditions. In one example, the binding pH can be between about 5 and 11 (*e.g.*, about 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, or any included decimal) and the release pH can be between about 6 and about 11 (*e.g.*,

6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11 or any included decimal). In another example, the binding pH can be between about 4 and about 8 (*e.g.*, about 4, 4.5, 5, 5.5., 6, 6.5, 7, 7.5, 8 or any included decimal) and the release pH can be between about 7 and about 11 (e.g., 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, or any included decimal). In another example, the binding pH is about 6 and the release pH is about 9. In another example, the binding pH is about 9 and the release pH is about 6.

[0040] A pH sensitive switchable affinity reagent can be characterized by having a pKa of between about 1 and 14; for example, a pKa of about 1-14, 1-13, 1-12, 1-11, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-14, 2-13, 2-12, 2-11, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-14, 3-13, 3-12, 3-11, 3-10, 3-9, 3-8, 3- 7, 3-6, 3-5, 3-4, 4-14, 4-13, 4-12, 4-11, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-14, 5-13, 5-12, 5-11, 5-10, 5-9, 5-8, 5-7, 5-6, 6-14, 6-13, 6-12, 6-11, 6-10, 6-9, 6-8, 6-7, 7-14, 7-13, 7-12, 7-11, 7-10, 7-9, 7-8, 8-14, 8-13, 8-12, 8-11, 8-10, 8-9, 9-14, 9-13, 9-12, 9-11, 9-10, 10-14, 10-13, 10-12, 10-11, 11-14, 11-13, 11-12, 12-14, 12-13, 13-14, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 10.1, 10.2, 10.3, 10.4, 10.5, 10.6, 10.7, 10.8, 10.9, 11, 11.1, 11.2, 11.3, 11.4, 11.5, 11.6, 11.7, 11.8, 11.9, 12, 12.1, 12.2, 12.3, 12.4, 12.5, 12.6, 12.7, 12.8, 12.9, 13, 13.1, 13.2, 13.3, 13.4, 13.5, 13.6, 13.7, 13.8, 13.9, 14. In one example, the pH sensitive switchable affinity reagent has a pKa of about 7 and is neutrally charged at a pH of about 9 and positively charged at a pH of about 5.

A peptoid switchable reagent

[0041] In some instances the subunit is comprised of a peptoid backbone. The peptoid backbone is comprised of N peptoid subunits (sometimes referred to as oligo-N-substituted glycines), where the peptoid backbone subunit can be comprised of:



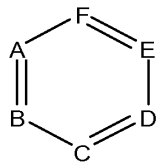
Formula I.

[0042] The peptoid backbone subunit is comprised of peptoid submonomers. A peptoid submonomer can refer to the primary amine-R group conjugate (NH_2-R), which is reacted with a

carbonyl group of the preceding backbone subunit to form a new peptide backbone subunit. In some instances, the peptoid submonomer can be referred to as a synthon.

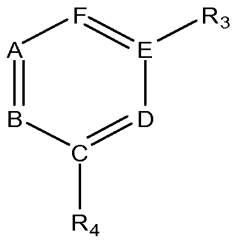
[0043] In some instances the N subunits is at least 1, 2, 3, 4, 5 or more. In some instances the N subunits is 2.

[0044] Conjugated to the N peptoid subunits is an aromatic or non-aromatic ring structure where position A is always a carbon, but positions B-F can be either carbon or nitrogen. The aromatic ring can be comprised of:



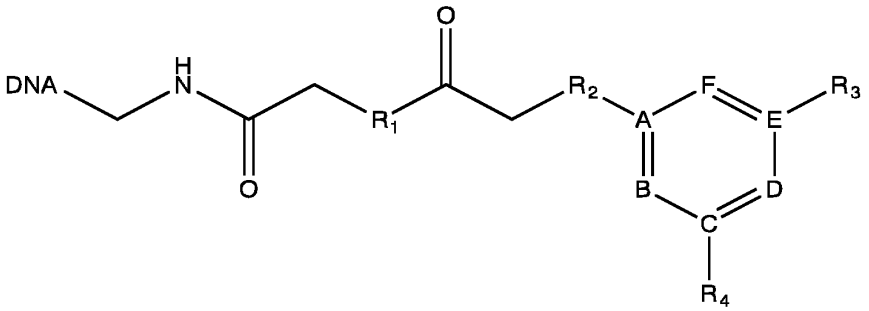
Formula II.

[0045] In some instances the aromatic ring can be a 1, 3, 5- triazine ring with B, D, and F positions being nitrogens. In some instances the triazine ring is di-substituted at the C and E positions.



Formula III.

[0046] In some instances the switchable affinity reagent has a backbone structure comprised of:



Formula IV.

[0047] In some embodiments the switchable reagents comprises the backbone structure with R groups as described in Table 5. The R1-R4 groups in Table 5 correspond to the R1-R4 groups in Formulas I, III, or IV where one or more of the N-H bond(s) from the compounds in the table are converted to C-N bonds in the Formula. Accordingly, in various embodiments, the switchable reagents are the compounds described in Formulas I, III, or IV with the identity of the R groups as described in Table 5.

[0048] The peptoid switchable reagent can be used to purify target proteins from a mixture. In some instances the mixture is derived from a cellular lysate. In some instances the mixture of proteins is 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 particular embodiments, the one or more target proteins are derived from plant sources. Any suitable plant can be used as a source of one or more target proteins for purification using one or more switchable affinity reagents. Non-limiting examples of plants that can be a source of the target proteins include abaca (Manila hemp); alfalfa ; almond; anise seeds; apple; apricot; areca (betel nut); arracha; arrowroot; artichoke; asparagus; avocado; bajra (pearl millet); bambara groundnut; banana; barley; beans; beet, red; beet, sugar; bergamot; betel nut; black pepper; black wattle; blackberries of various species; blueberry; brazil nut; breadfruit; broad bean; broccoli; broom millet; broom sorghum; brussels sprouts; buckwheat; cabbage (red, white, savoy); cabbage, chinese; cacao (cocoa); cantaloupe; caraway seeds; cardamom; cardoon; carob; carrot; cashew nuts; cassava (manioc); castor bean; cauliflower; celeriac; celery; chayote; cherry (all varieties); chestnut; chickpea (gram pea); chicory; chili; cinnamon; citron; citronella; clementine; clove; clover (all varieties); cocoa (cacao); coconut; cocoyam; coffee; cola nut (all varieties); colza (rapeseed); corn (maize); corn (sweet); cotton (all varieties); cottonseed (all varieties); cowpea; cranberry; cress; cucumber; currants (all varieties); custard apple; dasheen; dates; drumstick tree; durra (sorghum); durum wheat; earth pea; edo (eddoe); eggplant; endive; fennel; fenugreek; fig; filbert (hazelnut); fique; flax ; flax seed oil (linseed); formio (New Zealand flax); garlic; geranium; ginger; gooseberry (all varieties); gourd; gram pea (chickpea); grape; grapefruit; grass esparto; grass, orchard; grass, sudan; groundnut (peanut); guava; guinea corn (sorghum); hazelnut (filbert); hemp ; hempseed; henequen; henna; hop; horse bean; horseradish; hybrid maize; indigo; jasmine; jerusalem artichoke; jowar (sorghum); jute; kale; kapok; kenaf; kohlrabi; lavender; leek; lemon; lemon grass; lentil; lespedeza (all varieties); lettuce; lime, sour; lime, sweet; linseed ; liquorice; litchi; loquat; lupine (all varieties); macadamia (Queensland nut);

mace; maguey; maize ; mandarin; mangel (fodder beet); mango; manioc (cassava); maslin (mixed cereals); medlar; melon ; millet broom; millet, bajra; millet, bulrush; millet, finger; millet, foxtail; millet, japanese; millet, pearl (bajra, bulrush); millet, proso; mint (all varieties); mulberry (all varieties); mushrooms; mustard; nectarine; New Zealand flax (formio); niger seed; nutmeg; oats; oil palm; okra; olive; onion, green; opium; orange; ornamental plants; palm palmyra; palm, kernel oil; palm, oil; palm, sago; papaya (pawpaw); parsnip; pea; peach; peanut (groundnut); pear; pecan nut; pepper, black; persimmon; pigeon pea; pineapple; pistachio nut; plantain; plum; pomegranate; pomelo; poppy seed; potato; potato, sweet; prune; pumpkin; pyrethum; quebracho; queensland nut; quince; quinine; quinoa; radish; ramie; rapeseed (colza); raspberry (all varieties); red beet; redtop; rhea; rhubarb; rice; rose; rubber; rutabaga (swede); rye; ryegrass seed; safflower; sainfoin; salsify; sapodilla; satsuma (mandarin/tangerine); scorzonera — black salsify; sesame; shea butter (nut); sisal; sorghum; sorghum, broom; sorghum, durra; sorghum, guinea corn; sorghum, jowar; sorghum, sweet; soybean; soybean hay; spelt wheat; spinach; squash; strawberry; sugar beet; sugarcane; sunflower ; sunhemp; swede; sweet corn; sweet lime; sweet pepper; sweet potato; sweet sorghum; tangerine; tannia; tapioca (cassava); taro; tea; teff; timothy; tobacco; tomato; trefoil; triticale for fodder; tung tree; tree (all types) turnip; arena (Congo jute); vanilla; vetch for grain; walnut; watermelon; wheat; yam; yerba mate; 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. One of skill in the art will understand that any organism in the plant kingdom may be used in the present invention.

[0049] In some instances the peptoid switchable reagent is used to purify Rubisco. Rubisco (Ribulose-1,5-bisphosphate carboxylase oxygenase) is an enzyme involved in the process of carbon fixation and is one of the most abundant proteins in plants. 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.

[0050] The peptoid pH switchable reagents can bind Rubisco in base and release Rubisco in acid. In some embodiments the switchable reagents comprises the backbone structure with R groups as described in Table 1. The R1-R4 groups in Table 1 correspond to the R1-R4 groups in Formulas I, III, or IV where one or more of the N-H bond(s) from the compounds in the table are converted to C-N bonds in the Formula. Accordingly in various embodiments the switchable reagents are the compounds described in Formulas I, III, or IV with the identity of the R groups as described in Table 1.

Table 1. Peptoid pH switchable reagents that bind Rubisco in base and release Rubisco in acid comprise the backbone structure with R groups comprising:

	R1	R2	R3	R4
pH switchable reagent 1	4-IODOBENZYLAMINE	4-nitro-benzylamine	ISOAMYLAMINE	BENZYLAMINE
pH switchable reagent 2	4-PYRROLIDINOBUTYLAMINE	3-ISOPROPOXYPROPYLAMINE	2-amino-N-cyclopropyl-Acetamide	cyclopropylamine
pH switchable reagent 3	1-(3-AMINOPROPYL) PYRROLIDINE	N1-(2-aminoethyl)-N1-methylethane-1,2-diamine	DL-HOMOCYSTEINE THIOLACTONE	N-(3'-AMINOPROPYL)-2-PYRROLIDINONE
pH switchable reagent 4	2-(2-AMINOETHOXY)ETHANOL	3-CHLOROANILINE	9-AMINOFLUORENE	2-amino-3-hydroxy-Butanoic acid
pH switchable reagent 5	3-(AMINOMETHYL)PYRIDINE	(1R,2R)-(-)-2-AMINO-1-PHENYL-1,3-PROPANEDIOL	3,5-dimethyl-Phenylalanine	3-(METHYLTHIO) PROPYLAMINE
pH switchable reagent 6	4-(2-AMINOETHYL)-1-BENZYLPIPERIDINE	2-(aminomethyl)pyridine	HOMOTAURINE	HOMOTAURINE
pH switchable reagent 7	4-(2-AMINOETHYL) MORPHOLINE	D-PHENYLALANINOL	BUTYLAMINE	2-METHYLBUTYLAMINE

[0051] The peptoid pH switchable reagents can bind Rubisco in acid and release Rubisco in base. In some embodiments the switchable reagents comprises the backbone structure with R groups as described in Table 2. The R1-R4 groups in Table 2 correspond to the R1-R4 groups in Formulas I, III, or IV where one or more of the N-H bond(s) from the compounds in the table are converted to C-N bonds in the Formula. Accordingly, in various embodiments, the switchable reagents are the compounds described in Formulas I, III, or IV with the identity of the R groups as described in Table 2.

Table 2. Peptoid pH switchable reagents that bind Rubisco in acid and release Rubisco in base comprise the backbone structure with R groups comprising:

pH switchable reagent 1	N-(3-AMINOPROPYL)MORPHOLINE	(R)-(+)-1-PHENYLETHYLAMINE	2-ETHOXYETHYLAMINE	2-METHYLBUTYLAMINE
pH switchable reagent 2	N-(3-AMINOPROPYL)MORPHOLINE	(1R,2R)-(-)-2-BENZYLOXYCYCLOPENTYLAMINE	2-AMINO-1-METHOXYBUTANE	2-METHYLBUTYLAMINE
pH switchable reagent 3	(R)-1-(3-METHOXYPHENYL)ETHYLAMINE	TRANS-4-AMINOCYCLOHEXANOL	BUTYLAMINE	2-METHYLBUTYLAMINE
pH switchable reagent 4	piperazine	2-(dimethylamino)-4-Pyridinemethanamine	2-(2-CHLOROPHENYL)ETHYLAMINE	2-AMINOETHYL ISOPROPYL ETHER
pH switchable reagent 5	3-AMINO-1-PROPANOL	2-(dimethylamino)-4-Pyridinemethanamine	3,4,5-TRIMETHOXYBENZYLAMINE	2-METHYLBUTYLAMINE

[0052] In some instances the peptoid switchable reagent is used to purify Leghemoglobin. Leghemoglobin is a nitrogen or oxygen carrier and is produced in response to plant roots being infected with nitrogen-fixing bacteria. Leghemoglobin,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.

[0053] 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).

[0054] The peptoid pH switchable reagents can bind leghemoglobin in base and release leghemoglobin in acid. In some embodiments the switchable reagents comprises the backbone structure with R groups as described in Table 3. The R1-R4 groups in Table 3 correspond to the R1-R4 groups in Formulas I, III, or IV where one or more of the N-H bond(s) from the compounds in the table are converted to C-N bonds in the Formula. Accordingly in various embodiments the switchable reagents are the compounds described in Formulas I, III, or IV with the identity of the R groups as described in Table 3.

Table 3. Peptoid pH switchable reagents that bind Leghemoglobin in base and release Leghemoglobin in acid comprise the backbone structure with R groups comprising:

pH switchable reagent 1	N,N-bis(2-aminoethyl)ethane-1,2-diamine	2-(4-CHLOROPHENYL)ETHYLAMINE	N-(3-AMINOPROPYL)MORPHOLINE	2-(2-AMINOETHOXY)ETHANOL
pH switchable reagent 2	3,4-DICHLOROBENZYLAMINE	1-(3-AMINOPROPYL)PYRROLIDINE	1-phenylbutan-3-amine	2-CHLOROBENZYLAMINE
pH switchable reagent 3	3-FLUORO-5-(TRIFLUOROMETHYL)BENZYLAMINE	2,4,6-TRIMETHYLANILINE	3-FLUORO-5-(TRIFLUOROMETHYL)BENZYLAMINE	indan-5-ylamine
pH switchable reagent 4	2-METHOXYETHYLAMINE	5-METHOXYTRYPTAMINE	N1-(2-aminoethyl)-N1-methylethane-1,2-diamine	N,N,2,2-TETRAMETHYL-1,3-PROPANEDIAMINE

[0055] The peptoid pH switchable reagents can bind leghemoglobin in acid and release leghemoglobin in base. In some embodiments the switchable reagents comprises the backbone structure with R groups as described in Table 4. The R1-R4 groups in Table 4 correspond to the R1-R4 groups in Formulas I, III, or IV where one or more of the N-H bond(s) from the compounds in the table are converted to C-N bonds in the Formula. Accordingly in various embodiments the switchable reagents are the compounds described in Formulas I, III, or IV with the identity of the R groups as described in Table 4.

Table 4. Peptoid pH switchable reagents that bind Leghemoglobin in acid and release Leghemoglobin in base comprise the backbone structure with R groups comprising:

pH switchable reagent 1	4-PYRROLIDINOBUTYLAMINE	2-(1H-indol-3-yl)ethanamine (tryptamine)	2-CHLOROBENZYLAMINE	2-AMINOETHYL ISOPROPYL ETHER
pH switchable reagent 2	N1-(2-aminoethyl)-N1-methylethane-1,2-diamine	2-(4-CHLOROPHENYL)ETHYLAMINE	8-Quinolinemethanamine	2-AMINOETHYL ISOPROPYL ETHER
pH switchable reagent 3	3-FLUOROPHENETHYLAMINE	TRANS-4-AMINOCYCLOHEXANOL	2-(2-AMINOETHYL)-1-METHYLPYRROLIDINE	3-CHLORO-2,6-DIFLUOROBENZYLAMINE
pH switchable reagent 4	N,N,2,2-TETRAMETHYL-1,3-PROPANEDIAMINE	D-PHENYLALANINOL	4-PYRROLIDINOBUTYLAMINE	5-AMINOMETHYL-2-CHLOROPYRIDINE
pH switchable reagent 5	N,N-DIMETHYL-1,3-PROPANEDIAMINE	5-AMINOMETHYL-2-CHLOROPYRIDINE	2-(4-nitro-phenyl)-ethylamine hydrochloride salt	HOMOTAURINE

DNA-programmed Combinatorial Chemistry (DPCC)

[0056] A library of switchable affinity reagents can be produced using a process termed DNA-programmed combinatorial chemistry; for example, as disclosed in Wrenn, *et al.* J Am Chem Soc. 2007 October 31; 129(43): 13137-13143 or U.S. Patent No. 7479472, each of which is hereby incorporated by reference in its entirety. DNA-programmed combinatorial chemistry can enable selection of an affinity reagent that has high and selective affinity for native features of a target protein from a combinatorial library of small molecules.

[0057] DNA-programmed combinatorial chemistry (DPCC) can be used to synthesize diverse combinatorial libraries of affinity reagents, wherein the affinity reagents comprise a small molecule compound attached to an encoding polynucleotide sequence that encodes a program for the synthesis of the small molecule compound from an array of precursors. The term "combinatorial library" can refer to a library of molecules containing a large number (*e.g.*, between about 10^3 and 10^{12}) of different compounds. The compounds can be characterized by different sequences of subunits or precursors, or a combination of different sequences of side chains and linkages. The term "small molecule" can refer to small organic molecules that can have a common parent structure (*e.g.*, a ring structure, a triazine, a peptoid, a peptide, *etc.*) and a plurality of different R group substituents or ring-structure modifications, each of which takes a variety of forms, *e.g.*, different R groups. In one embodiment, small molecule compounds are non-oligomeric (that is, do not consist of sequences of repeating similar subunits). In one example, small molecule compounds can be similar in terms of basic structure and functional groups, but vary in such aspects as chain length, ring size or number, or patterns of substitution. In one embodiment, the precursors are amino acids. In another embodiment, the precursors are peptoids. In another embodiment, the precursors are L-peptides. In another embodiment, the precursors are D-peptides.

Strategy for Synthesis of Combinatorial Libraries

[0058] Encoded combinatorial chemical libraries can comprise a plurality of species of candidate affinity reagents. Affinity reagents can be bifunctional molecules comprising a small molecule compound and an encoding polynucleotide sequence, wherein the encoding polynucleotide sequence defines, and directs the synthesis of the corresponding small molecule compound .

Encoding Polynucleotide Sequence

[0059] In one embodiment, an affinity reagent comprises a small molecule compound and an encoding polynucleotide sequence that encodes a program for the synthesis of the small molecule compound. The encoding polynucleotide sequence can comprise one or more homology sequences. Each homology sequence can specify the addition of a specific precursor molecule. Homology sequences can be characterized by the following properties: (i) homology sequences can bind to their corresponding complementary nucleotide sequences (hereafter "complementary sequences") at a specified melting temperature (e.g. about 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C, 75°C, 80°C, 85°C, 90°C, 95°C, 100°C, or any included integer or fraction) under specified solution conditions and (ii) the

homology sequences do not cross-hybridize efficiently with another of the 1536 sequences, or with the complement to any of the other 1536 sequences, at the specified melting temperature and specified solution conditions.

[0060] The number of homology sequences in an encoding polynucleotide sequence can be selected based upon a desired length of the small molecule compound. The number of homology sequences in the encoding polynucleotide sequence can be between about 1 and about 50; for example, about 1-50, 1-40, 1-30, 1-25, 1-20, 1-15, 1-10, 1-5, 5-50, 5-40, 5-30, 5-25, 5-20, 5-15, 5-10, 10-50, 10-40, 10-30, 10-25, 10-20, 10-15, 15-50, 15-40, 15-30, 15-25, 15-20, 20-50, 20-40, 20-30, 20-25, 25-50, 25-40, 25-30, 30-50, 30-40, 40-50, 3-6, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 homology sequences. In one example, the encoding polynucleotide sequence comprises about 5 homology sequences. In another example, the encoding polynucleotide comprises about 10 homology sequences. In another example, the encoding polynucleotide comprises about 15 homology sequences. The encoding polynucleotide sequence can comprise between about 3 and about 6 homology sequences; for example, about 3, 4, 5, or 6 homology sequences.

[0061] In one embodiment, a homology sequence can comprise between about 1 and about 50 nucleotides; for example about 1-50, 1-40, 1-30, 1-25, 1-20, 1-15, 1-10, 1-5, 5-50, 5-40, 5-30, 5-25, 5-20, 5-15, 5-10, 10-50, 10-40, 10-30, 10-25, 10-20, 10-15, 15-50, 15-40, 15-30, 15-25, 15-20, 20-50, 20-40, 20-30, 20-25, 25-50, 25-40, 25-30, 30-50, 30-40, 40-50, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides. In one embodiment, the homology sequences comprise about 10 nucleotides. In another embodiment, the homology sequences comprise about 20 nucleotides. In one embodiment, each homology sequence in the encoding polynucleotide sequence comprises a unique sequence. In another embodiment, two or more homology sequences in the encoding peptide can comprise the same sequence.

[0062] An encoding polynucleotide sequence can further comprise one or more spacer sequences. The number of spacer sequences can vary according to the number of homology sequences. In one embodiment, each homology sequence is separated by a spacer sequence. The spacer sequences can comprise between 1 and 50 nucleic acids; for example, about 1-50, 1-40, 1-30, 1-25, 1-20, 1-15, 1-10, 1-5, 5-50, 5-40, 5-30, 5-25, 5-20, 5-15, 5-10, 10-50, 10-40, 10-30, 10-25, 10-20, 10-15, 15-50, 15-40, 15-30, 15-25, 15-20, 20-50, 20-40, 20-30, 20-25, 25-50, 25-40, 25-30, 30-50, 30-40, 40-50, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45,

46, 47, 48, 49, or 50 nucleotides. In one embodiment, the spacer sequences comprise about 5 nucleic acids. In another embodiment, the spacer sequences comprise about 10 nucleic acids. In another embodiment, the spacer sequences comprise about 15 nucleic acids. In one embodiment, each spacer sequence comprises an identical nucleotide sequence. In another embodiment, two or more unique spacer sequences are used.

[0063] An encoding polynucleotide sequence can further comprise a chemical reaction site. In one embodiment, the chemical reaction site is located at the end, e.g. the 5' end, of the encoding polynucleotide sequence. In another embodiment, the chemical reaction site is located at the end, e.g. the 3' end, of the polynucleotide sequence. In one embodiment, the 5' alcohol of the 5' base of the nucleic acid tag is modified with a commercially available reagent which introduces a phosphate group tethered to a linear spacer, *e.g.*, a 12-carbon and terminated with a primary amine group (e.g., Glen Research catalog #10-1912-xx or numerous other reagents which are available for introducing thiols or other chemical reaction sites into synthetic DNA). In this embodiment, the primary amine represents the chemical reaction site on which a small molecule compound is synthesized. Many different types of chemical reaction sites (in addition to primary amines) can be introduced at the 5' terminus of the encoding polynucleotide sequence. Exemplary chemical reaction sites include, but are not limited to, chemical components capable of forming amide, ester, urea, urethane, carbon-carbonyl bonds, carbon-nitrogen bonds, carbon-carbon single bonds, olefin bonds, thioether bonds, and disulfide bonds. In the case of enzymatic synthesis, co-factors may be supplied as are required for effective catalysis; for example, the phosphopantetheinyl group useful for polyketide synthesis.

Synthesis of Small Molecule Compounds

[0064] A split-and-recombine strategy, comprising two or more synthetic steps, can be utilized for synthesis of combinatorial libraries. A traditional split-and-recombine strategy for synthesis of combinatorial libraries has been described. Chen, et al., Methods in Enzymology 267:211-9 (1996); Ellman and Gallop, Curr Opin Chem Biol 2:317-319 (1998). For example, in a combinatorial synthesis consisting of i steps, for which j different chemical coupling reactions are performed at each step, j^i compounds will be present in the final library. The traditional split and-recombine strategy is carried out using the following steps; (i) at the beginning of each of the i steps, the pool of solid tags is randomly split into j subsets, (ii) each of the j subsets of solid tags is subjected to a different chemical coupling step, and (iii) after the chemical coupling step, the subsets are recombined into a single pool. This recombined pool is again randomly divided into j subsets (specifically as in (i) above) at the beginning of the next step in the library

synthesis. In the synthesis of peptide libraries, for example, the coupling step is the addition of an amino-acid active ester to a free amine group on the solid tag. Each of the j subsets is coupled to a different amino acid (e.g. alanine coupled to subset #1, arginine to subset #2, cysteine to subset #3 etc.). For example, a split-and-recombine synthesis of 10 synthetic steps, with 10 coupling reactions at each step, would yield a final library size of 10^{10}.

[0065] A compound library can be split into subsets at each step of the split-and-recombine combinatorial synthesis by differential hybridization of an encoding polynucleotide sequence comprising one or more homology sequences to complementary sequences bound to a solid support (*e.g.*, polystyrene beads). The complementary sequences to each homology sequence of the encoding polynucleotide sequence can be synthesized. In one embodiment, a 5' alcohol of the 5' base of the each complementary sequence is modified with a commercially available reagent that introduces a phosphate group tethered to a linear spacer (*e.g.*, a linear spacer having six carbons and terminated with a thiol group (Glen Research catalog #10-1926-xx)). Each of the thiol-bearing complementary sequences can be immobilized, for example, through a thioether linkage to a macroporous resin (*e.g.*, polystyrene, MPS; Biopore catalog #NH-2CM, L-970317) bearing electrophilic bromoacetamide groups. Thus a number of affinity resins result, each bearing a unique complementary sequence. Each of the affinity resins can be loaded into its own column. The columns can comprise luer-lock fittings at either end. The columns can be connected in a linear sequence.

[0066] A first split can be performed by contacting a library of encoding polynucleotide sequences with the previously described affinity resins. In one embodiment, the contacting can comprise pumping a high-salt aqueous solution containing the entire library of encoding polynucleotide sequences cyclically over a linear sequence of affinity columns under high stringency conditions see, *e.g.*, Southern, E M et al., Nucl Acids Res. 22(8) 1368-1373 (1994), using a peristaltic pump and for a time sufficient for all of the encoding polynucleotide sequences to hybridize to the complementary sequences bound to the columns. The split can be completed by breaking the luer-lock linkages between the affinity columns. At this point the different encoding polynucleotide sequences have been divided into physically separate subsets on the basis of homology sequences contained with the encoding polynucleotide sequences.

[0067] Each subset of encoding polynucleotide sequences formed by hybridization as described *supra* can then be subjected to a different synthetic coupling reaction. The methods employed in the synthetic coupling reaction can vary according to the desired small molecule compound. For example, an amino terminus blocking group, *e.g.*, fluorenylmethoxycarbonyl group (FMOC),

which can be added and removed, can be used in the synthesis of a small molecule compound comprising peptide subunits.

[0068] An exemplary procedure to synthesize small molecule compounds comprising peptide or amino acid subunits or precursors is as follows: The encoding polynucleotide sequences bound to the affinity columns can be eluted off of the affinity columns with 10mM NaOH and 0.005% Triton X-100. The polynucleotide sequences are transferred onto chemistry columns, *e.g.*, hydroxyapatite resin columns (Bio-Rad Macro-Prep Ceramic Hydroxyapatite TYPE II catalog #1588200) with binding in 300 mM $CaC1_2$ or DEAE Sepharose fastflow (Pharmacia 17-0709-01) with binding in 10 mM acetate at pH 5.0 with 0.005% triton. The encoding polynucleotide sequences can remain non-covalently bound to the hydroxyapatite or sepharose resin in numerous organic solvents (*e.g.*, DMF, acetonitrile, ethanol, and mixtures of those solvents with water). Thus organic reagents can be flowed over the columns and reacted with chemical reaction sites on the encoding nucleotide sequences in the same manner that conventional solid phase chemical synthesis is carried out. Accordingly, a different Fmoc-protected amino-acid preactivated with N[(1H-benzotriazol-1-y1) (dimethylamino) methylene]-N-methylmethanaminium tetrafluoroborate (TBTU) or as an N-hydroxy succinimide ester in DMF can be flowed over each hydroxyapatite or sepharose column, resulting in the acylation of the primary amines of the chemical reaction site on each of the hydroxyapatite or sepharose columns with an Fmoc-protected amino acid [Albericio, F. and Carpino L A, Methods in Enymology 289:104-26 (1997)]. Following acylation, the Fmoc group can removed from the newly added amino acid by flowing a piperidine/DMF solution over the hydroxyapatite or sepharose columns, thus presenting a new primary amine ready for the next coupling step.

[0069] An exemplary procedure to synthesize small molecule compounds comprising peptoid subunits or precursors is as follows: The encoding polynucleotide sequences bound to the affinity columns are transferred onto chemistry columns (*e.g.*, DEAE-sepharose columns). Chemistry columns can be washed with DEAE bind buffer (10 mM acetic acid, 0.005% Triton-X100), water, methanol, or a combination thereof. The chemistry columns can then be incubated one or more times (*e.g.*, 2 times) with 150 mM DMT-MM (4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4- methylmorpholinium chloride) and 100 mM sodium chloroacetate in distilled methanol for a period of time of about 20 minutes. The chemistry columns can then be washed with methanol, and incubated with a solution containing a desired peptoid subunit. The column can be microwaved for about 20 seconds one or more times during the peptoid incubation; for example, 1, 2, 3, 4, 5, 6, or more times. The chemistry column can then be washed, for example, with DMSO (dimethylsulfoxide), DEAE bind buffer, or a combination thereof.

[0070] An entire affinity reagent library can be synthesized by carrying out alternate rounds of library splitting and chemical and/or biochemical coupling to each subset of encoding nucleotide sequences.

Modifications to generate Switchable Affinity Reagents

[0071] The described methods of generating affinity reagent libraries can be modified in order to generate switchable affinity reagents, wherein the switchable affinity reagents comprise a small molecule compound and an encoding polynucleotide sequence. The small molecule compound can be synthesized from subunit or precursors, as described supra. In one embodiment, the precursors can be chosen to bias the synthesis toward a family of molecules with a pKa about 7, so that they can be neutrally charged at about pH 9 and positively charged at about pH 5. The affinity reagents can be incubated with a crude mixture of non-specific proteins (*e.g.*, cell lysates from *Escherichia coli*), which can eliminate molecules with non-specific affinity for proteins. The remaining library can then be incubated at a binding pH (*e.g.*, about pH 9) with the target protein, attached to a solid support. Affinity reagents that fail to bind to the target protein can be washed away. Then the buffer pH can be adjusted to a release pH (*e.g.*, about pH 5) and the bound affinity reagents that elute can be collected. It should be understood that the stated pH values are provided merely as examples and that a variety of binding and release pH combinations are contemplated.

[0072] This selection can be reiterated to obtain progressively greater enrichment of the affinity reagents that perform best in the selection (*e.g.*, binding at about pH 9 and eluting at about pH 5). The selected pool of affinity reagents can be identified by sequencing the attached DNA molecules, resynthesized, and further characterized for their specificity to the target protein and performance under actual purification conditions. Any number of reiterations can be employed to select the affinity reagents; for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more reiterations can be performed.

[0073] Note that the specific properties of the affinity reagents, including the manner in which affinity for the targeted protein is controlled (either by pH or other changes in conditions) may vary from the above ¬the methods for discovering them and for using them to purify a target protein can be indifferent to the specifics of the affinity switch.

Target Proteins, Compounds, or Molecules

[0074] A target protein, compound, or molecule can be any protein, compound, or molecule that has industrial value, clinical effect, or environmental effect. Target proteins, compounds, or molecules can be used as food ingredients. Target proteins can be enzymes (*e.g.*, industrial enzymes). Target proteins, compounds, or molecules can be used as therapeutic agents; for

example, target proteins can include antibodies or other proteins that can be used to treat a disease or disorder such as cancer. Target proteins, compounds, or molecules can include analytes for a diagnostic test or other clinical use.

[0075] A target protein, compound, or molecule can also be a protein, compound or molecule whose presence in a mixture is undesirable. For example, a target protein, compound or molecule can be a toxin or an allergen; for example the target protein could be hordein in a fermented barley beverage whose removal would render the beer "gluten free". A target protein, compound, or molecule can contribute to an undesirable taste, odor, or visual appearance in a product such as a food product. A target protein, compound, or molecule can be target protein, compound, or molecule (*e.g.*, enzyme) whose activity decreases the stability of a desired material; for example, the target protein could be an RNAse in a cell extract wherein RNA is the desired material. A switchable affinity reagent can be used to remove undesirable target proteins, compounds or molecules from a mixture comprising a desired material.

[0076] The target proteins or molecules can be used to produce a consumable; for example, a meat substitute food product. The consumable can be for human consumption. The consumable can be approved by suitable regulatory authorities. The consumables can be sold in grocery stores or prepared in restaurants, similar to already existing human foods. The consumable can be for animal consumption. The consumable can be food for domestic animals; for example, farm animals (*e.g.*, cows, sheep, pigs, goats, horses, chickens, turkeys, *etc.*) or pets (*e.g.*, cats, dogs, ferrets, rabbits, hamsters, guinea pigs, mice, fish, birds, *etc.*). The consumable can be food for wild animals (*e.g.*, foxes, wolves, bears, great cats, primates, rodents, crocodiles, alligators, iguanas, lizards, snakes, tortoises, turtles, frogs, fish, *etc.*).

[0077] Target proteins can comprise animal proteins. Non-limiting examples of animal sources of proteins include humans, monkeys, farm animals, mammals, birds, reptiles and insects.

[0078] Target proteins can comprise proteins 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.

[0079] Target proteins can comprise plant proteins. Target proteins can be purified from crude solutions derived from plant material. Non-limiting examples of plant proteins include RuBisCO (ribulose-1,5-bisphosphate carboxylase oxygenase), leghemoglobin, non-symbiotic hemoglobin, ribosomes, cytoplasmic actin, and seed storage proteins. In one example, the target proteins comprise RuBisCO (alternatively "Rubisco" or "rubisco"). In another example, the target proteins comprise a leghemoglobin. In another example, the target proteins comprise a non-

symbiotic hemoglobin. In one example, the target proteins comprise a ribosomal protein. In another example, the target proteins comprise a cytoplasmic actin. In another example, the target protein comprise seed storage proteins; for example, an albumin, a conalbumin, a glutenin, a gliadin, a glutelin, a gluten, a hordein, a prolamine, a phaseolin, a secalin, a triticeae gluten, or a zein. In another example, the target proteins are selected from the group consisting of leghemoglobin, non-symbiotic hemoglobin, hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, 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, pea globulins, pea albumins, or combinations therof

[0080] In another example the target proteins are selected from the group consisting of leghemoglobin, non-symbiotic hemoglobin, hemoglobin, myoglobin, chlorocruorin, erythrocruorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, 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, pea globulins, pea albumins, or combinations therof

[0081] Any mixture of proteins can be used as a source of one or more target proteins for purification using one or more switchable affinity reagents. In some instances the mixture is derived from a cellular lysate. In some instances the mixture of proteins is 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 particular embodiments, the one or more target proteins are derived from plant sources.. Any suitable plant can be used as a source of one or more target proteins for purification using one or more switchable affinity reagents. Non-limiting examples of plants that can be a source of the target proteins include abaca (Manila hemp); alfalfa ; almond; anise seeds; apple; apricot; areca (betel nut); arracha; arrowroot; artichoke; arundo donax: asparagus; avocado; bajra (pearl millet); bambara groundnut; banana; barley; beans; beet, red; beet, sugar; bergamot; betel nut; black pepper; black wattle; blackberries of various species; blueberry; brazil nut; breadfruit; broad bean; broccoli; broom millet; broom sorghum; brussels sprouts; buckwheat; cabbage (red, white, savoy); cabbage, chinese; cacao (cocoa); cantaloupe; caraway seeds; cardamom; cardoon; carob; carrot; cashew nuts; cassava (manioc); castor bean; cauliflower; celeriac; celery; chayote; cherry (all varieties); chestnut; chickpea (gram pea); chicory; chili; cinnamon; citron; citronella; clementine; clove; clover (all varieties); cocoa (cacao); coconut; cocoyam; coffee; cola nut (all varieties); colza (rapeseed); corn (maize); corn (sweet); cotton (all varieties); cottonseed (all varieties); cowpea; cranberry; cress; cucumber; currants (all varieties); custard apple; dasheen; dates; drumstick tree; durra (sorghum); durum wheat; earth pea; edo (eddoe); eggplant; endive; fennel; fenugreek; fig; filbert (hazelnut); fique; flax ; flax seed oil (linseed); formio (New Zealand flax); garlic; geranium; ginger; gooseberry (all varieties); gourd; gram pea (chickpea); grape; grapefruit; grass esparto; grass, orchard; grass, sudan; groundnut (peanut); guava; guinea corn (sorghum); hazelnut (filbert); hemp ; hempseed; henequen; henna; hop; horse bean; horseradish; hybrid maize; indigo; jasmine; jerusalem artichoke; jowar (sorghum); jute; kale; kapok; kenaf; kohlrabi; lavender; leek; lemon; lemon grass; lentil; lespedeza (all varieties); lettuce; lime, sour; lime, sweet; linseed ; liquorice; litchi; loquat; lupine (all varieties); macadamia (Queensland nut); mace; maguey; maize ; mandarin; mangel (fodder beet); mango; manioc (cassava); maslin (mixed cereals); medlar; melon ; millet broom; millet, bajra; millet, bulrush; millet, finger; millet, foxtail; millet, japanese; millet, pearl

(bajra, bulrush); millet, proso; mint (all varieties); miscanthus; mulberry (all varieties); mushrooms; mustard; nectarine; New Zealand flax (formio); niger seed; nutmeg; oats; oil palm; okra; olive; onion, green; opium; orange; ornamental plants; palm palmyra; palm, kernel oil; palm, oil; palm, sago; papaya (pawpaw); parsnip; pea; peach; peanut (groundnut); pear; pecan nut; pepper, black; persimmon; pigeon pea; pineapple; pistachio nut; plantain; plum; pomegranate; pomelo; poppy seed; potato; potato, sweet; prune; pumpkin; pyrethum; quebracho; queensland nut; quince; quinine; quinoa; radish; ramie; rapeseed (colza); raspberry (all varieties); red beet; redtop; rhea; rhubarb; rice; rose; rubber; rutabaga (swede); rye; ryegrass seed; safflower; sainfoin; salsify; sapodilla; satsuma (mandarin/tangerine); scorzonera — black salsify; sesame; shea butter (nut); sisal; sorghum; sorghum, broom; sorghum, durra; sorghum, guinea corn; sorghum, jowar; sorghum, sweet; soybean; soybean hay; spelt wheat; spinach; squash; strawberry; sugar beet; sugarcane; sunflower ; sunhemp; swede; sweet corn; sweet lime; sweet pepper; sweet potato; sweet sorghum; tangerine; tannia; tapioca (cassava); taro; tea; teff; timothy; tobacco; tomato; trefoil; triticale for fodder; tung tree; tree (all types) turnip; arena (Congo jute); vanilla; vetch for grain; walnut; watermelon; wheat; yam; yerba mate; 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. One of skill in the art will understand that any organism in the plant kingdom may be used in the present invention.

Methods for Purifying Target Proteins, Compounds, or Molecules

[0082] The present disclosure provides numerous methods for the purification of one or more target proteins, compounds or molecules using one or more switchable affinity reagents. The methods can comprise the step of (a) applying a composition comprising one or more target proteins, compounds, or molecules to a substrate comprising one or more switchable affinity reagents at a binding pH such that the target proteins, compounds, or molecules bind to the

switchable affinity reagents; and (b) applying a solution at a release pH to the substrate comprising the one or more switchable affinity reagents such that the one or more target proteins, compounds, or molecules are released from the one or more switchable affinity reagents. The methods can further comprise a washing step wherein a wash solution is applied to the substrate comprising the one or more switchable affinity reagents bound to the one or more target proteins, wherein the wash solution is at or near the binding pH. The wash step can be used to remove one or more undesirable components (*e.g.*, undesirable proteins, contaminants, *etc.*). The methods disclosed herein can be used to obtain a pure, concentrated solution of the one or more target proteins.

Immobilization of pH switchable reagent on substrate

[0083] The switchable affinity reagents can be immobilized on a substrate (*e.g.*, on beads, a solid support or a semi-solid support). The switchable affinity reagents can be packed into a column or other suitable container. The pH switchable reagent can be conjugated to a substrate such as a bead. Substrate, e.g. beads, can be made of styrene, polystyrene, iron oxide, agarose, or sepharose. Substrate, e.g. beads, can be magnetic or paramagnetic. Substrate, e.g. beads, can be coated with silica or high density functional groups, such as carboxyl groups, aldehyde groups, or *N*-hydroxysuccinimide –ester (NHS) groups. A collection of conjugated substrate, e.g. beads, in a slurry can form a resin.

[0084] The pH switchable reagent can be conjugated to a substrate, e.g. beads. pH switchable reagents can be conjugated to substrate using chemicals such as N-ethylmaleimide (NEM), *N*-hydroxysuccinimide (NHS) and other imidoesters, other chemical crosslinkers such as 1-ethyl-3-(-3-dimethylaminopropyl) (EDC, EDAC) carbodiimide hydrochloride, and N',N'-dicyclohexyl carbodiimide (DCC), iodoacetate and alpha-halocarbonyl compounds or any chemicals which react with primary amines or thiols.

[0085] Resins can comprise cross-linked beaded agarose, cross-linked bisacrylamide and azlactone, or polyethylene glycol. Resins can be microporous or macroporous. The binding capacity of resins can be measure in terms of the milligram amount of protein bound per milliliter amount of resin. Resins can be packed into columns for large scale affinity chromatography purification of a target protein from a cellular lysate.

[0086] In one example, a column purification can be performed wherein a crude solution comprising the target proteins, compounds, or molecules are run through a column comprising beads or resin comprising one or more switchable affinity reagents. The crude solution can be at a binding pH (*e.g.*, between about 7 and about 11, *e.g.*, about 9). In this example, other components of the crude solution can pass through the column and can be collected and/or

disposed. The crude solution can be run over the column one or more times. Running the crude solution over the column two or more times can increase the amount of the target proteins, compounds, or molecules that are purified from the crude solution. In another example, a batch purification can be performed wherein beads comprising one or more switchable affinity reagents can be added to a crude solution comprising one or more target proteins, compounds, or molecules. The crude solution can be at a binding pH (*e.g.*, between about 7 and about 11, *e.g.*, about 9). Following an incubation period with optional mixing, the beads can be collected and removed from the crude solution, along with any target proteins bound to the switchable affinity reagents. Both column and batch purification methods can comprise one or more wash steps, wherein the wash steps are performed with a solution that is at or near the binding pH (*e.g.*, between about 7 and about 11, *e.g.*, about 9). In both column and batch purifications, the one or more target proteins, compounds, or molecules can be released from the switchable affinity reagents using a solution at a release pH (*e.g.*, between about 3 and about 7, *e.g.*, about 5). Both column and batch purification methods can be used to produce pure concentrated solutions of the target proteins, compounds, or molecules.

[0087] Purification of plant-derived target proteins using a pH switchable reagent is designed to occur on massively large scale. Isolation of high fructose corn syrup, antibiotics or industrial proteins can occur in columns >4m in diameter. These columns are often used for process development and manufacturing. Columns used for purifying plant target proteins using pH switchable reagents can be up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times the size of current largest columns. The amount of target protein recovered can be at least 0.1, 0.5, 1, 5, 10, 20, 50, 100, 200, 500, 1000, 5000, 10000, 50000, 100000 or more grams. The concentration of the recovered target protein can be at least 0.1mg/mL, 0.5 mg/mL, 1 mg/mL, 5 mg/mL, 10 mg/mL, 20 mg/mL, 50 mg/mL, 100 mg/mL, 200 mg/mL, 500 mg/mL.

[0088] Purity of the target sample will be assessed with standard protein purity detection techniques such as SDS page, mass spectrometry, spectroscopy and other molecular biology techniques well known to those skilled in the art.

[0089] In some instances the pH switchable reagent will leach off the column and be carried into the target protein sample which is used in downstream processes to produce a consumable food product, suggesting that the consumable food product may contain minute quantities of the pH switchable reagent. In some instances, the amount of leached pH switchable reagent and/or pH switchable reagent resin will be at most 1, 100, 500, 1000, 2000, 5000 parts per million target protein. In some instances the amount of leached pH switchable reagent and/or pH switchable reagent resin will represent at most 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 0.01, 0.05, 0.1, 0.5, 1, 2,

3, 4, 5, 6, 7, 8, 9, 10 percent weight/volume of the purified target protein composition. In some instances, the amount of leached pH switchable reagent and/or pH switchable reagent resin will represent at most 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 1000 parts per billion (ppb). In some instances the purified target protein will represent more than 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 98.5, 99, 99.5, 99.9, 99.95 percent of the weight/volume of the purified target protein composition. In some instances the purified target protein is increased 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.

[0090] A pure target protein can be substantially free of celluloses and complex polysaccharides. Cellulose is the polysaccharide responsible for the structural integrity of the plant cell wall. Celluloses are only partially digestible by humans and may not be desirable in a consumable product. Celluloses can comprise lignin, hemicellulose, and cellulose. Complex polysaccharides can also be found in the walls of plant cells. Complex polysaccharides may not be desirable in a consumable product. Complex polysaccharides can comprise arabinose, xylose, arabinoxylans, cellulose, lignin, hemicellulose, chitin, pectin, amylose, or amylopectins.

[0091] A pure target protein can be substantially free of colorants and/or odorants, or contain trace amounts of colorants and/or odorants. Colorants may include anions, cations, salts, metals, alkali metals, metalloids, or transition elements. Colorants may be of a type related to chlorophylls such as chlorins, chlorophyll a, chlorophyll b, chlorophyll c1, chlorophyll c2, chlorophyll d, and chlorophyll f. In some instances, the amount colorant and/or odorant will be at most 1, 100, 500, 1000, 2000, 5000, 10000, 100000 parts per million target protein. In some instances the amount of colorant and/or odorant will represent at most 0.05, 0.1, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 percent weight/volume of the purified target protein composition. In some instances the purified target protein will represent more than 90, 91, 92, 93, 94, 95, 96, 97, 98, 98.5, 99, 99.5, 99.9, 99.95 percent of the weight/volume of the purified target protein composition.

Methods for Removing Contaminants or Hazards from a Mixture

[0092] The present disclosure also provides numerous methods for removing one or more undesirable components (*e.g.*, contaminants, toxins, hazards, *etc.*) from a mixture using switchable affinity reagents, wherein the switchable affinity reagents specifically bind to the undesirable components under a first set of conditions (*e.g.*, a binding pH) and do not bind or bind less strongly under a second set of conditions. The switchable affinity reagents can be bound to a solid support or substrate such as a bead or a resin. Beads or resins comprising one or

more switchable affinity reagents can be used in column or batch purifications. In one example, a mixture comprising one or more undesirable components is applied to a column, wherein the column is packed with beads or resin comprising one or more switchable affinity reagents, wherein the switchable affinity specifically bind to the one or more undesirable components under a first set of conditions (*e.g.*, a binding pH) and do not bind, or bind less strongly under a second set of conditions (*e.g.*, a release pH). Prior to running the mixture over the column, it can be adjusted to the first set of conditions (*e.g.* the binding pH) and then the mixture that passes through the column will have all, substantially all, or a portion of the undesired components removed. The mixture can be passed through the column one or more additional times in order to further deplete the mixture of the undesirable components. A second solution under the second set of conditions (*e.g.*, the release pH) can be passed over the column to regenerate the beads or resin by releasing the undesirable components from the switchable affinity reagents. Such method can be useful, for example, for removing toxins from an environment. Such methods can also be useful for removing allergens or other undesirable components from a mixture.

[0093] 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.

EXAMPLES

EXAMPLE 1: PREPARATION OF DNA LIBRARIES

[0094] To make DNA Libraries 3072 40mer oligonucleotides were synthesized using standard phosphoramidite chemistry. The 5' 20 bases of each oligonucleotide consisted of a noncoding region sequence, while the 3' 20 bases consisted of a codon sequence. 1536 of the oligos were "forward" reads, 1536 of the oligos were "anti-sense". The oligonucleotides were divided into 8 subpools from each of 5 sets exclusive to A-type codons (A001–A384 forward, and A001'-A384' antisense), B-type codons (B1–B384 forward, and B001'-B384' antisense), and so forth through E-type codons. Oligos were diluted to 4.5 μM concentration in 1x T4 polynucleotide kinase (PNK) buffer (Fermentas). Oligos were heated to 95 °C 5 min to denature, and then cooled to room temperature over an hour to hybridize. The 8 subpools from each of the 5 sets

were pooled to make 5 pools. The resulting constructs are comprised of 2 oligos that have annealed along the first 20 bases of their 3' ends but are un-annealed at their 5' 20 bases. The constructs are phosphorylated with T4 PNK (Fermentas) with 3.2 μM oligonucleotide, 1 mM ATP, 1x PNK buffer, 0.11 units/μl PNK in 750 μl volume, reacted for 8 hours at 37 °C. The reactions are concentrated to 250ul by n-butanol extraction and ten ethanol precipitated with $MgCl_2$ to 10mM and 1.25ml ethanol. Pellets are resuspended in 200ul TAE and purified by agarose gel electrophoresis. Equimolar amounts from each coding region are combined for ligation. Ligation reactions were conducted on 15ug of purified constructs with 5% PEG4000, 1x T4 ligase buffer, 0.1units of DNA ligase /ul in 500ul at 37C overnight. The reaction was phenol/chloroform extracted, concentrated by n-butanol extraction to 300ul and precipitated by isopropanol. Pellets were dissolved in 30ul H2O and 30ul formamide loading dye were added. The ligation reactions of assembled library were purified on a 5% TBE formamide (40%) – urea (7M) denaturing gel. After gel purification, the library was PCR amplified.

EXAMPLE 2: CONSTRUCTION OF HYBRIDIZATION ARRAY AND ATTACHMENT OF OLIGOS

[0095] Hybridization arrays were constructed using Delrin polymer. Holes were cut into the array using an Epilog36 ext laser cutter. The array contains azido-sepharose which allows the oligos to be clicked onto the array. To attach oligos to the array, the array was placed into a chembot (Array-to-384-well plate adapter) to facilitate performing reactions on the array in a feature-specific manner. Attachment of the oligos to the array was effected by a click chemistry reagent solution which was added rapidly to the oligos and then the mixture of click reagents and oligos were rapidly added to the appropriate wells on the chembot. The reaction was run for 15min at room temp, whereupon the reaction solution was spun through the array, out of the chembot, into a receiver plate. The reaction mix was re-added to the chembot wells, and reacted for another 15min. During that 15min reaction, a second aliquot of ascorbate was added to each well of the receiver plate. The reaction mix was spun through the array, out of the chembot, and into the receiver plate where it mixed with the fresh ascorbate. Two more 15 minute reactions were done, with spin-outs and reloads between them. After the reaction completed, the array was washed by pouring a "quench" buffer into the shallow reservoir atop the chembot and pulling the buffer through on the vacuum manifold 6 or 7 times. The "quench" buffer is 100mM Tris pH 8, 10mM EDTA pH8, 0.005% triton x-100, 0.02% SDS. The array was stored in Array Storage Buffer: 10mM Tris, 1mM EDTA, 150mM NaCl, 0.01% azide, 0.005% triton x-100.

EXAMPLE 3: CONVERSION OF dsDNA INTO HYBRIDIZATION-READY DNA (hrDNA)

[0096] PCR was used to amplify the dsDNA library. The PCR products were in vitro transcribed using T7 RNA polymerase. RNA was purified using lithium chloride precipitation. Library RNA was reverse transcribed. The cDNA was purified using sodium acetate precipitation. The library was incubated with Zip oligos that are complementary to the conserved regions of DNA. Hydrolysis of the RNA leaves ssDNA, and annealing of Zip oligos results in a construct that is intermittently single- and double-stranded, and which is ready for sorting by hybridization on an array. hrDNA is recovered using centrifugal concentrators.

EXAMPLE 4: BINDING OF LIBRARY hrDNA TO HYBRIDIZATION ARRAY

[0097] The library of hrDNA was sorted on the array by hybridization in 2xSSC, +15mM Tris pH7.4 + 0.005% Triton X100, 0.02% SDS, 0.05% sodium azide for 60 hours at 37°C. Unhybridized DNA was eluted off the arrays by washing in 40ml hybridization buffer for 20-30 minutes. A second wash buffer of 12.5% Hybridization Buffer in 40ml dH2O + 0.005% triton + 0.02% SDS was incubated with the array at 37°C for 20-30 minutes. DNA was eluted into 384-well plates placing the array in the chembot, adding 30ul 10mM NaOH + 0.005% TritonX100 + 0.02% SDS, to each well of the chembot, incubating for 8-10 minutes and spinning out of the chembot into a 384-well plate. The elute buffer was neutralized with 9ul of 1M HOAc and 9ul of 1M Tris pH 7.4 to each well.

EXAMPLE 5: PEPTOID COUPLING CHEMICAL REACTION

[0098] The array-bound hrDNA library was chemically translated via a peptoid coupling step. 20-30 ul of settled DEAE resin was added to each well of a filter plate and washed with a bind buffer of 10mM acetic acid, 0.005% Triton X-100 mixed 1:1 with water. Eluted hrDNA was added to the plate, which was then washed with 1x80ul bind buffer, 2x80ul dH2O, and 3x80ul dry MeOH. Acetylation reagents were prepared immediately before use as below (the exact amounts will vary depending on how much settled DEAE resin was used to capture the DNA. Use at least 125% as much reaction solution volume as settled resin. The numbers below give >40ul of acetylation reaction mix per well and are sufficient for any amount of resin less than ~35ul.): Sodium chloroacetate, 10ml of 200mM stock solution (233mg) DMT-MM-Cl, (4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride)10ml of 300mM stock solution (830mg). Equal volumes of the 2 stock solutions were rapidly combined, vortexed, and aliquoted into the wells with alacrity, 30ul per well, reacted for 15minutes, and drained on the

vacuum manifold. The samples were washed twice with 1x80ul dry MeOH and once with 3x80ul dry MeOH, 1x80ul dH2O, 3x80ul DMSO.

EXAMPLE 6: TRIAZINE COUPLING CHEMICAL REACTION

[0099] 5ul saturated $K_2B_4O_7$ (pH9.2) was added to a 384-well plate. Array was washed in hybridization buffer 3x 20 min at 37°C. DNA was eluted with 3x30 ul of elute buffer into the library elution plate. 6 ul of ~250mM cyanuric chloride was added to a separate 384-well plate. The reaction was initiated by transferring DNA from the Library Elution Plate to the plate containing the cyanuric chloride and was reacted for 1hr at 4°C. The triazine adduct was reacted with amine synthons. A filter plate was loaded with 25 ul DEAE resin and washed with 4x80 ul DEAE bind buffer. 11 ul of synthons from the R1 or R2 sections of Table 5 were added to a separate, synthon mix plate. 56 ul of the cyanuric chloride reactions was added to each well of the filter plate, mixed, incubated for 5 minutes, and drained. This was repeated until all the material from the cyanuric chloride reaction plate was transferred to the filter plate. The DEAE resin in the filter plate was immediately washed with 2x80ul ACN, 2x80ul dH2O. The filter plate was stoppered and 45ul 75mM $K_2B_4O_7$ pH 9.5 was added to it. Synthon mixes from the synthon mix plate were added to the filter plate and reacted for 16hrs at 4°C. The DEAE resin was washed with 3x80ul ACN, 2x80ul dH2O. DNA was eluted by adding 6 ul of 1M Tris pH7.4 to each well of a new plate labeled DEAE Elution Plate and 4x33ul DEAE Elute Buffer (containing 50mM NaOH) with 4 min incubations between elutions. The eluents were concentrated in centrifugal concentrators. The hrDNA was then re-sorted by hybridization as described above. The second synthesis step was performed by adding 9 ul saturated borate, the eluted hrDNA, and 30 ul of synthon mixes (such as synthons drawn from the R3 section of Table 5) to each well of a receiver plate. The plate was reacted for 6 hours at 80°C. Synthons with lower solubilities were precipitated with 45 ul of dH2O via mixing for 60 seconds, and centrifugation at 569xg for 30 minutes. Supernatants were removed from the plate and combined. DNA was eluted three times from the receiver plate with 33ul of Elution Buffer (1.5M NaCl + 0.05M NaOH + 0.005% triton)

EXAMPLE 7: CONVERTING hrDNA TO DOUBLE-STRANDED DNA

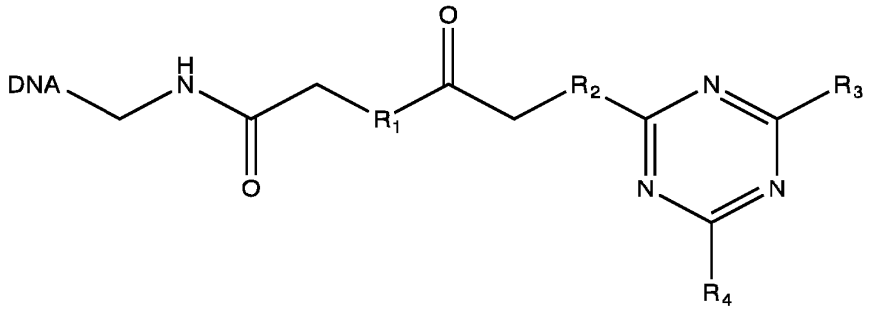
[00100] The eluted hrDNA was desalted by alcohol precipitation and made double stranded in a single cycle PCR reaction. The cycle conditions included an initial denaturation of 7min at 95°C, an anneal step of 1min at 57°C, and an extension of 10min at 72°C. PCR products were purified by alcohol precipitations.

EXAMPLE 8: SELECTIONS OF SWITCHABLE AFFINITY REAGENT

[00101] A 10 mM Bis-Tris-Propane buffer was made. To each pH of buffer Tween (to 0.5%) (and 0.05% triton), tRNA (60ng/ul), and BSA (0.2mg/ml) was added. 50ul streptavidin beads were added to 500ul biotinylated *E.coli* Lysate. Separately, 12ul of streptavidin beads were added to biotinylated rubisco and leghemoglobin. The lysate was passed through a column, washed with 4 mL of the appropriate pH buffer and eluted with 60 ul of the appropriate pH buffer. The pH of library was adjusted by adding BTP to 20mM, and KCl to 100mM. The library was added to the lysate beads, incubated for 15 minutes, and poured on the column. The flow-through was collected. 3 mM EDTA and protease inhibitor was added to 380 ul of the lysate mix (66 uM, ~250 fold over library). Then the library was added and incubated for 5 minutes. The mixes were pH'd as appropriate with 200mM BTP with the addition of KCl to 100mM. Separately, target-bead mixes were poured through a column and washed with 1ml appropriate pH buffer. Bead-bound targets were eluted with 100ul appropriate buffer (5 column volumes) and added to a mix of library and *E.coli* Lysate, and incubated for 20 min. Mixes were poured through columns, washed with 6000ul of appropriate buffer, and collected in 1200ul aliquots. A final wash was performed with 60 ul and collected separately. Elution was performed with a 1x 60 ul aliquot, a 1x 40ul aliquot, and a 1x 60ul aliquot. Beads were pressed off the column with 60 ul pH7.5 buffer.

EXAMPLE 9: IDENTITY OF THE SWITCHABLE REAGENT

[00102] The libraries were screened for binding to two proteins: leghemoglobin and rubisco. The ligands were selected for binding at low pH and release at high pH and also for high pH binding and low pH release. After screening a number of molecules were enriched and the following ligand structures were chosen for further testing.



EXAMPLE 10: SEQUENCING OF LIBRARY MEMBERS SURVIVING SELECTION AND ANALYSIS OF SELECTION PRODUCTS.

[00103] Those library members surviving the selection were analyzed by deep sequencing. The selected library was amplified by PCR. The number of rounds of PCR required varied with the stringency of the selection, more stringently selected libraries requiring more cycles. Sequencing was performed by commercial providers of DNA sequencing services using the Illumina MiSeq instrument. Libraries were prepared in accordance with the individual provider's instructions. Sequencing yielded ~15 million DNA sequences per experiment.

[00104] The DNA sequences were analyzed statistically to determine which library members in the selections were capable of binding the target proteins in a pH dependent manner. Library members were considered to be selected if they met the following criteria: the relative abundance of sequences encoding a library member must be enriched after a selection, the number of different genes encoding a library member must be greater than 1, the library member must be part of a family of library members with similar chemical structures that were also enriched, the library member must not also be enriched in a negative control selection performed in the absence of a desired target, the library member must not also be enriched in selections for other targets, the library member must not be enriched in selections for the same target under other condition (eg. a library member enriched in a selection binding Rubisco in acid and eluting in base, is not considered if it is also seen to be enriched in a selection binding Rubisco in base and eluting in acid). Exemplary results are summarized in Tables 1-4 above.

PROPHETIC EXAMPLE 11: GENERATION OF SWITCHABLE REAGENT FOR PURIFICATION OF TARGET PROTEIN

[00105] Selected molecules will be conjugated to a set of beads and will be mixed to create four different resins. The resins will be packed into four large columns which can have a column cross section diameter of greater than 4 meters. These columns are often used for process development and manufacturing.

PROPHETIC EXAMPLE 12: PURIFICATION OF RUBISCO AND LEGHEMOGLOBIN

[00106] The resins described in Example 11 will be used to purify a target protein by the column purification method. Columns containing pH switchable reagents that bind to the target protein Rubisco will be used to purify rubisco. Similarly, columns containing pH switchable reagents that bind to the target protein leghemoglobin will be used to purify leghemoglobin. The mixture of proteins will be flowed onto the column in a buffer with a first pH conducive for binding Rubisco or Leghemoglobin to the resin. The column will be washed in a similar buffer to remove non-specifically binding proteins. To elute the protein from the column, the column will be washed in a buffer with a second pH conducive for releasing the target protein from the resin. The collected protein will be subjected to further processes downstream for producing a consumable food product.

Table 5. Synthon table

Chemical Diversity Elements For R_1

2-PHENYLPROPYLAMINE, 4-METHYLBENZYLAMINE, 2-PHENYLETHANAMINE, BENZYLAMINE, 3-PHENYLPROPYLAMINE, 4-(2-AMINOMETHYL)BENZENESULFONAMIDE, 2-METHYLBUTYLAMINE, ISOAMYLAMINE, ISOBUTYLAMINE, BUTYLAMINE, PROPARGYLAMINE, PIPERAZINE, ETHYLAMINE, 1-AMINO-3,3-DIETHOXYPROPANE, 4-AMINO-2-METHYL-1-BUTANOL, 5-AMINO-1-PENTANOL, ACETAMIDE, 2-AMINO-N-CYCLOPROPYL-1-AMINO-2-BUTANOL, N,N-BIS(2-AMINOETHYL)ETHANE-1,2-DIAMINE, N,N,2,2-TETRAMETHYL-1,3-PROPANEDIAMINE, N,N-DIETHYL-1,3-PROPANEDIAMINE, N1-(2-AMINOETHYL)-N1-METHYLETHANE-1,2-DIAMINE, N,N-DIMETHYL-1,3-PROPANEDIAMINE, 4-(DIFLUOROMETHOXY)BENZYLAMINE, 2-N-PROPOXYETHYLAMINE, 2-METHOXYETHYLAMINE, N-TERT-BUTOXYCARBONYL-3-AMINOPROPANAMINE, 3-ISOPROPOXYPROPYLAMINE, 3-(METHYLTHIO)PROPYLAMINE, 2-AMINOETHYL ISOPROPYL ETHER, 4-BROMO-2-FLUOROBENZYLAMINE , 4-IODOBENZYLAMINE, 4-BROMOBENZYLAMINE, 2-(4-NITRO-PHENYL)-ETHYLAMINE, 3-FLUORO-5-(TRIFLUOROMETHYL)BENZYLAMINE, 4-NITRO-BENZYLAMINE, 3-BROMOBENZYLAMINE, 3-CHLORO-2,6-DIFLUOROBENZYLAMINE, 3,4-DICHLOROBENZYLAMINE, 4-(TRIFLUOROMETHYL)BENZYLAMINE, 2-(3-CHLOROPHENYL)ETHYLAMINE, 2-(4-CHLOROPHENYL)ETHYLAMINE, 3-CHLOROBENZYLAMINE, 2-

CHLOROBENZYLAMINE, 3-FLUOROPHENETHYLAMINE, 4-FLUOROPHENETHYLAMINE, 3-FLUOROBENZYLAMINE, TETRAHYDROFURFURYLAMINE, 4-(2-AMINOETHYL)-1-BENZYLPIPERIDINE, (2,2-DIMETHYL-[1,3]-DIOXOLAN-4-YL)-METHYLAMINE, N-(3-AMINOPROPYL)MORPHOLINE, 4-(2-AMINOETHYL)MORPHOLINE, 2-(2-AMINOETHYL)-1-METHYLPYRROLIDINE, 4-(AMINOMETHYL)PIPERIDINE, 2-(AMINOMETHYL)-1-ETHYLPYRROLIDINE, 4-PYRROLIDINOBUTYLAMINE, 1-(3-AMINOPROPYL)PYRROLIDINE, 1-(3-AMINOPROPYL)-4-METHYLPIPERAZINE, N-(3'-AMINOPROPYL)-2-PYRROLIDINONE, ETHYL 4-AMINO-1-PIPERIDINECARBOXYLATE, ETHANONE, 2-AMINO-1-(4-MORPHOLINYL)-3-AMINOPROPANE-1,2-DIOL, 3-AMINOPROPIONITRILE, 3-AMINO-1-PROPANOL, 2-(2-AMINOETHOXY)ETHANOL, 2-(1H-INDOL-3-YL)ETHANAMINE (TRYPTAMINE), 3-(2-AMINOETHYL)PYRIDINE, 1H-INDOLE-2-METHANAMINE, ACETAMIDE, 2-AMINO-N-(1-METHYLETHYL)-, 2-(2-METHOXYPHENYL)ETHYLAMINE, 2-(AMINOMETHYL)PYRIDINE, 4-(2-AMINOETHYL)PYRIDINE, 2-THIOPHENEMETHYLAMINE, 4-(AMINOMETHYL)PYRIDINE, 3-(AMINOMETHYL)PYRIDINE, 3-BROMO-4-METHOXYPHENETHYLAMINE, 3-FURANMETHANAMINE, 2-QUINOLINEMETHANAMINE, HOMOPIPERAZINE, PROPANAMIDE, 3-AMINO-N-METHYL-, 2-(2-FURYLMETHYLTHIO)ETHANAMINE, 4-(2-AMINOETHYL)BENZENESULFONAMIDE, 5-AMINOMETHYL-2-CHLOROPYRIDINE, 5-METHOXYTRYPTAMINE, N-ACETYLETHYLENEDIAMINE, S-(2-AMINOETHYL)ISOTHIOURONIUM BROMIDE, HOMOTAURINE, (R)-1-(3-METHOXYPHENYL)ETHYLAMINE, 2-(3,4-DIMETHOXYPHENYL)ETHYLAMINE, 3-METHOXYPHENETHYLAMINE, 2-(4-METHOXYPHENYL)ETHYLAMINE, 4-METHOXYBENZYLAMINE, 2-PHENOXYETHYLAMINE, 2-METHOXYBENZYLAMINE, 3-METHOXYBENZYLAMINE

Chemical Diversity Elements for R_2

2-PHENYLPROPYLAMINE, 4-METHYLBENZYLAMINE, 2-PHENYLETHANAMINE, BENZYLAMINE, 3-PHENYLPROPYLAMINE, 4-(2-AMINOMETHYL)BENZENESULFONAMIDE, 2-METHYLBUTYLAMINE, ISOAMYLAMINE, ISOBUTYLAMINE, BUTYLAMINE, PROPARGYLAMINE, PIPERAZINE, ETHYLAMINE, 1-AMINO-3,3-DIETHOXYPROPANE, 4-AMINO-2-METHYL-1-BUTANOL, 5-AMINO-1-PENTANOL, ACETAMIDE, 2-AMINO-N-CYCLOPROPYL-, 1-AMINO-2-BUTANOL, N,N-BIS(2-AMINOETHYL)ETHANE-1,2-

DIAMINE, N,N,2,2-TETRAMETHYL-1,3-PROPANEDIAMINE, N,N-DIETHYL-1,3-PROPANEDIAMINE, N1-(2-AMINOETHYL)-N1-METHYLETHANE-1,2-DIAMINE, N,N-DIMETHYL-1,3-PROPANEDIAMINE, 4-(DIFLUOROMETHOXY)BENZYLAMINE, 2-N-PROPOXYETHYLAMINE, 2-METHOXYETHYLAMINE, N-TERT-BUTOXYCARBONYL-3-AMINOPROPANAMINE, 3-ISOPROPOXYPROPYLAMINE, 3-(METHYLTHIO)PROPYLAMINE, 2-AMINOETHYL ISOPROPYL ETHER, 4-BROMO-2-FLUOROBENZYLAMINE , 4-IODOBENZYLAMINE, 4-BROMOBENZYLAMINE , 2-(4-NITRO-PHENYL)-ETHYLAMINE, 3-FLUORO-5-(TRIFLUOROMETHYL)BENZYLAMINE, 4-NITRO-BENZYLAMINE , 3-BROMOBENZYLAMINE, 3-CHLORO-2,6-DIFLUOROBENZYLAMINE, 3,4-DICHLOROBENZYLAMINE, 4-(TRIFLUOROMETHYL)BENZYLAMINE, 2-(3-CHLOROPHENYL)ETHYLAMINE, 2-(4-CHLOROPHENYL)ETHYLAMINE, 3-CHLOROBENZYLAMINE, 2-CHLOROBENZYLAMINE, 3-FLUOROPHENETHYLAMINE, 4-FLUOROPHENETHYLAMINE, 3-FLUOROBENZYLAMINE, TETRAHYDROFURFURYLAMINE, 4-(2-AMINOETHYL)-1-BENZYLPIPERIDINE, (2,2-DIMETHYL-[1,3]-DIOXOLAN-4-YL)-METHYLAMINE, N-(3-AMINOPROPYL)MORPHOLINE, 4-(2-AMINOETHYL)MORPHOLINE, 2-(2-AMINOETHYL)-1-METHYLPYRROLIDINE, 4-(AMINOMETHYL)PIPERIDINE, 2-(AMINOMETHYL)-1-ETHYLPYRROLIDINE, 4-PYRROLIDINOBUTYLAMINE, 1-(3-AMINOPROPYL)PYRROLIDINE, 1-(3-AMINOPROPYL)-4-METHYLPIPERAZINE, N-(3'-AMINOPROPYL)-2-PYRROLIDINONE, ETHYL 4-AMINO-1-PIPERIDINECARBOXYLATE, ETHANONE, 2-AMINO-1-(4-MORPHOLINYL)-, 3-AMINOPROPANE-1,2-DIOL, 3-AMINOPROPIONITRILE, 3-AMINO-1-PROPANOL, 2-(2-AMINOETHOXY)ETHANOL, 2-(1H-INDOL-3-YL)ETHANAMINE (TRYPTAMINE), 3-(2-AMINOETHYL)PYRIDINE , 1H-INDOLE-2-METHANAMINE, ACETAMIDE, 2-AMINO-N-(1-METHYLETHYL)-, 2-(2-METHOXYPHENYL)ETHYLAMINE, 2-(AMINOMETHYL)PYRIDINE, 4-(2-AMINOETHYL)PYRIDINE, 2-THIOPHENEMETHYLAMINE, 4-(AMINOMETHYL)PYRIDINE, 3-(AMINOMETHYL)PYRIDINE, 3-BROMO-4-METHOXYPHENETHYLAMINE, 3-FURANMETHANAMINE, 2-QUINOLINEMETHANAMINE, HOMOPIPERAZINE, PROPANAMIDE, 3-AMINO-N-METHYL-, 2-(2-FURYLMETHYLTHIO)ETHANAMINE, 4-(2-AMINOETHYL)BENZENESULFONAMIDE, 5-AMINOMETHYL-2-CHLOROPYRIDINE, 5-METHOXYTRYPTAMINE, N-ACETYLETHYLENEDIAMINE, S-(2-AMINOETHYL)ISOTHIOURONIUM BROMIDE , HOMOTAURINE, (R)-1-(3-

METHOXYPHENYL)ETHYLAMINE, 2-(3,4-DIMETHOXYPHENYL)ETHYLAMINE, 3-METHOXYPHENETHYLAMINE, 2-(4-METHOXYPHENYL)ETHYLAMINE, 4-METHOXYBENZYLAMINE, 2-PHENOXYETHYLAMINE, 2-METHOXYBENZYLAMINE, 3-METHOXYBENZYLAMINE, (Z)-4-CHLORO-2-BUTEN-1-AMINE , 2-(1-CYCLOHEXENYL)ETHYLAMINE, 3-METHOXYPROPYLAMINE, 3-ETHOXYPROPYLAMINE, 3-N-PROPOXYPROPYLAMINE, 2-ETHOXYETHYLAMINE, 3-IODOBENZYLAMINE, 3-(TRIFLUOROMETHYL)BENZYLAMINE, 4-(4-FLUOROPHENOXY)BENZYLAMINE, 3,4-METHYLENEDIOXYBENZYLAMINE, 8-QUINOLINEMETHANAMINE, 4-PYRIDINEMETHANAMINE, 2-(DIMETHYLAMINO)-, 2-AMINOETHYL HYDROGEN SULFATE, AMINOMETHANESULFONIC ACID, 3,4,5-TRIMETHOXYBENZYLAMINE, DL-1-(1-NAPHTHYL)ETHYLAMINE, (S)-(-)-1-(4-METHYLPHENYL)ETHYLAMINE, (S)-(-)-BETA-METHYLPHENETHYLAMINE, (R)-(-)-1-AMINOINDAN, (S)-(-)-1-PHENYLETHYLAMINE, (R)-(+)-1-PHENYLETHYLAMINE, 1-PHENYLBUTAN-3-AMINE, D-PHENYLALANINOL, (1R,2S)-1-AMINO-2-INDANOL, 2-AMINO-3,3-DIMETHYLBUTANE, 1,3-DIMETHYLBUTYLAMINE, SEC-BUTYLAMINE, ISOPROPYLAMINE, 2-AMINO-1-METHOXYBUTANE, (S)-2-AMINO-1-PHENYLETHANOL, (S)-(+)-2-AMINO-1-PENTANOL , (R)-(-)-2-AMINO-1-BUTANOL, L-LEUCINAMIDE , CYCLOPENTYLAMINE, CYCLOPROPYLAMINE, TRANS-4-AMINOCYCLOHEXANOL, (1S,2S)-(+)-2-BENZYLOXYCYCLOHEXYLAMINE, (1R,2R)-(-)-2-BENZYLOXYCYCLOPENTYLAMINE, 2-(2-CHLOROPHENYL)ETHYLAMINE, 2-FLUOROPHENETHYLAMINE, (1R,2R)-(-)-2-AMINO-1-(4-NITROPHENYL)-1,3-PROPANEDIOL, 4-AMINOTETRAHYDROPYRAN , DL-HOMOCYSTEINE THIOLACTONE, DL-α-AMINO-ε-CAPROLACTAM, INDAN-5-YLAMINE, 4-AMINOPYRIDINE, N-(3-AMINOPROPYL)-N-METHYLANILINE, 2-FLUOROETHANAMINE, ALPHA-PHENYLGLYCINONITRILE , (1R,2R)-(-)-2-AMINO-1-PHENYL-1,3-PROPANEDIOL, BETA ALANINE NAPTHYLAMIDE , L-LEUCINE-4-NITROANILIDE, 2-AMINO-3-METHYL-3-SULFANYLBUTANOIC ACID, (S)-(-)-1-(3-METHOXYPHENYL)ETHYLAMINE, (S)-(-)-1-(4-METHOXYPHENYL)ETHYLAMINE, TRANS-2,5-DIMETHYLPIPERAZINE, 9-AMINOFLUORENE , 2,4,6-TRIMETHYLANILINE, 2,4-DIMETHYLANILINE, 3,5-DIMETHYL ANILINE, 2,5-DIMETHYLANILINE, 2-METHYLANILINE, 2,6-DIETHYLANILINE, 3-ISOPROPYLANILINE, 4-ISOPROPYLANILINE, *m*-AMINOBENZOYLAMINE, 4-AMINOBENZAMIDE, 3-NITROANILINE, 2,6-DIFLUOROANILINE, 3',4'-DIFLUOROANILINE, 3-CHLOROANILINE, 2-CHLOROANILINE, 2-FLUORO-ANILINE,

4-FLUOROANILINE, 3,4-METHYLENEDIOXY-1-AMINOBENZENE, 2-AMINOPYRAZINE, 3-AMINOPYRIDINE, 2-AMINOPYRIDINE, 4-METHYL-1,3-THIAZOL-2-AMINE, 2-AMINOPYRIMIDINE, 5-AMINO-1H-TETRAZOLE, 4-ETHOXYANILINE, 2-(METHYLMERCAPTO)ANILINE, 2-ETHOXYANILINE, *o*-ANISIDINE, 4-AMINO-1-BUTANOL

Chemical Diversity Elements for R_3

2-PHENYLPROPYLAMINE, 4-METHYLBENZYLAMINE, 2-PHENYLETHANAMINE, BENZYLAMINE, 3-PHENYLPROPYLAMINE, 4-(2-AMINOMETHYL)BENZENESULFONAMIDE, 2-METHYLBUTYLAMINE, ISOAMYLAMINE, ISOBUTYLAMINE, BUTYLAMINE, PROPARGYLAMINE, PIPERAZINE, ETHYLAMINE, 4-AMINO-2-METHYL-1-BUTANOL, 5-AMINO-1-PENTANOL, ACETAMIDE, 2-AMINO-N-CYCLOPROPYL-, 1-AMINO-2-BUTANOL, N,N-BIS(2-AMINOETHYL)ETHANE-1,2-DIAMINE, N,N,2,2-TETRAMETHYL-1,3-PROPANEDIAMINE, N,N-DIETHYL-1,3-PROPANEDIAMINE, N1-(2-AMINOETHYL)-N1-METHYLETHANE-1,2-DIAMINE, N,N-DIMETHYL-1,3-PROPANEDIAMINE, 4-(DIFLUOROMETHOXY)BENZYLAMINE, 3-(METHYLTHIO)PROPYLAMINE, 4-BROMO-2-FLUOROBENZYLAMINE , 4-BROMOBENZYLAMINE , 2-(4-NITRO-PHENYL)-ETHYLAMINE, 3-FLUORO-5-(TRIFLUOROMETHYL)BENZYLAMINE, 4-NITRO-BENZYLAMINE , 3-BROMOBENZYLAMINE, 3-CHLORO-2,6-DIFLUOROBENZYLAMINE, 3,4-DICHLOROBENZYLAMINE, 4-(TRIFLUOROMETHYL)BENZYLAMINE, 2-(3-CHLOROPHENYL)ETHYLAMINE, 3-CHLOROBENZYLAMINE, 2-CHLOROBENZYLAMINE, 3-FLUOROPHENETHYLAMINE, 4-FLUOROPHENETHYLAMINE, TETRAHYDROFURFURYLAMINE, 4-(2-AMINOETHYL)-1-BENZYLPIPERIDINE, (2,2-DIMETHYL-[1,3]-DIOXOLAN-4-YL)-METHYLAMINE, N-(3-AMINOPROPYL)MORPHOLINE, 4-(2-AMINOETHYL)MORPHOLINE, 2-(2-AMINOETHYL)-1-METHYLPYRROLIDINE, 4-(AMINOMETHYL)PIPERIDINE, 2-(AMINOMETHYL)-1-ETHYLPYRROLIDINE, 4-PYRROLIDINOBUTYLAMINE, 1-(3-AMINOPROPYL)-4-METHYLPIPERAZINE, N-(3'-AMINOPROPYL)-2-PYRROLIDINONE, ETHYL 4-AMINO-1-PIPERIDINECARBOXYLATE, ETHANONE, 2-AMINO-1-(4-MORPHOLINYL)-, 3-AMINOPROPANE-1,2-DIOL, 3-AMINOPROPIONITRILE, 3-AMINO-1-PROPANOL, 2-(2-AMINOETHOXY)ETHANOL, 2-(1H-INDOL-3-YL)ETHANAMINE (TRYPTAMINE), 3-(2-AMINOETHYL)PYRIDINE , 1H-INDOLE-2-METHANAMINE, ACETAMIDE, 2-AMINO-N-

(1-METHYLETHYL)-, 2-(2-METHOXYPHENYL)ETHYLAMINE, 2-(AMINOMETHYL)PYRIDINE, 4-(2-AMINOETHYL)PYRIDINE, 4-(AMINOMETHYL)PYRIDINE, 3-(AMINOMETHYL)PYRIDINE, 3-BROMO-4-METHOXYPHENETHYLAMINE, 3-FURANMETHANAMINE, 2-QUINOLINEMETHANAMINE, HOMOPIPERAZINE, PROPANAMIDE, 3-AMINO-N-METHYL-, 2-(2-FURYLMETHYLTHIO)ETHANAMINE, 4-(2-AMINOETHYL)BENZENESULFONAMIDE, 5-METHOXYTRYPTAMINE, N-ACETYLETHYLENEDIAMINE, (R)-1-(3-METHOXYPHENYL)ETHYLAMINE, 2-(3,4-DIMETHOXYPHENYL)ETHYLAMINE, 2-(4-METHOXYPHENYL)ETHYLAMINE, 4-METHOXYBENZYLAMINE, 2-PHENOXYETHYLAMINE, 2-METHOXYBENZYLAMINE, 3-METHOXYBENZYLAMINE, (Z)-4-CHLORO-2-BUTEN-1-AMINE, 2-(1-CYCLOHEXENYL)ETHYLAMINE, 3-METHOXYPROPYLAMINE, 3-ETHOXYPROPYLAMINE, 3-N-PROPOXYPROPYLAMINE, 2-ETHOXYETHYLAMINE, 3-IODOBENZYLAMINE, 3-(TRIFLUOROMETHYL)BENZYLAMINE, 4-(4-FLUOROPHENOXY)BENZYLAMINE, 3,4-METHYLENEDIOXYBENZYLAMINE, 8-QUINOLINEMETHANAMINE, 4-PYRIDINEMETHANAMINE, 2-(DIMETHYLAMINO)-, 2-AMINOETHYL HYDROGEN SULFATE, AMINOMETHANESULFONIC ACID, 3,4,5-TRIMETHOXYBENZYLAMINE, DL-1-(1-NAPHTHYL)ETHYLAMINE, (S)-(-)-1-(4-METHYLPHENYL)ETHYLAMINE, 1-PHENYLBUTAN-3-AMINE, D-PHENYLALANINOL, (1R,2S)-1-AMINO-2-INDANOL, 2-AMINO-3,3-DIMETHYLBUTANE, 1,3-DIMETHYLBUTYLAMINE, 2-AMINO-1-METHOXYBUTANE, (S)-2-AMINO-1-PHENYLETHANOL, (S)-(+)-2-AMINO-1-PENTANOL , L-LEUCINAMIDE , CYCLOPENTYLAMINE, CYCLOPROPYLAMINE, TRANS-4-AMINOCYCLOHEXANOL, (1S,2S)-(+)-2-BENZYLOXYCYCLOHEXYLAMINE, (1R,2R)-(-)-2-BENZYLOXYCYCLOPENTYLAMINE, 2-(2-CHLOROPHENYL)ETHYLAMINE, 2-FLUOROPHENETHYLAMINE, (1R,2R)-(-)-2-AMINO-1-(4-NITROPHENYL)-1,3-PROPANEDIOL, 4-AMINOTETRAHYDROPYRAN, DL-HOMOCYSTEINE THIOLACTONE , DL-ALPHA-AMINO-EPSILON-CAPROLACTAM, INDAN-5-YLAMINE, 4-AMINOPYRIDINE, 2-(AMINOMETHYL)PIPERIDINE, 1-(3-AMINOPROPYL)-2-PIPECOLINE, BENZENEPROPANOIC ACID, β-(AMINOMETHYL)-, BENZENEACETIC ACID, A-(AMINOMETHYL)-, BENZOIC ACID, 2-(AMINOMETHYL)-, 6-AMINOCAPROIC ACID, PENTANOIC ACID, 5-AMINO-, CYCLOHEXANECARBOXYLIC ACID, 4-(AMINOMETHYL)-, (2S)-4-AMINO-2-HYDROXY-BUTYRIC ACID, N-(3-AMINOPROPYL)-N-METHYLANILINE, TYRAMINE, PHENOL, 3-(AMINOMETHYL)-, 3-

METHOXYTYRAMINE , 2-FLUOROETHANAMINE, 3-AMINOPYRROLIDINE, 4-AMINOPIPERIDINE, PHENYLALANINE, 3,5-DIMETHYL-, GLUTAMIC ACID, D-PHENYLALANINE, 3-FLUORO-, BENZENEACETIC ACID, A-AMINO-4-FLUORO-, TYROSINE, 3-FLUORO-, 4-PYRIDINEPROPANOIC ACID, A-AMINO-, (αR)-, ASPARAGINE, ALPHA-PHENYLGLYCINONITRILE , (1R,2R)-(-)-2-AMINO-1-PHENYL-1,3-PROPANEDIOL, BETA ALANINE NAPTHYLAMIDE , L-LEUCINE-4-NITROANILIDE, 2-AMINO-3-METHYL-3-SULFANYLBUTANOIC ACID, 1,2,3,4-TETRAHYDRO-1-NAPHTHYLAMINE, (S)-(-)-1-(3-METHOXYPHENYL)ETHYLAMINE, (S)-(-)-1-(4-METHOXYPHENYL)ETHYLAMINE, trans-2,5-DIMETHYLPIPERAZINE, cis-2, 6 DIMETHYLPIPERAZINE, 3-PYRROLIDINECARBOXYLIC ACID, 1H-IMIDAZOLE, 1-(3-PYRROLIDINYL)-, PYRROLIDINE, 3-(METHYLSULFONYL)-, 3-PYRROLIDINECARBOXAMIDE, 9-AMINOFLUORENE, 2,6-DIETHYLANILINE, META-AMINOBENZOYLAMINE, 3-NITROANILINE, 3,4-METHYLENEDIOXY-1-AMINOBENZENE, 2-AMINOPYRAZINE, 4-ETHOXYANILINE, 1H-INDOL-5-OL, 3-(2-AMINOETHYL)-, D-PHENYLALANINE, GLUTAMINE, BENZENEPROPANOIC ACID, B-AMINO-3,4-DIHYDROXY-, BUTANOIC ACID, 2-AMINO-4,4,4-TRIFLUORO-, BUTANOIC ACID, 2-AMINO-3-HYDROXY-, 3-THIOPHENEPROPANOIC ACID, α-AMINO-, 3-THIOPHENEACETIC ACID, α-AMINO-, 2-MORPHOLINEACETIC ACID, BENZOIC ACID, 4-AMINO-3-HYDROXY-, 1H-PYRAZOLE-3-CARBOXYLIC ACID, 5-AMINO-, 4-AMINO-1-BUTANOL, 4-DIMETHYLAMINOBENZYLAMINE

Chemical Diversity Elements for R_4

2-PHENYLPROPYLAMINE, 4-METHYLBENZYLAMINE, 2-PHENYLETHANAMINE, BENZYLAMINE, 3-PHENYLPROPYLAMINE, 4-(2-AMINOMETHYL)BENZENESULFONAMIDE, 2-METHYLBUTYLAMINE, ISOAMYLAMINE, ISOBUTYLAMINE, BUTYLAMINE, PROPARGYLAMINE, (Z)-4-CHLORO-2-BUTEN-1-AMINE , 2-(1-CYCLOHEXENYL)ETHYLAMINE, 3-METHOXYPROPYLAMINE, 3-ETHOXYPROPYLAMINE, 3-N-PROPOXYPROPYLAMINE, 2-ETHOXYETHYLAMINE, 3-IODOBENZYLAMINE, 3-(TRIFLUOROMETHYL)BENZYLAMINE, 4-(4-FLUOROPHENOXY)BENZYLAMINE, 3,4-METHYLENEDIOXYBENZYLAMINE, 8-QUINOLINEMETHANAMINE, 4-PYRIDINEMETHANAMINE, 2-(DIMETHYLAMINO)-, PIPERAZINE, ETHYLAMINE, 1-AMINO-3,3-DIETHOXYPROPANE, 4-AMINO-2-METHYL-1-BUTANOL, 5-AMINO-1-PENTANOL, ACETAMIDE, 2-AMINO-N-CYCLOPROPYL-, 1-AMINO-2-BUTANOL, N,N-BIS(2-AMINOETHYL)ETHANE-1,2-DIAMINE, N,N,2,2-TETRAMETHYL-1,3-

PROPANEDIAMINE, N,N-DIETHYL-1,3-PROPANEDIAMINE, N1-(2-AMINOETHYL)-N1-METHYLETHANE-1,2-DIAMINE, N,N-DIMETHYL-1,3-PROPANEDIAMINE, 3,4,5-TRIMETHOXYBENZYLAMINE, DL-1-(1-NAPHTHYL)ETHYLAMINE, (S)-(-)-1-(4-METHYLPHENYL)ETHYLAMINE, (S)-(-)-BETA-METHYLPHENETHYLAMINE, (R)-(-)-1-AMINOINDAN, (S)-(-)-1-PHENYLETHYLAMINE, (R)-(+)-1-PHENYLETHYLAMINE, 1-PHENYLBUTAN-3-AMINE, D-PHENYLALANINOL, (1R,2S)-1-AMINO-2-INDANOL, 2-AMINO-3,3-DIMETHYLBUTANE, 1,3-DIMETHYLBUTYLAMINE, 4-(DIFLUOROMETHOXY)BENZYLAMINE, 2-N-PROPOXYETHYLAMINE, 2-METHOXYETHYLAMINE, N-TERT-BUTOXYCARBONYL-3-AMINOPROPANAMINE, 3-ISOPROPOXYPROPYLAMINE, 3-(METHYLTHIO)PROPYLAMINE, 2-AMINOETHYL ISOPROPYL ETHER, 4-BROMO-2-FLUOROBENZYLAMINE , 4-IODOBENZYLAMINE, 4-BROMOBENZYLAMINE , 2-(4-NITRO-PHENYL)-ETHYLAMINE, 3-FLUORO-5-(TRIFLUOROMETHYL)BENZYLAMINE, SEC-BUTYLAMINE, ISOPROPYLAMINE, 2-AMINO-1-METHOXYBUTANE, (S)-2-AMINO-1-PHENYLETHANOL, (S)-(+)-2-AMINO-1-PENTANOL , (R)-(-)-2-AMINO-1-BUTANOL, L-LEUCINAMIDE , CYCLOPENTYLAMINE, CYCLOPROPYLAMINE, TRANS-4-AMINOCYCLOHEXANOL, (1S,2S)-(+)-2-BENZYLOXYCYCLOHEXYLAMINE, (1R,2R)-(-)-2-BENZYLOXYCYCLOPENTYLAMINE, 4-NITRO-BENZYLAMINE , 3-BROMOBENZYLAMINE, 3-CHLORO-2,6-DIFLUOROBENZYLAMINE, 3,4-DICHLOROBENZYLAMINE, 4-(TRIFLUOROMETHYL)BENZYLAMINE, 2-(3-CHLOROPHENYL)ETHYLAMINE, 2-(4-CHLOROPHENYL)ETHYLAMINE, 3-CHLOROBENZYLAMINE, 2-CHLOROBENZYLAMINE, 3-FLUOROPHENETHYLAMINE, 4-FLUOROPHENETHYLAMINE, 3-FLUOROBENZYLAMINE, 2-(2-CHLOROPHENYL)ETHYLAMINE, 2-FLUOROPHENETHYLAMINE, (1R,2R)-(-)-2-AMINO-1-(4-NITROPHENYL)-1,3-PROPANEDIOL, 4-AMINOTETRAHYDROPYRAN , DL-HOMOCYSTEINE THIOLACTONE, DL-α-AMINO-EPSILON-CAPROLACTAM, INDAN-5-YLAMINE, 4-AMINOPYRIDINE, 2-(AMINOMETHYL)PIPERIDINE, 1-(3-AMINOPROPYL)-2-PIPECOLINE, BENZENEPROPANOIC ACID, β-(AMINOMETHYL)-, BENZENEACETIC ACID, α -(AMINOMETHYL)-, TETRAHYDROFURFURYLAMINE, 4-(2-AMINOETHYL)-1-BENZYLPIPERIDINE, (2,2-DIMETHYL-[1,3]-DIOXOLAN-4-YL)-METHYLAMINE, N-(3-AMINOPROPYL)MORPHOLINE, 4-(2-AMINOETHYL)MORPHOLINE, 2-(2-AMINOETHYL)-1-METHYLPYRROLIDINE, 4-(AMINOMETHYL)PIPERIDINE, 2-(AMINOMETHYL)-1-ETHYLPYRROLIDINE, 4-PYRROLIDINOBUTYLAMINE, 1-(3-AMINOPROPYL)PYRROLIDINE, 1-(3-

AMINOPROPYL)-4-METHYLPIPERAZINE, N-(3'-AMINOPROPYL)-2-PYRROLIDINONE, BENZOIC ACID, 2-(AMINOMETHYL)-, 6-AMINOCAPROIC ACID, PENTANOIC ACID, 5-AMINO-, CYCLOHEXANECARBOXYLIC ACID, 4-(AMINOMETHYL)-, (2S)-4-AMINO-2-HYDROXY-BUTYRIC ACID, TYRAMINE, PHENOL, 2-(AMINOMETHYL)-, PHENOL, 4-(AMINOMETHYL)-, 3-METHOXYTYRAMINE , 2-FLUOROETHANAMINE , 3-AMINOPYRROLIDINE, 4-AMINOPIPERIDINE, ETHYL 4-AMINO-1-PIPERIDINECARBOXYLATE, ETHANONE, 2-AMINO-1-(4-MORPHOLINYL)-, 3-AMINOPROPANE-1,2-DIOL, 3-AMINOPROPIONITRILE, 3-AMINO-1-PROPANOL, 2-(2-AMINOETHOXY)ETHANOL, 2-(1H-INDOL-3-YL)ETHANAMINE (TRYPTAMINE), 3-(2-AMINOETHYL)PYRIDINE , 1H-INDOLE-2-METHANAMINE, ACETAMIDE, 2-AMINO-N-(1-METHYLETHYL)-, 2-(2-METHOXYPHENYL)ETHYLAMINE, 2-(AMINOMETHYL)PYRIDINE, PHENYLALANINE, 3,5-DIMETHYL-, GLUTAMIC ACID, BUTANOIC ACID, 2-AMINO-, D-PHENYLALANINE, 3-FLUORO-, BENZENEACETIC ACID, α -AMINO-4-FLUORO-, TYROSINE, 3-FLUORO-, 4-PYRIDINEPROPANOIC ACID, A-AMINO-, (α R)-, ASPARAGINE, BETA ALANINE NAPTHYLAMIDE , L-LEUCINE-4-NITROANILIDE, 2-AMINO-3-METHYL-3-SULFANYLBUTANOIC ACID, 1,2,3,4-TETRAHYDRO-1-NAPHTHYLAMINE, 4-(2-AMINOETHYL)PYRIDINE, 2-THIOPHENEMETHYLAMINE, 4-(AMINOMETHYL)PYRIDINE, 3-(AMINOMETHYL)PYRIDINE, 3-BROMO-4-METHOXYPHENETHYLAMINE, 3-FURANMETHANAMINE, 2-QUINOLINEMETHANAMINE, HOMOPIPERAZINE, PROPANAMIDE, 3-AMINO-N-METHYL-, 2-(2-FURYLMETHYLTHIO)ETHANAMINE, 4-(2-AMINOETHYL)BENZENESULFONAMIDE, 5-AMINOMETHYL-2-CHLOROPYRIDINE, (S)-(-)-1-(3-METHOXYPHENYL)ETHYLAMINE, (S)-(-)-1-(4-METHOXYPHENYL)ETHYLAMINE, CIS-2, 6 DIMETHYLPIPERAZINE, 3-PYRROLIDINECARBOXYLIC ACID, 3-PIPERIDINECARBOXYLIC ACID, 1H-IMIDAZOLE, 1-(3-PYRROLIDINYL)-, 5H-PYRROLO[3,4-B]PYRIDINE, 6,7-DIHYDRO-, PYRIDINE, 2-(3-PYRROLIDINYL)-, PYRROLIDINE, 3-(METHYLSULFONYL)-, 3-PYRROLIDINECARBOXAMIDE, PHENYLALANINE, 4-ETHYL-, 5-METHOXYTRYPTAMINE, N-ACETYLETHYLENEDIAMINE, S-(2-AMINOETHYL)ISOTHIOURONIUM BROMIDE , HOMOTAURINE, (R)-1-(3-METHOXYPHENYL)ETHYLAMINE, 2-(3,4-DIMETHOXYPHENYL)ETHYLAMINE, 3-METHOXYPHENETHYLAMINE, 2-(4-METHOXYPHENYL)ETHYLAMINE, 4-METHOXYBENZYLAMINE, 2-PHENOXYETHYLAMINE, 2-METHOXYBENZYLAMINE, 3-METHOXYBENZYLAMINE, PHENYLALANINE, 4-ETHYL-, BENZENEACETIC ACID,

α -AMINO-, GLUTAMINE, BENZENEACETIC ACID, A-AMINO-4-HYDROXY-, (α R)-, BUTANOIC ACID, 2-AMINO-3-HYDROXY-, 3-THIOPHENEACETIC ACID, A-AMINO-, BENZOIC ACID, 2-AMINO-5-HYDROXY-, 4-AMINO-1-BUTANOL, 4-DIMETHYLAMINOBENZYLAMINE

CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising a non-polypeptide pH switchable reagent which binds a target at a first pH and does not bind the target at a second pH.

2. The composition of claim 1, wherein the pH switchable reagent is a peptoid.

3. The composition of claim 2, wherein the peptoid is comprised of N subunits.

4. The composition of claim 3, wherein N subunits is 1, 2, 3, 4, 5 subunits.

5. The composition of claim 3, wherein N subunits is 2 subunits.

6. The composition of claim 1, wherein the peptoid is modified with multiple diversity points.

7. The composition of claim 6 wherein the multiple diversity points are at least 1, 2, 3, 4, 5, 6 diversity points.

8. The composition of claim 6 wherein the multiple diversity points are 4 diversity points.

9. The composition of claim 2, wherein the peptoid comprises a triazine.

10. The composition of claim 9, wherein the triazine is tri-substituted.

11. The composition of claim 1, wherein the pH switchable reagent has a backbone structure comprising Formula IV.

12. The composition of claim 11 wherein the switchable reagent is selected from the group consisting of the compounds described in Formula IV with the identity of the R groups as described in Table 5, wherein the R1-R4 groups in Table 5 correspond to the R1-R4 groups in Formula IV where one or more of the N-H bond(s) from the compounds in the table are converted to C-N bonds in Formula IV.

13. The composition of claim 11 wherein the switchable reagent is selected from the group consisting of the compounds described in Formula IV with the identity of the R groups as described in Table 1, wherein the R1-R4 groups in Table 1 correspond to the R1-R4 groups in Formula IV where one or more of the N-H bond(s) from the compounds in the table are converted to C-N bonds in Formula IV.

14. The composition of claim 13 wherein the switchable reagents can bind Rubisco in base and release Rubisco in acid.

15. The composition of claim 11 wherein the switchable reagent is selected from the group consisting of the compounds described in Formula IV with the identity of the R groups as described in Table 2, wherein the R1-R4 groups in Table 2 correspond to the R1-R4 groups in Formula IV where one or more of the N-H bond(s) from the compounds in the table are converted to C-N bonds in Formula IV.

16. The composition of claim 15 wherein the switchable reagents can bind Rubisco in acid and release Rubisco in base.

17. The composition of claim 11 wherein the switchable reagent is selected from the group consisting of the compounds described in Formulas IV with the identity of the R groups as described in Table 3, wherein the R1-R4 groups in Table 3 correspond to the R1-R4 groups in Formula IV where one or more of the N-H bond(s) from the compounds in the table are converted to C-N bonds in Formula IV.

18. The composition of claim 17 wherein the switchable reagents can bind leghemoglobin in base and release leghemoglobin in acid.

19. The composition of claim 11 wherein the switchable reagent is selected from the group consisting of the compounds described in Formula IV with the identity of the R groups as described in Table 4, wherein the R1-R4 groups in Table 4 correspond to the R1-R4 groups in Formula IV where one or more of the N-H bond(s) from the compounds in the table are converted to C-N bonds in Formula IV.

20. The composition of claim 19 wherein the switchable reagents can bind leghemoglobin in acid and release leghemoglobin in base.

21. The composition of claim 1, wherein the composition comprises a mixture of diastereomers.

22. The composition of claim 1, wherein the composition comprises a mixture of enantiomers.

23. The composition of claim 1, wherein the composition comprises an essentially pure diastereomer, wherein the essentially pure diastereomer refers to a diastereomer that is greater than 70% purified from other diastereomers.

24. The composition of claim 1, wherein the composition comprises an essentially pure enantiomer, wherein the essentially pure enantiomer refers to a enantiomer that is greater than 70% purified from other enantiomers.

25. The composition of claim 2, wherein the peptoid is non-degradable by proteases.

26. The composition of claim 1, wherein the first pH is greater than 7.

27. The composition of claim 1, wherein the first pH is less than 7.

28. The composition of claim 1, wherein the second pH is greater than 7.

29. The composition of claim 1, wherein the second pH is less than 7.

30. The composition of claim 1, wherein the pH switchable reagent is conjugated to a substrate.

31. The composition of claim 30, wherein the substrate is a bead that is selected from a group comprising agarose, sepharose, polystyrene, styrene, iron oxide, magnetic, or paramagnetic.

32. The composition of claim 30, wherein the substrate is coated with a coat selected from a group comprising silica, carboxyl functional groups, aldehyde functional groups, or N-hydroxysuccinimide functional groups.

33. The composition of claim 30, wherein the substrate is coated with a functional group able to conjugate primary amines and thiols.

34. The composition of claim 30, wherein the substrate is a component of a resin used for chromatography.

35. The composition of claim 34, wherein the resin is used for protein purification chromatography.

36. The composition of claim 34, wherein the resin is used for affinity protein purification chromatography.

37. A consumable product comprising a pH switchable reagent.

38. The consumable product of claim 37 wherein the switchable reagent is present at a concentration of less than 1,000 parts per million target protein.

39. The consumable product of claim 37 wherein at least 90% dry weight of the composition comprises plant based protein.

40. The consumable product of claim 37 substantially free of chlorophylls, chlorins, metalloids, transition metals, celluloses, or complex polysaccharides.

41. The consumable product of claim 37, wherein the consumable product is food-safe.

42. A composition comprising a protein content, wherein the protein content is obtained from a plant; wherein the composition is substantially free of odorants found in the plant.

43. The composition of claim 42, wherein the pH switchable reagent is present at a concentration of less than 1,000 parts per million target protein.

44. The composition of claim 42 wherein at least 90% of the composition comprises plant based protein.

45. The composition of claim 42 substantially free of chlorophylls, chlorins, metalloids, transition metals, celluloses, or complex polysaccharides.

46. The consumable product of claim 42, wherein the consumable product is food-safe.

47. A composition comprising a protein content, wherein the protein content is obtained from a plant; wherein the composition is substantially free of colorants found in the plant.

48. The composition of claim 47 wherein the pH switchable reagent is present at a concentration of less than 1,000 parts per million target protein.

49. The composition of claim 47 wherein at least 90% of the composition comprises plant based protein.

50. The composition of claim 47 substantially free of chlorophylls, chlorins, metalloids, transition metals, celluloses, or complex polysaccharides.

51. The consumable product of claim 47, wherein the consumable product is food-safe.

52. A method for identifying a non-polypeptide pH switchable reagent, the method comprising:

a. generating a library of non-polypeptides by tag-directed synthesis;

b. exposing at least a subset of the library to a protein in a solution at a first pH;

c. altering the pH of the solution to a second pH; and

d. identifying a non-polypeptide pH switchable peptoid reagent which binds a target at a first pH and does not bind the target at a second pH.

53. A method for isolating one or more target proteins comprising:

a. applying a composition comprising the one or more target proteins to a substrate; wherein the substrate comprises one or more non-polypeptide pH switchable reagents; wherein at least part of the applying step occurs at a first pH; wherein at the first pH at least one of the one or more target proteins binds to the one or more non-polypeptide pH switchable reagents;

b. releasing the target proteins by adjusting the pH of the solution to a second pH; wherein at the second pH at least one or more of the target proteins does not bind the non-polypeptide pH switchable reagent and;

c. collecting the target proteins that eluted from the one or more non-polypeptide pH switchable reagents.

54. The method of claim 53, wherein the pH switchable reagent is a peptoid.

55. The method of claim 54, wherein the peptoid is comprised of N subunits.

56. The method of claim 55, wherein N subunits is 1, 2, 3, 4, 5 subunits.

57. The method of claim 55, wherein N subunits is 2 subunits.

58. The method of claim 53, wherein the peptoid is modified with multiple diversity points.

59. The composition of claim 58, wherein the multiple diversity points are at least 1, 2, 3, 4, 5, 6 diversity points.

60. The method of claim 58 wherein the multiple diversity points are 4 diversity points.

61. The method of claim 54, wherein the peptoid comprises a triazine.

62. The method of claim 61, wherein the triazine is tri-substituted.

63. The method of claim 53, wherein the pH switchable reagent has a backbone structure comprising Formula IV.

64. The method of claim 53, wherein the switchable reagent is selected from the group consisting of the compounds described in Formula IV with the identity of the R groups as described in Table 5, wherein the R1-R4 groups in Table 5 correspond to the R1-R4 groups in Formula IV where one or more of the N-H bond(s) from the compounds in the table are converted to C-N bonds in Formula IV.

65. The method of claim 53, wherein the switchable reagent is selected from the group consisting of the compounds described in Formula IV with the identity of the R groups as described in Table 1, wherein the R1-R4 groups in Table 1 correspond to the R1-R4 groups in Formula IV where one or more of the N-H bond(s) from the compounds in the table are converted to C-N bonds in Formula IV.

66. The method of claim claim 65 wherein the switchable reagents can bind Rubisco in base and release Rubisco in acid.

67. The method of claim 53, wherein the switchable reagent is selected from the group consisting of the compounds described in Formula IV with the identity of the R groups as described in Table 2, wherein the R1-R4 groups in Table 2 correspond to the R1-R4 groups in Formula IV where the N-H bond(s) from the compounds in the table are converted to C-N bonds in Formula IV.

68. The method of claim 67 wherein the switchable reagents can bind Rubisco in acid and release Rubisco in base.

69. The method of claim 53, wherein the switchable reagent is selected from the group consisting of the compounds described in Formula IV with the identity of the R groups as described in Table 3, wherein the R1-R4 groups in Table 3 correspond to the R1-R4 groups in Formula IV where one or more of the N-H bond(s) from the compounds in the table are converted to C-N bonds in Formula IV.

70. The method of claim 69 wherein the switchable reagents can bind leghemoglobin in base and release leghemoglobin in acid.

71. The method of claim 53, wherein the switchable reagent is selected from the group consisting of the compounds described in Formula IV with the identity of the R groups as described in Table 4, wherein the R1-R4 groups in Table 4 correspond to the R1-R4 groups in Formula IV where one or more of the N-H bond(s) from the compounds in the table are converted to C-N bonds in Formula IV.

72. The method of claim 71 wherein the switchable reagents can bind leghemoglobin in acid and release leghemoglobin in base.

73. The method of claim 53, wherein the pH switchable reagent comprises a mixture of diastereomers.

74. The method of claim 53, wherein the pH switchable reagent comprises a mixture of enantiomers.

75. The composition of claim 53, wherein the pH switchable reagent comprises an essentially pure diastereomer, wherein the essentially pure diastereomer refers to a diastereomer that is greater than 70% purified from other diastereomers.

76. The composition of claim 53, wherein the pH switchable reagent comprises an essentially pure enantiomer, wherein the essentially pure enantiomer refers to a enantiomer that is greater than 70% purified from other enantiomers.

77. The method of claim 54, wherein the peptoid is non-degradable by proteases.

78. The method of claim 53, wherein the first pH is greater than 7.

79. The method of claim 53, wherein the first pH is less than 7.

80. The method of claim 53, wherein the second pH is greater than 7.

81. The method of claim 53, wherein the second pH is less than 7.

82. The method of claim 53, wherein the pH switchable reagent is conjugated to a substrate.

83. The method of claim 82, wherein the substrate is a bead that is selected from a group comprising agarose, sepharose, polystyrene, styrene, iron oxide, magnetic, or paramagnetic.

84. The method of claim 82, wherein the substrate is coated with a coat selected from a group comprising silica, carboxyl functional groups, aldehyde functional groups, or N-hydroxysuccinimide functional groups

85. The method of claim 82, wherein the substrate is coated with a functional group able to conjugate primary amines and thiols.

86. The method of claim 82, wherein the substrate is a component of a resin used for chromatography.

87. The method of claim 86, wherein the resin is used for protein purification chromatography.

88. The method of claim 87, wherein the resin is used for affinity protein purification chromatography.

89. The composition of claim 13 wherein rubsco binds at pH 9 and releases at pH 6.

90. The composition of claim 15 wherein rubsco binds at pH 6 and releases at pH 9

91. The composition of claim 17 wherein leghemoglobin binds at pH 9 and releases at pH 6.

92. The composition of claim 19 wherein leghemoglobin binds at pH 6 and releases at pH 9.

93. The method of claim 65 wherein rubsco binds at pH 9 and releases at pH 6.

94. The method of claim 67 wherein rubsco binds at pH 6 and releases at pH 9

95. The method of claim 69 wherein the leghemoglobin binds at pH 9 and releases at pH 6.

96. The method of claim 71wherein the leghemoglobin binds at pH 6 and releases at pH 9.

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Published:

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(54) Title: AFFINITY REAGANTS FOR PROTEIN PURIFICATION

(57) Abstract: Disclosed herein are methods and compositions for purifying proteins from crude solutions.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/32675

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/00 (2013.01)
USPC - 424/158.1; 536/25.32
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC -- 424/158.1; 536/25.32

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC -- 530/387.1; 424/130.1; 530/387.2; 530/391.7

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (USPT,PGPB,JPAB,EPAB); Google
Search Terms: Protein DNA purification isolation affinity target label tag chromatography reagent pH switchable agent peptoid triazine trisubstituted substrate bead coated magnetic paramagnetic isomer enantiomer diastereomer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	US 2008/0261202 A1 (Baker et al.) 23 October 2008 (23.10.2008), entire document especially Abstract, para [0006], [0029]-[0032], [0014], [0043], [0051] and [0126]	1, 21-24, 27, 28, 30-33 --- 2-10, 25, 26, 29, 34-36 --- 11-20
Y --- A	US 2003/0166002 A1 (Chang et al.) 04 September 2003 (04.09.2003), entire document especially Abstract, para [0020], Fig. 3-5	2-10, 25, 34-36 --- 11-20
Y --- A	WO 2005/049653 A1 (Fang et al.) 02 June 2005 (02.06.2005), pg 1, 27, 28	26, 29 --- 11-20
A	US 5,582,988 A (Backus et al.) 10 December 1996 (10.12.1996), col 1, ln 8-9; col 6, ln 63-col 7, ln 61	11-20
A	WO 2011/012302 A1 (Gottschall et al.) 03 February 2011 (03.02.2011), entire document	1-36
A	WO 2001/047921 A1 (Erickson et al.) 05 July 2001 (05.07.2001), entire document especially Abstract and claim 1	1-36
A	US 2010/0173889 A1 (Schunk et al.) 08 July 2010 (08.07.2010), entire document	1-36
A	US 2009/0004641 A1 (Gee et al.) 01 January 2009 (01.01.2009), entire document	1-36

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 July 2013 (25.07.2013)	05 AUG 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/32675

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

---------- See Supplemental Box ----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-36

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/32675

Box III: Observations where unity of invention is lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-36: drawn to a composition comprising a non-polypeptide pH switchable reagent which binds a target at a first pH and does not bind the target at a second pH.

Group II: claims 37-41: drawn to a consumable product comprising a pH switchable reagent.

Group III: claims 42-51: drawn to a composition comprising a protein content, wherein the protein content is obtained from a plant; wherein the composition is substantially free of odorants/colorants found in the plant.

Group IV: claim 52: drawn to a method for identifying a non-polypeptide pH switchable reagent, the method comprising:
a. generating a library of non-polypeptides by tag-directed synthesis;
b. exposing at least a subset of the library to a protein in a solution at a first pH;
c. altering the pH of the solution to a second pH; and
d. identifying a non-polypeptide pH switchable peptoid reagent which binds a target at a first pH and does not bind the target at a second pH.

Group V: claims 53-96: drawn to a method for isolating one or more target proteins comprising:
a. applying a composition comprising the one or more target proteins to a substrate; wherein the substrate comprises one or more non-polypeptide pH switchable reagents; wherein at least part of the applying step occurs at a first pH; wherein at the first pH at least one of the one or more target proteins binds to the one or more non-polypeptide pH switchable reagents;
b. releasing the target proteins by adjusting the pH of the solution to a second pH; wherein at the second pH at least one or more of the target proteins does not bind the non-polypeptide pH switchable reagent and;
c. collecting the target proteins that eluted from the one or more non-polypeptide pH switchable reagents.

The inventions listed as Groups I-V do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:
Group II includes the special technical feature of a consumable product, which is not required by Groups I and III-V.

Group III includes the special technical features of a protein content, wherein the protein content is obtained from a plant, wherein the composition is substantially free of odorants/colorants found in the plant, which is not required by Groups I-II and IV-V.

Group IV includes the special technical features of a method for identifying a non-polypeptide pH switchable reagent, the method comprising:
a. generating a library of non-polypeptides by tag-directed synthesis;
b. exposing at least a subset of the library to a protein in a solution at a first pH; and
d. identifying a non-polypeptide pH switchable peptoid reagent which binds a target at a first pH and does not bind the target at a second pH, which are not required by Groups I-III and V.

Group V includes the special technical features of a method for isolating one or more target proteins comprising:
a. applying a composition comprising the one or more target proteins to a substrate; wherein the substrate comprises one or more non-polypeptide pH switchable reagents; wherein at least part of the applying step occurs at a first pH; wherein at the first pH at least one of the one or more target proteins binds to the one or more non-polypeptide pH switchable reagents;
b. releasing the target proteins by adjusting the pH of the solution to a second pH; wherein at the second pH at least one or more of the target proteins does not bind the non-polypeptide pH switchable reagent and;
c. collecting the target proteins that eluted from the one or more non-polypeptide pH switchable reagents, which are not required by Groups I-IV.

Shared Technical Features:
Groups I-V share the technical features of a composition comprising a non-polypeptide pH switchable reagent which binds a target at a first pH and does not bind the target at a second pH.

Groups IV and V share the technical feature of altering the pH of a solution to a second pH.

However, these shared technical features do not represent a contribution over the prior art, as being anticipated by US 2008/0261202 A1 to Baker et al. (23 Oct 2008) (hereinafter "Baker").

Baker discloses a composition (Abstract; para [0006]) comprising a non-polypeptide pH switchable reagent (para [0006], [0029]-[0032]-Bis-Tris and PolyTris) which binds a target (para [0014]) at a first pH (para [0006], [0126]) and does not bind the target at a second pH (para [0126]), and releasing the target proteins (para [0006], [0014]) by adjusting the pH of the solution to a second pH (para [0006], [0126]).

As the common features were known at the time, these cannot be considered a special technical feature that would otherwise unify the groups.

Therefore, Groups I-V lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: CHEWING GUM CONTAINING WHEAT GLUTEN

(57) Abstract

A digestible, biodegradable chewing gum comprising wheat gluten and a texturizing agent such as calcium carbonate, rice flour or ascorbic acid. The chewing gum can be swallowed and digested, or it can be discarded in ordinary compost waste facilities.

FOR THE PURPOSES OF INFORMATION ONLY

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CHEWING GUM CONTAINING WHEAT GLUTEN

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

This invention is directed to chewing gum compositions comprising wheat gluten and to methods for manufacturing such compositions.

2. DESCRIPTION OF RELATED ART

Conventional chewing gums have achieved broad success in the marketplace. Such chewing gums typically comprise gum base and other components that provide pleasant chewing characteristics. Unfortunately, conventional chewing gums have several drawbacks. Conventional chewing gum bases are not "biodegradable," or digestible, and disposal of conventional chewing gums can cause unsightly litter.

Gluten has been tried as a digestible and biodegradable alternative to conventional chewing gum base. U.S. Patent No. 3,814,815 to Hashimoto et al., issued on June 4, 1974, for example, is directed to the use of a gum base of gluten denatured more than 10%.

Another attempt to use gluten as a gum base, U.S. Patent No. 2,469,861 to Cohoe, issued May 10, 1949, is directed to a chewing gum base comprising a combination of

zein and wheat gluten. United States Patent No. 2,586,675 to Lutz, issued February 19, 1952, is also directed to an edible chewing gum composition comprising wheat gluten.

Japanese Patent No. 52,096,771, published August 13, 1977, is directed to using freeze-dried wheat gluten in chewing gum. Japanese Patent No. 54,044,071, published April 7, 1979, is directed to a wheat gluten chewing gum base made by cross-linking wheat gluten with a protein, polysaccharides, saccharides and oligiosaccharides, such as starch, mannan, agar, gum arabic, curdlan, and dextran. The cross-linking is carried out by application of microwaves and is done in the presence of water.

Japanese Patent No. 52,120,168, published October 8, 1977, is directed to a chewing gum-like food containing 5-50% fibrous milk protein, 5-30% non-edible chewing gum base, 50-95% edible nougat (or both), sweetener and flavoring.

Wheat gluten has also been used an a oil absorbing agent in a conventional, chocolate, chewing gum in Japanese Patent No. 59,055,148, published March 30, 1984, and gluten has been used as a pigment retention agent in conventional chewing gum in Japanese Patent No. 58,094,350, published June 4, 1983.

Gluten has also found use in a variety of fields. U.S. Patent No. 3,409,440 to Hobl, issued November 5, 1968 is directed to a heat stable wheat protein suspension by bringing denatured wheat gluten into an aqueous suspension and establishing a pH of 3.7 to 4.9 by the addition of a food acid, for use in bakery goods. United States Patent No. 2,461,829 to Lowen is directed to the use of wheat gluten in medicinal capsules.

Despite the use of gluten in other fields, gluten's use in the chewing gum field has been hampered by a number of organoleptic factors, and no gluten based chewing gum has gained wide acceptance in the American market, regardless of the advantages of a degradable, edible chewing gum.

SUMMARY OF THE INVENTION

It is an object of the invention to provide a digestible, degradable, gluten-based chewing gum having acceptable flavor, sweetness and organoleptic qualities.

It is also an object of the invention to provide unique texturizing agents for a protein and carbohydrate based chewing gum.

It is another object of the invention to provide a method for making a chewing gum from digestible and degradable materials.

Additional objects and advantages of the invention will be apparent in part from the following description of the invention.

To achieve the objects of the invention, as broadly set out herein, the invention comprises a long-chewing composition prepared from hydrated grain protein and other ingredients to mimic the texture and chew properties of chewing gum. Gluten, particularly wheat gluten, is elastic and long chewing, but it is difficult to hydrate uniformly, is prone to clumping and has a blubbery texture that makes it unsatisfactory as a chewing gum ingredient. The chew texture of gluten is softened by the addition of calcium carbonate or glutinous rice flour to wheat gluten. Whey protein, rice protein concentrate and polydextrose or glycols such as glycerin and propylene glycol, may also be used. Other suitable softening agents include ascorbic acid and other ingredients commonly used as dough conditioners or that enter into redox reactions with wheat gluten.

The invention also provides controlled flavor release over an extended duration. As a result, a lower level of flavor may be used compared to conventional chewing gums.

DESCRIPTION OF PREFERRED EMBODIMENTS

The presently preferred embodiments of the invention will now be set forth.

The invention comprises gluten as a substantial portion of the chewing gum base. Preferably, the gluten is wheat gluten, and most preferably the wheat gluten is vital wheat gluten or some other nondenatured wheat gluten. The gluten may comprise from about 15% to about 75% by weight of the final composition, preferably from about 20% to about 65% by weight, and most preferably from about 30% to about 55% by weight. Higher amounts of gluten will make the chewing gum composition unacceptably tough, due to not enough water being present in the composition to hydrate the matrix formed by the gluten, and too little gluten will prevent the chewing gum from forming an adequate matrix to make a chewing gum bolus.

A blend of spray dried and flash dried wheat glutens combine to produce a texture which more closely mimics that of chewing gum than does either gluten type alone. The spray dried gluten is chemically treated to permit the atomization required for spray drying.

Increasing the ratio of flash dried to spray dried gluten increases the piece hardness and chew texture.

Higher levels of spray dried gluten produce a smoother, more evenly hydrated product. The particular ratio desired depends upon other formulation ingredient levels, notably those of water, glycerin and the texture modifiers outlined below.

Hydrated wheat gluten forms an elastic bonding network that can be used as a chewing gum. Gluten alone, however, does not provide an organoleptically acceptable chewing gum. It is prone to crumbling and is difficult to hydrate uniformly to form an acceptable chewing gum bolus in the mouth.

Chewing gum compositions of the invention typically include water as an ingredient. Water may comprise from about 10% to about 40% by weight of the chewing gum composition, preferably from about 15% to about 30%, and most preferably from about 20% to about 25%.

In order to overcome the limitations of using gluten alone as a gum base, the chewing gum of the invention may additionally comprise a texturizing agent. The texturizing agent should be degradable and edible like wheat gluten, and should act to modify the elastic bonding network of the gluten without disrupting the matrix and assist in uniform hydration of the gluten.

Calcium carbonate is a preferred texture modifier. Other salts such as dicalcium phosphate, tricalcium

phosphate, magnesium stearate and the like are also appropriate. Preferably, the calcium carbonate comprises from about 3 to about 20% by weight of the chewing gum composition, more preferably from about 3 to about 10% and most preferably from about 5 to about 8%.

Certain carbohydrates have proven to be acceptable texturizing agents. One such agent is glutinous rice flour, a mixture of carbohydrates and proteins. Preferably, the rice flour comprises from about 5% to about 20% by weight of the chewing gum composition, more preferably from about 6% to about 18%, and most preferably from about 7% to about 15%. The most preferred level is 9.2%. The addition of rice flour improves the texture of the chewing gum. Other grain flours, such as bread flour and corn starch, that improve the texture of the chewing gum can also be used. Some other flours, however, can make the gluten matrix disintegrate.

Polydextrose may also act as a texturizing agent for gluten-based chewing gums. Glucose, sucrose and polyols such as sorbitol or mannitol, however, are not acceptable at levels used in conventional chewing gums. Preferably, the polydextrose may comprise from about 5% to about 20% by weight of the chewing gum composition, more preferably from about 6% to about 18%, and most preferably from about 7% to about 15%.

Certain commercially available proteins may also be used as texturizing agents. Whey protein and rice protein concentrates are especially preferred for use as texturizing agents. Not all proteins, however, are useful as texturizing agents. Soy proteins and pea proteins cause the gluten network to disintegrate. Preferably the texturizing protein should comprise from about 5% to about 20% by weight of the chewing gum composition, more preferably from about 6% to about 18%, and most preferably from about 7% to about 15%.

When used together, the texturizing protein and the texturizing carbohydrate should comprise from about 5% to about 20% by weight of the chewing gum composition, more preferably from about 6% to about 18%, and most preferably from about 7% to about 15%.

Another texturizing agent that may be used in conjunction with the texturizing agents discussed above is ascorbic acid. Ascorbic acid produces a less lumpy, noticeably smoother texture to the chewing gum. Preferably the ascorbic acid should comprise up to about 1% of the chewing gum, by weight. Other ingredients commonly used as dough conditioners or ingredients that enter into oxidation-reduction (redox) reactions with wheat gluten may also be used.

Acidulants may also be added to the chewing gum in limited amounts. Acidulants include, but are not limited to, edible acids typically used in food products for flavor. Preferred acids include acetic, citric and lactic acid.

Flavors and intense sweeteners may also be added in appropriate amounts. One advantage of the invention is that flavors and sweeteners are more fully and more evenly released by the chewing gum of the invention than is the case with conventional chewing gums. Flavors and intense sweeteners may comprise up to about 1% by weight of the chewing gum.

Other agents may be added to the chewing gum to bring about various refinements in the organoleptic or processing qualities of the chewing gum. Hydrocolloids, such as agar, acacia, guar, carrageenan, pectin and alginates; amino acids, such as cysteine and protein hydrolysates; processing aids such as magnesium stearate and microcrystalline cellulose; antimicrobial agents, such as sorbates, benzoates and propionates; antioxidants such as BHA and BHT; acidulants such as citric acid; colors and dyes, such as carotenoids, certified dyes and lakes and natural color; celluloses, such as cellulose gum; processing agents, such as chelating agents, dough conditioners and release agents; emulsifiers, such as acetylated monoglycerides, glycerol esters and lecithin; enzymes, such as amylases, cellulases

and proteases; grains and flours such as wheat, rice, barley, buckwheat and pea; proteins such as lactalbumin, ovalbumin, and milk solids; carbohydrates, such as maltodextrins, dextrins and hydrogenated starch hydrolysates; bulk fillers such as dietary fiber, yeast cell walls; calcium carbonate, talc, dicalcium phosphate and the like; humectants such as glycerin, sorbitol, propylene glycol, 1-3 hexylene glycol and triacetin; starches such as corn, wheat and modified starches; fruits, dried fruits and fruit concentrates; and zein and other film forming agents such as carboxymethyl cellulose.

The method for preparing the chewing gum of the invention begins with blending wheat gluten and texturizing agents such as calcium carbonate or glutinous rice flour along with other ingredients such as potassium sorbate, sweetener and flavors. Any conventional blending process can be used, but dry blending is preferred. Liquids, such as flavor oils, may be dispersed onto the blend of dry powders.

The flavored blend is then placed in a mixing kettle, and glycerin, ascorbic acid and water are added in the mixer.

In alternative embodiments, the liquid components, including the glycerin and water, may be added to the powder in a low-shear mixer, such as a planetary mixer, and then

the mixing may be completed in a higher-shear mixer. Or, the dry-blend powder may be combined with the liquids in a low-shear mixer, and the resulting mixture may be extruded through an extruder, preferably a twin screw extruder, with sufficient energy or shear to form the gluten bonding network. A continuous extrusion process, in which the dry powders and liquids are metered into and mixed in an extruder barrel, is another acceptable method for preparing the chewing gum of the invention.

The different techniques set out above for making the inventive chewing gum affect the extent of gluten bonding. The site of water and glycerin introduction and the amount of shear or heat given to the product affect the amount of gluten bonding, which in turn affects the organoleptic qualities of the chewing gum. Too much shear or heat will destroy the matrix, while not enough will give a lumpy texture. One advantage of the addition of the ingredients directly to the extruder is that gluten bonding and piece formation may take place simultaneously.

The following examples will demonstrate some aspects of the invention, but they should not be considered as limiting the scope or spirit of the invention.

Example 1

The following ingredients were combined in a mixing kettle and blended until a uniform product was obtained:

Ingredient	Weight Percent
Wheat Gluten	37.48%
Rice Flour	4.70%
Unmodified Corn Starch	4.69%
Calcium Carbonate	3.00%
Titanium Dioxide	0.50%
Potassium Acesulfame (ACE-K)	0.28%
Potassium Sorbate	0.30%
Peppermint Flavor	0.55%
Ascorbic Acid	0.50%
Glycerin	33.00%
Water	15.00%
Total	100.00%

The kettle was operated at room temperature and blending took from 5 to 10 minutes. The product has a soft, desirable texture and a pleasing taste.

Examples 2-3

The composition of Example 1 was prepared by using a low shear "dough" and strengthening the bonding matrix by shear extrusion. The composition of Example 1 was also prepared by adding the liquid ingredients (along with ascorbic acid) to the blended dry ingredients through different ports in a mixing extruder.

Examples 4-6

The effect of calcium carbonate was evaluated by preparing the following compositions using the method of Example 1.

Ingredient	Example 4 Wt. Percent	Example 5 Wt. Percent	Example 6 Wt. Percent
Wheat Gluten	31.67%	33.67%	29.67%
Rice Flour	14.70%	14.70%	-
Bread Flour	-	-	14.70%
Calcium-Carbonate	3.00%	1.00%	5.00%
Ace-K	0.28%	0.28%	0.28%
Potassium-Sorbate	0.30%	0.30%	0.30%
Flavor	0.55%	0.55%	0.55%
Ascorbic Acid	0.50%	0.50%	0.50%
Glycerin	33.00%	33.00%	33.00%
Water	16.00%	16.00%	16.00%
Total:	100.00%	100.00%	100.00%

The chewing gum containing 3.00% Calcium Carbonate was preferred, although the other chewing gums were satisfactory.

Examples 7-8

Dicalcium phosphate is a salt often used in chewing gum. A comparative test was made to determine whether this salt was preferable to Calcium Carbonate at 5.00% levels in the chewing gum of the invention. The following compositions were prepared using the method of Example 1.

Ingredient	Example 7 Wt. Percent	Example 8 Wt. Percent
Wheat Gluten	30.87%	30.87%
Rice/whey-protein blend	13.50%	13.50%
Calcium-Carbonate	5.00%	-
Dicalcium-phosphate	-	5.00%
Ace-K	0.28%	0.28%
Potassium-Sorbate	0.30%	0.30%
Flavor	0.55%	0.55%
Ascorbic Acid	0.50%	0.50%
Glycerin	33.00%	33.00%
Water	16.00%	16.00%
Totals:	100.00%	100.00%

Although both calcium carbonate and dicalcium phosphate provided functional chewing gums, the calcium carbonate-containing chewing gum had better characteristics.

Examples 9-11

Ascorbic acid proved to be a suitable texture modifier for wheat gluten and produced a smoother, more consistent texture. Three chewing gum compositions were prepared as in Example 1.

Ingredient	Example 9 Wt. Percent	Example 10 Wt. Percent	Example 11 Wt. Percent
Wheat Gluten	34.67%	34.92%	34.42%
Rice/milk-protein blend	15.00%	15.00%	15.00%
Ace-K	0.28%	0.28%	0.28%
Flavor	0.55%	0.55%	0.55%
Ascorbic Acid	0.50%	0.25%	0.75%
Glycerin	33.00%	33.00%	33.00%
Water	16.00%	16.00%	16.00%
Totals:	100.00%	100.00%	100.00%

Of these formulations, 0.5% ascorbic acid was preferable to 0.25% and 0.75%. At higher levels, such as 1.5% to 4%, the taste of the product was not as good as at

lower levels. The observed effect of ascorbic acid was not as an acidulant alone, because a comparative example made with 0.5% citric acid did not provide the same texturizing benefit as ascorbic acid.

Examples 12-17

Sugar or sugar alcohols are used in chewing gum for bulk sweetness and to soften the gum base. These carbohydrates are typically used at between 50 and 75% by weight of the chewing gum. In order to test the compatibility of sugar and sugar alcohols with the invention, the following compositions were prepared using the method of Example 1.

Ingredient	Example 12 Wt. Percent	Example 13 Wt. Percent	Example 14 Wt. Percent
Wheat Gluten	30.87%	27.40%	23.90%
Rice/whey-protein blend	13.50%	11.97%	10.47%
Mannitol	5.00%	10.00%	15.00%
Ace-K	0.28%	0.28%	0.28%
Potassium Sorbate	0.30%	0.30%	0.30%
Flavor	0.55%	0.55%	0.55%
Ascorbic Acid	0.50%	0.50%	0.50%
Glycerin	33.00%	33.00%	33.00%
Water	16.00%	16.00%	16.00%
Totals:	100.00%	100.00%	100.00%

Ingredient	Example 15 Wt. Percent	Example 16 Wt. Percent	Example 17 Wt. Percent
Wheat Gluten	30.87%	27.40%	23.90%
Rice/whey-protein blend	13.50%	11.97%	10.47%
Sucrose	5.00%	10.00%	15.00%
Ace-K	0.28%	0.28%	0.28%
Potassium Sorbate	0.30%	0.30%	0.30%
Flavor	0.55%	0.55%	0.55%
Ascorbic Acid	0.50%	0.50%	0.50%
Glycerin	33.00%	33.00%	33.00%
Water	16.00%	16.00%	16.00%
Totals:	100.00%	100.00%	100.00%

The texture of the inventive composition was compatible only with lower levels (e.g., 5%) of sugar and sugar alcohols. At these levels the added sweetness from the sugar or sugar alcohol was not significant.

Examples 18-20

Sugars and sugar alcohols likely interrupt the gluten matrix by more strongly bolding the available water at the expense of the proteins in gluten. This competition for water is strongly influenced by the difference in molecular weight between sugars and proteins. A higher molecular weight carbohydrate would be expected to have better performance. Polydextrose was tested as a texturizing agent by adding it as an ingredient in the following examples made by the process of Example 1.

Ingredient	Example 18 Wt. Percent	Example 19 Wt. Percent	Example 20 Wt. Percent
Wheat Gluten	30.87%	27.40%	23.90%
Rice/whey-protein blend	13.50%	11.97%	10.47%
Polydextrose	5.00%	10.00%	15.00%
Ace-K	0.28%	0.28%	0.28%
Potassium Sorbate	0.30%	0.30%	0.30%
Flavor	0.55%	0.55%	0.55%
Ascorbic Acid	0.50%	0.50%	0.50%
Glycerin	33.00%	33.00%	33.00%
Water	16.00%	16.00%	16.00%
Totals:	100.00%	100.00%	100.00%

Polydextrose proved to have acceptable performance over the tested range.

Examples 21-22

Glycerin was compared to sorbitol as a humectant. The following compositions were prepared by the method of Example 1.

Ingredient	Example 21 Wt. Percent	Example 22 Wt. Percent
Wheat Gluten	44.00%	45.15%
Whey protein	9.65%	10.00%
Ace-K	0.28%	0.28%
Flavor	0.55%	0.55%
Glycerin	28.02%	14.01%
Sorbitol Solution (70%)	-	19.01%
Water	17.50%	11.00%
Totals:	100.00%	100.00%

Glycerin was found to be the preferred humectant. In Example 22, the gluten matrix was destroyed.

Examples 23-25

Various proteins were tested as texture modifiers for wheat gluten. The following compositions were prepared in accordance with the procedure set out in Example 1.

Ingredient	Example 23 Wt. Percent	Example 24 Wt. Percent	Example 25 Wt. Percent
Wheat Gluten	45.45%	40.45%	38.65%
Whey protein	10.00%	10.00%	10.00%
Soy protein	-	5.00%	-
Pea protein isolate	-	-	5.00%
Ace-K	0.28%	0.28%	0.28%
Flavor	0.55%	0.55%	0.55%
Glycerin	28.02%	28.02%	28.02%
Water	15.70%	15.70%	17.50%
Totals:	100.00%	100.00%	100.00%

The presence of pea protein or soy protein as a partial replacement for whey protein destroyed the matrix.

It will be apparent to those skilled in the art that various modifications can be made to the formulations set out above.

Examples 26-30

Higher water activity systems were investigated. The following compositions were prepared in accordance with the procedure set out in Example 1.

Ingredient	Example 26 Wt. Percent	Example 27 Wt. Percent	Example 28 Wt. Percent	Example 29 Wt. Percent	Example 30 Wt. Percent
Wheat Gluten	57.12%	49.12%	49.12%	54.12%	51.37%
Calcium Carbonate	10.00%	8.00%	8.00%	3.00%	3.00%
Potassium Sorbate	0.30%	0.30%	0.30%	0.30%	0.30%
Ace-K	0.35%	0.35%	0.35%	0.35%	0.35%
Peppermint Flavor	1.20%	1.20%	1.20%	1.20%	1.20%
Titanium Dioxide	0.50%	0.50%	0.50%	0.50%	0.50%
Ascorbic Acid	0.50%	0.50%	0.50%	0.50%	0.50%
Water	30.00%	25.00%	20.00%	20.00%	16.00%
Glycerin	-	15.00%	20.00%	20.00%	26.75%
Totals:	100.00%	100.00%	100.00%	100.00%	100.00%

In general, tighter, tougher and more chewy textures resulted, with higher water activity, but higher water activity systems require microbiological control and stabilization.

WHAT IS CLAIMED IS:

1. A chewing gum base composition comprising:

(a) gluten; and

(b) a texturizing agent.

2. The chewing gum of claim 1, wherein said gluten is wheat gluten.

3. The chewing gum of claim 2, wherein said wheat gluten is vital wheat gluten.

4. The chewing gum of claim 1, wherein said texturizing agent is selected from the group consisting of rice flour, corn starch, polydextrose and calcium carbonate.

5. The chewing gum of claim 1, further comprising an additional texturizing agent.

6. The chewing gum of claim 5, wherein said additional texturizing agent is selected from the group consisting of whey proteins, rice proteins and ascorbic acid.

7. A chewing gum base composition comprising:

(a) gluten; and

(b) a protein-based texturizing agent.

8. The chewing gum of claim 1, wherein said gluten is wheat gluten.

9. The chewing gum of claim 2, wherein said wheat gluten is vital wheat gluten.

10. The chewing gum of claim 7, wherein said protein-based texturizing agent is selected from the group consisting of whey proteins and rice proteins.

11. The chewing gum of claim 7, further comprising an additional texturizing agent.

12. The chewing gum of claim 11, wherein said additional texturizing agent is selected from the group consisting of rice flour, polydextrose and ascorbic acid.

INTERNATIONAL SEARCH REPORT

Int. onal Application No
PCT/US 94/01175

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A23G3/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A23G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,1 700 387 (L.W. STETSON) 29 January 1929 see the whole document ---	1-3,8,9
X	US,A,2 586 675 (W.J. LUTZ) 19 February 1952 cited in the application see claims ---	1-3,8,9
X	US,A,2 469 861 (W.P. COHOE) 10 May 1949 cited in the application see claims; examples ---	1-3,5, 7-9,11

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 April 1994	04.05.94
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Lepretre, F

INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR,A,2 156 530 (THE EZAKI GLICO KK) 1 June 1973 see page 3, line 10 - line 16 see page 5, line 30 - page 6, line 27 & US,A,3 814 815 (HASHIMOTO) 4 June 1974 cited in the application ---	1-3,5, 7-9,11
A	DATABASE FSTA INTERNATIONAL FOOD INFORMATION SERVICE (IFIS), FRANFURT/MAIN, DE AN=91:111153 August 1991, J.M. HESSER 'World food uses of vital wheat gluten' & PROCEEDINGS OF THE WORLD CONGRESS ON VEGETABLE PROTEIN UTILIZATION IN HUMAN FOODS AND ANIMAL FEEDSTUFFS October 1988 , SINGAPORE, SG ---	1-3,8,9
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2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 94/01175

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-1700387		NONE	
US-A-2586675		NONE	
US-A-2469861		NONE	
FR-A-2156530	01-06-73	JP-A- 48056864	09-08-73
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		FR-A,B 2156530	01-06-73
		GB-A- 1334332	17-10-73
		NL-A- 7205956	25-04-73

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

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

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Application Number:	14796970
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Confirmation Number:	8501
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First Named Inventor/Applicant Name:	Marija Vrljic
Customer Number:	26191
Filer:	M. Angela Parsons/Nikki Krahler
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Payment Type	Deposit Account
Payment was successfully received in RAM	$180
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Deposit Account	061050
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The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	IDS_Transmittal.pdf	61409 c45b7a39076f9da83d37acfd6538fbbdb69fc84d	no	1
Warnings:					
Information:					
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2	Information Disclosure Statement (IDS) Form (SB08)	IDS-1449.pdf	184184 e0837c65f7640afefe1d6359b26fbb145521c37a	no	12
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3	Foreign Reference	CN1252231X.pdf	2703526 5bf1b4fefda0980d812f211d165ec4a4d5a2aad0	no	6
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4	Foreign Reference	CN1301811.pdf	2068718 60373b258143e4867e1365b32279c498b5a9edfe	no	10
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Information:					
5	Foreign Reference	CN1407108X.pdf	7022678 f9b565d6c38b0807603d640abf72a88585f5d2ff	no	15
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6	Foreign Reference	CN1593223X.pdf	3924247 456f9c206f7de402a08eb6df390e3741822b14ca	no	7
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7	Foreign Reference	CN1634524.pdf	851412 dbcd343e4d6498cb29bf2dae2374c11a39341c2e	no	7
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8	Foreign Reference	CN101138405.pdf	414361 35e77f637117d9941f09e648a7fab8ab9e7a8937	no	6
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9	Foreign Reference	CN101541187x.pdf	4683360 177b5108abb6bfcc347d4fdfa83fd6be1f21957a	no	40
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10	Foreign Reference	CN101606574X.pdf	6539332 4b379ca6029cf9483481c038672a3cfe5ff34fcd	no	14
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14	Foreign Reference	EP1166653.pdf	6184536 c795d0921a0bf25069b5839e09a16ac51c7ebb28	no	12
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15	Foreign Reference	EP1254601.pdf	2577348 d631eb9b20e5cd841149baf631f0c6dbb42b3f3d	no	22
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16	Foreign Reference	EP1361264.pdf	332209 9fe19a1d3acd2e0ced9929b41018912cc9de3056	no	40
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17	Foreign Reference	EP1952695.pdf	307183 0886fd7dbb7e1dd0c5e6db978bb171e34826d68d	no	13
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18	Foreign Reference	EP2138052.pdf	970942 d0482c0203d4e0902f8daf25af464605dd574b7b	no	11
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19	Foreign Reference	GB2016255.pdf	952070 050c781bf7236501c9ec24435ded1bb20aea48b4	no	6
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20	Foreign Reference	JP52156962.pdf	341029 7a2c12141626bc852556d1ad71f55ccf579f3a19	no	8
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Information:					
21	Foreign Reference	WO9853698A1x.pdf	6767424 d4b8c26578e0d6d80c8596489db6241fd4b8cd88	no	37
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Information:					
22	Foreign Reference	WO1993025697.pdf	13996560 84cb303e6ab47070aed343bc2073630f4f48f98c	no	122
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23	Foreign Reference	WO1996017981.pdf	2701242 0214fd4069c3f0b4d190e4246728986fe032d0ee	no	24
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24	Foreign Reference	WO1998012913.pdf	5670099 7e4a0c71d4b20e108136fc9f599c4d8c39c551b1	no	44
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25	Foreign Reference	WO2001022829.pdf	1437787 4f2ceba3884a472407073525ce314cdf37863666	no	13
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26	Foreign Reference	WO2001022830.pdf	2437428 444f691f89f87571e96de0721783898e82e2f6b1	no	20
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27	Foreign Reference	WO2003070172x.pdf	5680553 7a2b50b93ddb2b64c2feb104e632aaef1352c72d	no	38
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Information:					
28	Foreign Reference	WO2004113543.pdf	6818220 d382814b70bb15b082bfba9ad2b0685a7fa9c939	no	53
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29	Foreign Reference	WO2005013713.pdf	5132974 75955eeb9092d116187e443ea4afffc36a262985	no	46
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Information:					
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Information:					
31	Foreign Reference	WO2006042608.pdf	2451974 659d42409763893e4aeb7e28086d02d798fd567c	no	21
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Warnings:					
Information:					

35	Foreign Reference	WO2008017499.pdf	4994307 1ce1aaa6a18bd3fe5b4a6ec3be5a8d2e673992ae	no	43
Warnings:					
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36	Foreign Reference	WO2008030089.pdf	3634328 87994d83addeed446af173efe35ab6bf88cee478	no	33
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37	Foreign Reference	WO2008083117.pdf	10076684 734c53f3b98386f419403962118ba7209aac4d8c	no	65
Warnings:					
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38	Foreign Reference	WO2009060678.pdf	4864493 32dab6d4d837489ba2c221ff55676cfb77f89cd2	no	35
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39	Foreign Reference	WO2010101625.pdf	1798921 f015bceece61bbebcc126d04899b211a69e8bd41	no	98
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40	Foreign Reference	WO2012106751.pdf	6732876 b0a0e64f73c18d347a9e02d0d5331b05561d7f2a	no	56
Warnings:					
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41	Foreign Reference	WO2012110797.pdf	2758976 f448cfc8c74492379befb2d7af90fe8e2833b041	no	27
Warnings:					
Information:					
42	Foreign Reference	WO2012116703.pdf	4775014 48e89c10a5870b2a404bd408c5b803c7fde2858b	no	49
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43	Foreign Reference	WO2013010037.pdf	7689982 3da0e3b4f95c4b0a794c765c5e8ccc36c6186ad1	no	59
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44	Foreign Reference	WO2013010042.pdf	11300376 47f986633cbd1184d8be9afecc86265fcf58f961	no	81
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45	Foreign Reference	WO2013138793.pdf	3356751 ee240bb048aa454efb538fe414eac833a98a3731	no	60
Warnings:					
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46	Non Patent Literature	Acidified_Milk_Products.pdf	925471 27e6fafcafe9db112cdd24828b1cdf8e0ef3a5e6	no	15
Warnings:					
Information:					
47	Non Patent Literature	Ba_etal.pdf	762226 a59f1c619ae6544ffdb9ae1fe70a68e5a0845c33	no	32
Warnings:					
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48	Non Patent Literature	Baek_ProcessFlavors.pdf	109284 9d155f7dafb62760abaf1e21bfb96abfb639b524	no	14
Warnings:					
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49	Non Patent Literature	Battaglia_etal.pdf	1446620 be4f5342f12731a74d9822d95fdd9e99abb2cf2d	no	19
Warnings:					
Information:					
50	Foreign Reference	WO1994017673.pdf	2882130 ce78f2493fdf7b67c7bdaa3a6d076d2816bc9923	no	28
Warnings:					
Information:					
51	Non Patent Literature	Ice_Cream_and_Ice_Cream_Desserts.pdf	125807 1062b5a62b3997eeef37ecfe546872400180bb8	no	5
Warnings:					
Information:					
52	Non Patent Literature	Innovation_at_Its_Best_Food_Valley.pdf	6261273 bfc619d0098123cfd08a0bc9622c94e6ba649df0	no	51
Warnings:					
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53	Non Patent Literature	Low_Methylester_Amidated_Pectins.pdf	418741 62dfdb0cf1f291b8c3f2e29ff04705db8de1e39f	no	13
Warnings:					
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54	Non Patent Literature	Silcon_valley_gets.pdf	4583384 53813e3e3b451743c033002e7ac7a6354a5f2521	no	3
Warnings:					
Information:					
55	Non Patent Literature	Stabillisation_of_Whey.pdf	200426 252eb9cdd3734c91b9454798008b28e6d113fdfb	no	6
Warnings:					
Information:					
56	Non Patent Literature	Texturising_of_Ferm_Milk_Products.pdf	125342 30d035811d903dfbe1418a204967e44d2603faa3	no	6
Warnings:					
Information:					
57	Non Patent Literature	Beuchat_Fermentation_of_Peanut.pdf	870072 633223a6858cffdc8a4a2f4bcf69b7cd5008969b	no	4
Warnings:					
Information:					
58	Non Patent Literature	BeyondBetter_orderpage.pdf	832325 dfb06b607ccf2e00ac4bcdf808d2e0093b657a0d	no	8
Warnings:					
Information:					
59	Non Patent Literature	beyondmeat.pdf	53864 e4960f87eb329621e0da17c66236ef6b958ddeea	no	2
Warnings:					
Information:					
60	Non Patent Literature	Boca_Bruschetta.pdf	91599 ab1a5aa32ece4c96b32112e3fe732a05970875b8	no	1
Warnings:					
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61	Fee Worksheet (SB06)	fee-info.pdf	30809 243535f0e55bc7b74d3fcb7ddcd395741f11153f	no	2
Warnings:					
Information:					

Total Files Size (in bytes):	193643121

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

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: Monica McCormick Graham
Fish & Richardson P.C.
P.O. Box 1022
Minneapolis, Minnesota 55440-1022
United States of America

PCT

INVITATION TO PAY ADDITIONAL FEES
AND, WHERE APPLICABLE, PROTEST FEE

(PCT Article 17(3)(a) and Rules 40.1 and 40.2(e))

	Date of mailing *(day/month/year)* 10 APR 2014
Applicant's or agent's file reference 387670010WO1	**PAYMENT DUE** within **ONE MONTH** from the above date of mailing
International application No. PCT/US14/11361	International filing date *(day/month/year)* 13 January 2014 (13.01.2014)
Applicant MARAXI, INC.	

1. This International Searching Authority

   (i) considers that there are 12+ *(number of)* inventions claimed in the international application covered by the claims indicated below/on an extra sheet:

   -***-Please See Below-***-

   (ii) therefore considers that **the international application does not comply with the requirement of unity of invention** (Rules 13.1, 13.2 and 13.3) for the reasons indicated below/on an extra sheet:

   -***-Please See Below-***-

   (iii) ☐ has carried out a partial international search (see Annex) ☒ will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.:

   Group I: Claims 1-9, 17-22, 52-54

   (iv) will establish the international search report on the other parts of the international application only if, and to the extent to which, additional fees are paid.

2. Consequently, the applicant is hereby **invited to pay**, within the time limit indicated above, additional fees in the amount indicated below:

   $2,060.00 (Fee per additional invention) x 12+ (number of additional inventions) = $24,960.00+ (total amount of additional fees/currency) (See Item 2 of annex)

3. The applicant is informed that, according to Rule 40.2(c), **the payment of any additional fees may be made under protest**, that is, a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fees is excessive, where applicable, subject to the payment of a protest fee.

   Where the applicant pays additional fees under protest, the applicant is hereby invited, within the time limit indicated above, to pay a protest fee (Rule 40.2(e)) in the amount of _____ *(amount/currency)*

   Where the applicant has not, within the time limit indicated above, paid the required protest fee, the protest will be considered not to have been made and the International Searching Authority will so declare.

4. ☒ Claim(s) Nos. -***-Please See Supplemental Page-***- have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300

Form PCT/ISA/206 (April 2005)

INVITATION TO PAY ADDITIONAL FEES
AND, WHERE APPLICABLE, PROTEST FEE

International application No.
PCT/US14/11361

Item 2 (continued). For International Applications filed on or after 01 January 2014, Applicant is reminded that the search fee per additional invention indicated in item 2 is the undiscounted fee per additional invention. An Applicant may pay the search fee per additional invention fee reduced by 50% (small entity assertion) or 75% (micro entity certification), as appropriate. See 37 CFR 1.27 and 1.29.

-***-Continued from 4.: Claim(s) Nos. 10-16, 23-27, 40-46, 50, 55-58, 66, 67, 79, 80, 84-90 have been found to be unsearchable under Article 17(2)(b) because defects under Article 17(2)(a) and thereof have not been included with any invention.

-Continued from 1. (i):

Group I: Claims 1-9, 17-22, 52-54; Group II: Claims 28-31, 37-39, 68-70, 81-83; Group III: Claims 32-36, 61-65; Group IV: Claims 47-49; Group V: Claim 51; Groups VI+: Claims 59, 60, SEQ ID NO: 1 (Vigna radiata amino acid sequence); Groups VII+: Claims 71-73, SEQ ID NO: 1 (vigna radiata amino acid sequence); Group VIII: Claims 74-78; Group IX: Claims 91-93; Group X: Claim 94; Group XI: Claims 95-99; Group XII: Claims 100-104

-Continued from 1. (ii):

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-9, 17-22 and 52-54 are directed toward a consumable product comprising an isolated and purified plant protein, wherein the isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2 degrees C and about 32 degress C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM, and a method of making said consumable product.

Group II: Claims 28-31, 37-39, 68-70 and 81-83 are directed toward a meat replica comprising a muscle replica, a connective tissue replica; an adipose tissue replica, and a coacervate comprising one or more isolated and purified proteins, said adipose tissue replica including one or more isolated plant proteins, one or more plant or algal derived oils, optionally a phospholipid, and a three-dimensional matrix comprising fibers derived from non-animal sources, wherein said lipid and said protein are dispersed in said three-dimensional matrix, and wherein said three-dimensional matrix stabilizes the structure of the adipose tissue replica; and wherein the connective tissue replica comprises proteins assembled into fibrous structures by a solution spinning process.

Group III: Claims 32-36 and 61-65 are directed toward a consumable product comprising a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt, and a method of making said consumable product.

Group IV: Claims 47-49 are directed toward a consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, wherein said consumable product does not comprise meat.

Group V: Claim 51 is directed toward a consumable product comprising a gelled emulsion, said gelled emulsion comprising: a) an isolated and purified protein; b) a first lipid that when not in said consumable product is solid at a selected temperature range; and c) a second lipid that when not in said consumable product is liquid at said selected temperature range; wherein the melting temperature of the mixture of said first and second lipids is similar to the melting temperature of lipids found in meat, and wherein said first and second lipids are plant derived lipids or microbial derived lipids.

Groups VI+: Claims 59, 60 and SEQ ID NO: 1 are directed toward a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions. (SEQ ID NO: 1-27). + type Markush group.

The method for extending the shelf-life of a meat-free consumable product comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions can be searched to the extent that it encompasses a sequence at least 70% identical to SEQ ID NO: 1 (Vigna radiata amino acid sequence). Additional sequences will searched upon the payment of additional fees. It is believed that Claims 59 and 60 (in-part) encompass this first named invention and thus these claims can be searched, upon appropriate payment, to the extent that they encompass SEQ ID NO: 1 (Vigna radiata amino acid sequence). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group (s) may result in only the first claimed invention to be searched/examined. An exemplary election would be SEQ ID NO: 2 (Methylacidiphilum infernorum amino acid sequence).

Groups VII+: Claims 71-73 and SEQ ID NO: 1 are directed toward a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

Group VIII: Claims 74-78 are directed toward a method of making a coacervate, said method comprising: a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of sodium chloride; and b) isolating said coacervate from said solution.

Group IX: Claims 91-93 are directed toward methods of minimizing undesirable odors in a composition comprising plant proteins.

-***-Continued on Next Supplemental Page-***-

INVITATION TO PAY ADDITIONAL FEES
AND, WHERE APPLICABLE, PROTEST FEE

International application No.
PCT/US14/11361

-***-Continued from Previous Supplemental Page:

Group X: Claim 94 is directed toward a chocolate flavored spread comprising: a) sugar; b) a chocolate flavoring; and c) a cream fraction from a plant based milk.

Group XI: Claims 95-99 are directed toward a method for altering the texture of a consumable during or after cooking comprising incorporating within the consumable one or more plant proteins with a low denaturation temperature.

Group XII: Claims 100-104 are directed toward tissue replicas comprising a freeze-aligned non-animal protein.

The inventions listed as Groups I-XII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2 degrees C and about 32 degrees C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM, not present in any other groups; the special technical features of Group II including a meat replica comprising a muscle replica, a connective tissue replica; an adipose tissue replica, or a coacervate, which are not present in any other group; the special technical features of Group III including a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt, which are not present in any other Group; the special technical features of Group IV including a consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, not present in any other group; the special technical features of Group V including a consumable product comprising a gelled emulsion, said gelled emulsion comprising: a) an isolated and purified protein; b) a first lipid that when not in said consumable product is solid at a selected temperature range; and c) a second lipid that when not in said consumable product is liquid at said selected temperature range; wherein the melting temperature of the mixture of said first and second lipids is similar to the melting temperature of lipids found in meat, which are not not present in any other group; the special technical features of Groups VI+ including a method for extending the shelf-life of a meat-free consumable product, which are not present in any other group; the special technical features of Groups VII+ including a method for imparting a beef like flavor to a consumable product, which are not present in any other group; the special technical features of Group VIII including a method of making a coacervate comprising: a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, which are not present in any other group; the special technical features of Group IX including methods of minimizing undesirable odors in a composition comprising plant proteins, which are present in any other group; the special technical features of Group X including a chocolate flavored spread, which are not present in any other Group; the special technical features of Group XI including a method for altering the texture of a consumable, which not present in any other group; the special technical features of Group XII including a freeze-aligned non-animal protein.

Groups I-XII share the technical features including consumables. Groups I, II, V, VIII, IX, X and XI share the technical features including plants. Groups I, II, VIII, IX and XI share the technical features including plant proteins. Groups I-V, VI+, VII+, VIII, IX, XI and XII share the technical features including proteins. Groups I, III, V, VII+, XI and XII share the technical features including temperature. Groups I and VIII share the technical features including pH. Groups I, III and VIII share the technical features including sodium chloride. Groups I-III, VI+, VII+, VIII and XI share the technical features including a method of making a consumable product. Groups II, III and XII share the technical features including a meat replica. Groups II and VIII share the technical features including a coacervate. Groups II and V share the technical features including plant or algal derived oils or lipids. Groups II and V share the technical features comprising an emulsion. Groups III and V share the technical features including a gel. Groups IV, VI+ and VII+ share the technical features including a heme-containing protein. Groups IV and VI+ share the technical features including meat-free products. Groups VI+ and VII+ share the technical features including a heme-containing protein comprising a sequence at least 70% identical to any of SEQ ID NOs: 1-27.

Groups VI+ share the technical features including a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions.

Groups VII+ share the technical features including a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

However, these shared technical features are previously disclosed by WO 2001/015714 A1 (ZLOTKIN) in view of US 2010/0074998 A1 to Espeleta-Vega et al. (hereinafter 'Espeleta-Vega'), US 2011/0083681 A1 to Sengupta, et al. (hereinafter 'Sengupta'), the publication entitled 'Comparative Analysis Of Autoxidation Of Haemoglobin' by Jensen (hereinafter 'Jensen'), US 4,986,998 A to Yoo, et al. (hereinafter 'Yoo') and GenBank Accession AFK42304 by Krishnakumar, et al. (hereinafter 'Krishnakumar').

Zlotkin discloses consumables (compositions including micronutrients (consumables); abstract) including plants (chicory fructooligosaccharide (plants); page 11, lines 15-19); proteins (proteins; page 15, lines 20-28); temperature (temperature; page 17, lines 10-25); a method of making a consumable product (a process for producing a composition comprising micronutrients (a method of making a consumable product); page 8, lines 22-27); plant or algal derived oils or lipids (soybean oil (plant or algal derived oils or lipids); page 15, lines 1-4); meat-free products (cereals; purees of fruit, vegetables, milk, yogurt, cottage cheese (meat-free products); page 18, lines 1-11), and a method for preventing or reducing the discoloration and taste alteration of a food due to the addition of an iron compound thereto (a method for preventing or reducing the discoloration and taste alteration of a food due to the addition of an iron compound thereto; page 14, lines 21-28).

Zlotkin does not disclose plant proteins; pH, sodium chloride; a meat replica; a coacervate; an emulsion; a gel; a heme-containing protein; a heme-containing protein comprising a sequence at least 70% identical to any of SEQ ID NOs: 1-27; a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions; and a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

-***-Continued on Next Supplemetnal Page-***-

INVITATION TO PAY ADDITIONAL FEES
AND, WHERE APPLICABLE, PROTEST FEE

International application No.
PCT/US14/11361

-***-Continued from Previous Supplemental Page:

Espeleta-Vega discloses plant proteins (vegetable protein (plant proteins); abstract), pH (a pH modifier; paragraph [0010]), sodium chloride (salt (sodium chloride); paragraph [0038]), a meat replica (substitute meat food product (a meat replica); abstract); an emulsion (an emulsion; paragraph [0011]); a gel (a gel; paragraph [0011]).

Sengupta discloses a process for preparing coacervate gel particles comprising plant materials including proteins (a process for preparing coacervate gel particles comprising plant materials including proteins; paragraph [0015]).

Jensen discloses a heme-containing protein (hemoglobin; abstract) wherein the oxidation of hemoglobin and myoglobin is significantly slower than free heme (oxidation of hemoglobin and myoglobin is significantly slower than free heme; page 2029, column 1, paragraph 2), and wherein the oxidation of tetrameric hemoglobin is significantly slower than either monomeric hemoglobin or myoglobin (wherein the oxidation of tetrameric hemoglobin is significantly slower than either monomeric hemoglobin or myoglobin; page 2030, column 1, paragraph 1).

Yoo discloses a method for imparting (flavoring (imparting); abstract) a beef (cow (beef); column 4, lines 26-32) like flavor to a consumable product (flavor to a consumable product; abstract, column 2, lines 49-50), comprising adding to said consumable composition a heme-containing protein (comprising adding to said consumable a powdered blood product (comprising adding to said consumable composition a heme-containing protein); abstract), wherein after cooking, a beef-like flavor is imparted to said consumable (providing a meat-like flavor (beef-like flavor is imparted to said consumable); column 3, lines 1-6).

Krishnakumar discloses a heme-containing protein comprising a sequence at least 70% identical to SEQ ID NO: 1 (a globin (a heme-containing protein comprising a sequence at least 70% identical to SEQ ID NO: 1); page 1).

It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin by combining the consumable disclosed by Zlotkin with a meat replica, such as that disclosed by Espeleta-Vega, for providing the micronutrients in the consumable in a form acceptable to a consumer, such as in a meat replica, for enhancing the nutrient content of a meat replica, particularly the iron content of said replica, providing the previous disclosure of Zlotkin. Also, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin regarding micronutrient-comprising consumables with the coacervate preparation process of Sengupta, for enabling the preparation of particulates comprising the micronutrients disclosed by Zlotkin using a plant polysaccharide, such as pectin, as disclosed by Sengupta, for providing micronutrients in a consumable and easily digestible form to enable a consumer to absorb the nutrients. Furthermore, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin, for including the use of hemoglobin in preference to free iron or other forms of iron, in order to provide the iron to the consumer in a biologically useful form and oxidation state. Moreover, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have therefore modified the disclosure of Zlotkin, for providing a method for extending the shelf-life of a meat-free consumable product, such as the consumables disclosed by Zlotkin, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions, such as adding hemoglobin, based on the disclosure of Jensen that tetrameric hemoglobin oxidizes more slowly than other forms of heme-containing proteins. Also, it would have been obvious to a person of ordinary skill in the art to have modified the disclosure of Zlotkin, for including a meat-like flavoring including an iron-containing protein based on the disclosure of Yoo, in order to provide a beef-like flavor to a consumable food, including a poultry or fish composition, to provide a beef-flavored product not containing beef, but with lower fat, such as a consumable comprising a plant protein, fish, or poultry protein. Finally, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin regarding micronutrient-comprising consumables comprising iron, for including a plant-based iron-comprising proteins, such as the globin disclosed by Krishnakumar, in order to provide iron from non-animal sources having high absorbability and utilization by a consumer.

Since none of the special technical features of the Groups I-XII inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Zlotkin, Espeleta-Vega, Sengupta, Jensen, Yoo and Krishnakumar references, unity of invention is lacking.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: Monica McCormick Graham
Fish & Richardson P.C.
P. O. Box 1022
Minneapolis, MN 55440-1022
United States of America

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day/month/year)* 16 JUN 2014
Applicant's or agent's file reference 387670010WO1	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US14/11361	International filing date *(day/month/year)* 13 January 2014 (13.01.2014)
Applicant Maraxi, Inc.	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

   For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2010)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 387670010WO1	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US14/11361	International filing date *(day/month/year)* 13 January 2014 (13.01.2014)	(Earliest) Priority Date *(day/month/year)* 11 January 2013 (11.01.2013)
Applicant Maraxi, Inc.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 7 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of:
      ☒ the international application in the language in which it was filed.
      ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      ☐ as suggested by the applicant.
      ☐ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☒ none of the figures is to be published with the abstract.

International application No.
PCT/US14/11361

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 10-16, 23-27, 40-46, 50, 55-58, 66, 67, 79, 80, 84-90
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Group I: Claims 1-9, 17-22, 52-54

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/11361

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A23L 1/212 (2014.01)
USPC - 424/478, 479
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A23L 1/212, 1/10, 1/216; A23K 1/100 (2014.01)
USPC: 424/615, 481, 479, 478, 574, 531

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; Google Patents; Science Direct; ProQuest; consumable, plant, 'plant protein,' peptide, coacervate, 'food product,' 'meat substitute,' 'sodium chloride,' soluble, mayo, beverage

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 3,870,801 (TOMBS, MP) March 11, 1975; abstract; column 1, lines 64-65; column 3, lines 12-13; column 6, lines 20-21; column 8, lines 56-57; column 14, lines 3-5; column 19, lines 32-34	1, 2, 8, 9/1, 9/2, 9/8 ----- 3-7, 9/3-9/7, 18, 52, 53, 54/52, 54/53
Y	EP 1361264 B1 (TSUBAKI, K et al.) April 18, 2007; paragraphs [0001], [0029], [0038], [0039], [0044], [0045]	3-7, 9/3-9/7, 19-21, 22/20, 22/21, 52, 53, 54/52, 54/53
X --- Y	US 2013/0004617 A1 (ZHANG, N et al.) January 03, 2013; paragraphs [0004], [0006]	17 ----- 18-21, 22/20, 22/21, 53, 54/53

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 May 2014 (28.05.2014)	16 JUN 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

-***-Continued from Box No. III: Lack of Unity of Invention:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-9, 17-22 and 52-54 are directed toward a consumable product comprising an isolated and purified plant protein, wherein the isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2 degrees C and about 32 degress C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM, and a method of making said consumable product.

Group II: Claims 28-31, 37-39, 68-70 and 81-83 are directed toward a meat replica comprising a muscle replica, a connective tissue replica; an adipose tissue replica, and a coacervate comprising one or more isolated and purified proteins, said adipose tissue replica including one or more isolated plant proteins, one or more plant or algal derived oils, optionally a phospholipid, and a three-dimensional matrix comprising fibers derived from non-animal sources, wherein said lipid and said protein are dispersed in said three-dimensional matrix, and wherein said three-dimensional matrix stabilizes the structure of the adipose tissue replica; and wherein the connective tissue replica comprises proteins assembled into fibrous structures by a solution spinning process.

Group III: Claims 32-36 and 61-65 are directed toward a consumable product comprising a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt, and a method of making said consumable product.

Group IV: Claims 47-49 are directed toward a consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, wherein said consumable product does not comprise meat.

Group V: Claim 51 is directed toward a consumable product comprising a gelled emulsion, said gelled emulsion comprising: a) an isolated and purified protein; b) a first lipid that when not in said consumable product is solid at a selected temperature range; and c) a second lipid that when not in said consumable product is liquid at said selected temperature range; wherein the melting temperature of the mixture of said first and second lipids is similar to the melting temperature of lipids found in meat, and wherein said first and second lipids are plant derived lipids or microbial derived lipids.

Groups VI+: Claims 59, 60 and SEQ ID NO: 1 are directed toward a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions. (SEQ ID NO: 1-27). + type Markush group.

The method for extending the shelf-life of a meat-free consumable product comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions can be searched to the extent that it encompasses a sequence at least 70% identical to SEQ ID NO: 1 (Vigna radiata amino acid sequence). Additional sequences will searched upon the payment of additional fees. It is believed that Claims 59 and 60 (in-part) encompass this first named invention and thus these claims can be searched, upon appropriate payment, to the extent that they encompass SEQ ID NO: 1 (Vigna radiata amino acid sequence). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) may result in only the first claimed invention to be searched/examined. An exemplary election would be SEQ ID NO: 2 (Methylacidiphilum infernorum amino acid sequence).

Groups VII+: Claims 71-73 and SEQ ID NO: 1 are directed toward a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

Group VIII: Claims 74-78 are directed toward a method of making a coacervate, said method comprising: a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, wherein said solution comprises 100 mM or less of sodium chloride; and b) isolating said coacervate from said solution.

Group IX: Claims 91-93 are directed toward methods of minimizing undesirable odors in a composition comprising plant proteins.

Group X: Claim 94 is directed toward a chocolate flavored spread comprising: a) sugar; b) a chocolate flavoring; and c) a cream fraction from a plant based milk.

Group XI: Claims 95-99 are directed toward a method for altering the texture of a consumable during or after cooking comprising incorporating within the consumable one or more plant proteins with a low denaturation temperature.

Group XII: Claims 100-104 are directed toward tissue replicas comprising a freeze-aligned non-animal protein.

-***-Continued Within the Next Supplemental Box-***-

-***-Continued from Previous Supplemental Page:

The inventions listed as Groups I-XII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2 degrees C and about 32 degrees C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM, not present in any other groups; the special technical features of Group II including a meat replica comprising a muscle replica, a connective tissue replica; an adipose tissue replica, or a coacervate, which are not present in any other group; the special technical features of Group III including a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt, which are not present in any other Group; the special technical features of Group IV including a consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, not present in any other group; the special technical features of Group V including a consumable product comprising a gelled emulsion, said gelled emulsion comprising: a) an isolated and purified protein; b) a first lipid that when not in said consumable product is solid at a selected temperature range; and c) a second lipid that when not in said consumable product is liquid at said selected temperature range; wherein the melting temperature of the mixture of said first and second lipids is similar to the melting temperature of lipids found in meat, which are not not present in any other group; the special technical features of Groups VI+ including a method for extending the shelf-life of a meat-free consumable product, which are not present in any other group; the special technical features of Groups VII+ including a method for imparting a beef like flavor to a consumable product, which are not present in any other group; the special technical features of Group VIII including a method of making a coacervate comprising: a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, which are not present in any other group; the special technical features of Group IX including methods of minimizing undesirable odors in a composition comprising plant proteins, which are present in any other group; the special technical features of Group X including a chocolate flavored spread, which are not present in any other Group; the special technical features of Group XI including a method for altering the texture of a consumable, which not present in any other group; the special technical features of Group XII including a freeze-aligned non-animal protein.

Groups I-XII share the technical features including consumables. Groups I, II, V, VIII, IX, X and XI share the technical features including plants. Groups I, II, VIII, IX and XI share the technical features including plant proteins. Groups I-V, VI+, VII+, VIII, IX, XI and XII share the technical features including proteins. Groups I, III, V, VII+, XI and XII share the technical features including temperature. Groups I and VIII share the technical features including pH. Groups I, III and VIII share the technical features including sodium chloride. Groups I-III, VI+, VII+, VIII and XI share the technical features including a method of making a consumable product. Groups II, III and XII share the technical features including a meat replica. Groups II and VIII share the technical features including a coacervate. Groups II and V share the technical features including plant or algal derived oils or lipids. Groups II and V share the technical features comprising an emulsion. Groups III and V share the technical features including a gel. Groups IV, VI+ and VII+ share the technical features including a heme-containing protein. Groups IV and VI+ share the technical features including meat-free products. Groups VI+ and VII+ share the technical features including a heme-containing protein comprising a sequence at least 70% identical to any of SEQ ID NOs: 1-27.

Groups VI+ share the technical features including a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions.

Groups VII+ share the technical features including a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

However, these shared technical features are previously disclosed by WO 2001/015714 A1 (ZLOTKIN) in view of US 2010/0074998 A1 to Espeleta-Vega et al. (hereinafter 'Espeleta-Vega'), US 2011/0083681 A1 to Sengupta, et al. (hereinafter 'Sengupta'), the publication entitled 'Comparative Analysis Of Autoxidation Of Haemoglobin' by Jensen (hereinafter 'Jensen'), US 4,986,998 A to Yoo, et al. (hereinafter 'Yoo') and GenBank Accession AFK42304 by Krishnakumar, et al. (hereinafter 'Krishnakumar').

Zlotkin discloses consumables (compositions including micronutrients (consumables); abstract) including plants (chicory fructooligosaccharide (plants); page 11, lines 15-19); proteins (proteins; page 15, lines 20-28); temperature (temperature; page 17, lines 10-25); a method of making a consumable product (a process for producing a composition comprising micronutrients (a method of making a consumable product); page 8, lines 22-27); plant or algal derived oils or lipids (soybean oil (plant or algal derived oils or lipids); page 15, lines 1-4); meat-free products (cereals; purees of fruit, vegetables, milk, yogurt, cottage cheese (meat-free products); page 18, lines 1-11), and a method for preventing or reducing the discoloration and taste alteration of a food due to the addition of an iron compound thereto (a method for preventing or reducing the discoloration and taste alteration of a food due to the addition of an iron compound thereto; page 14, lines 21-28).

Zlotkin does not disclose plant proteins; pH, sodium chloride; a meat replica; a coacervate; an emulsion; a gel; a heme-containing protein; a heme-containing protein comprising a sequence at least 70% identical to any of SEQ ID NOs: 1-27; a method for extending the shelf-life of a meat-free consumable product, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions; and a method for imparting a beef like flavor to a consumable product, including a poultry or fish composition, comprising adding to said consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to said consumable.

-***-Continued Within the Next Supplemental Box-***-

-***-Continued from Previous Supplemental Page:

Espeleta-Vega discloses plant proteins (vegetable protein (plant proteins); abstract), pH (a pH modifier; paragraph [0010]), sodium chloride (salt (sodium chloride); paragraph [0038]), a meat replica (substitute meat food product (a meat replica); abstract); an emulsion (an emulsion; paragraph [0011]); a gel (a gel; paragraph [0011]).

Sengupta discloses a process for preparing coacervate gel particles comprising plant materials including proteins (a process for preparing coacervate gel particles comprising plant materials including proteins; paragraph [0015]).

Jensen discloses a heme-containing protein (hemoglobin; abstract) wherein the oxidation of hemoglobin and myoglobin is significantly slower than free heme (oxidation of hemoglobin and myoglobin is significantly slower than free heme; page 2029, column 1, paragraph 2), and wherein the oxidation of tetrameric hemoglobin is significantly slower than either monomeric hemoglobin or myoglobin (wherein the oxidation of tetrameric hemoglobin is significantly slower than either monomeric hemoglobin or myoglobin; page 2030, column 1, paragraph 1).

Yoo discloses a method for imparting (flavoring (imparting); abstract) a beef (cow (beef); column 4, lines 26-32) like flavor to a consumable product (flavor to a consumable product; abstract, column 2, lines 49-50), comprising adding to said consumable composition a heme-containing protein (comprising adding to said consumable a powdered blood product (comprising adding to said consumable composition a heme-containing protein); abstract), wherein after cooking, a beef-like flavor is imparted to said consumable (providing a meat-like flavor (beef-like flavor is imparted to said consumable); column 3, lines 1-6).

Krishnakumar discloses a heme-containing protein comprising a sequence at least 70% identical to SEQ ID NO: 1 (a globin (a heme-containing protein comprising a sequence at least 70% identical to SEQ ID NO: 1); page 1).

It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin by combining the consumable disclosed by Zlotkin with a meat replica, such as that disclosed by Espeleta-Vega, for providing the micronutrients in the consumable in a form acceptable to a consumer, such as in a meat replica, for enhancing the nutrient content of a meat replica, particularly the iron content of said replica, providing the previous disclosure of Zlotkin. Also, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin regarding micronutrient-comprising consumables with the coacervate preparation process of Sengupta, for enabling the preparation of particulates comprising the micronutrients disclosed by Zlotkin using a plant polysaccharide, such as pectin, as disclosed by Sengupta, for providing micronutrients in a consumable and easily digestible form to enable a consumer to absorb the nutrients. Furthermore, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin, for including the use of hemoglobin in preference to free iron or other forms of iron, in order to provide the iron to the consumer in a biologically useful form and oxidation state. Moreover, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have therefore modified the disclosure of Zlotkin, for providing a method for extending the shelf-life of a meat-free consumable product, such as the consumables disclosed by Zlotkin, said method comprising adding a heme-containing protein to said consumable product, wherein said heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions, such as adding hemoglobin, based on the disclosure of Jensen that tetrameric hemoglobin oxidizes more slowly than other forms of heme-containing proteins. Also, it would have been obvious to a person of ordinary skill in the art to have modified the disclosure of Zlotkin, for including a meat-like flavoring including an iron-containing protein based on the disclosure of Yoo, in order to provide a beef-like flavor to a consumable food, including a poultry or fish composition, to provide a beef-flavored product not containing beef, but with lower fat, such as a consumable comprising a plant protein, fish, or poultry protein. Finally, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Zlotkin regarding micronutrient-comprising consumables comprising iron, for including a plant-based iron-comprising proteins, such as the globin disclosed by Krishnakumar, in order to provide iron from non-animal sources having high absorbability and utilization by a consumer.

Since none of the special technical features of the Groups I-XII inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Zlotkin, Espeleta-Vega, Sengupta, Jensen, Yoo and Krishnakumar references, unity of invention is lacking.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Monica McCormick Graham
Fish & Richardson P.C.
P. O. Box 1022
Minneapolis, MN 55440-1022
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*) 16 JUN 2014

Applicant's or agent's file reference	FOR FURTHER ACTION
387670010WO1	See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US14/11361	13 January 2014 (13.01.2014)	11 January 2013 (11.01.2013)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A23L 1/212 (2014.01)
USPC - 424/478, 479

Applicant Maraxi, Inc.

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [x] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [x] Box No. IV Lack of unity of invention
- [x] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	28 May 2014 (28.05.2014)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11361

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

4. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11361

Box No. III ***Non-establishment of opinion with regard to novelty, inventive step and industrial applicability***

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 10-16, 23-27, 40-46, 50, 55-58, 66, 67, 79, 80, 84-90

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. -***-Please See Below-***- are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 10-16, 23-27, 40-46, 50, 55-58, 66, 67, 79, 80 and 84-90 are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. -***-Please See Above-***-

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11361

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the appicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

-***-Please See Supplemental Page-***-

4. Consequently, this opinion has been established in respect of the following parts of the international application:

   ☐ all parts

   ☒ the parts relating to claims Nos. 1-9, 17-22, 52-54

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/11361

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	3-7, 9/3-9/7, 18-21, 22/20, 22/21, 52, 53, 54/52, 54/53	YES
	Claims	1, 2, 8, 9, 9/1, 9/2, 9/8, 17	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-8, 9/1-9/8, 17-21, 22/20, 22/21, 52, 53, 54/52, 54/53	NO
Industrial applicability (IA)	Claims	1-8, 9/1-9/8, 17-21, 22/20, 22/21, 52, 53, 54/52, 54/53	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1, 2, 8, 9/1, 9/2 and 9/8 lack novelty under PCT Article 33(2) as being anticipated by US 3,870,801 A (TOMBS).

As per claim 1, Tombs discloses consumable product comprising an isolated and purified plant protein (a plant protein valuable in food products (consumable) such as arachin, a purified plant protein, can be obtained (isolated); abstract; column 6, lines 20-21), wherein the isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2 degrees C and about 32 degrees C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM (mesophases (proteins) of the invention comprise undenatured plant protein in a dissolved state (soluble), dissolved in aqueous solution of 0.1 N (0.1 N = 0.1 M = 100 mM) NaCl (sodium chloride) and specifically 500 g rape protein is extracted in 5 L water (at least 1 mg/ml) adjusted to pH 7.1 and the temperature required to set the mesophase is generally set by heating at 90 degrees C to 100 degrees C; column 1, lines 64-65; column 3, lines 12-13; column 8, lines 56-57; column 19, lines 32-34).

As per claim 2, Tombs discloses the consumable product of claim 1, and Tombs further discloses wherein said product is a beverage, a protein supplement, a baked good, a condiment, a meat product, or a meat substitute product (it is possible to produce a dried meat substitute formed wholly from vegetable protein using unset mesophase binder; column 14, lines 3-5).

As per claim 8, Tombs discloses the consumable product of claim 2, and Tombs further discloses wherein said meat product is a pate, a sausage replica, or a meat substitute (it is possible to produce a dried meat substitute formed wholly from vegetable protein using unset mesophase binder; column 14, lines 3-5).

As per claims 9/1, 9/2 and 9/8, Tombs discloses the consumable product of claims 1, 2 and 8, and Tombs further discloses wherein said isolated and purified plant protein is at least 10 kDa in size (a plant protein valuable in food products (consumable), such as arachin (21 kDa protein), a purified plant protein, can be obtained (isolated); abstract; column 6, lines 20-21).

Claim 17 lacks novelty under PCT Article 33(2) as being anticipated by US 2013/0004617 A1 to Zhang, et al. (hereinafter 'Zhang').

As per claim 17, Zhang discloses a consumable product comprising a coacervate (abstract) comprising one or more isolated and purified proteins (an aqueous dispersion of complex coacervates in stable form suitable for use in foods (consumable products) is provided and can include whey protein, hydrolyzed protein, lauric arginate, polylysine, casein or any combination thereof; paragraphs [0004], [0006]).

Claims 3-7, 9/3-9/7, 52 and 54/52 lack an inventive step under PCT Article 33(3) as being obvious over Tombs in view of EP 1,361,264 B1 to Tsubaki, et al. (hereinafter 'Tsubaki').

As per claim 3, Tombs discloses the consumable product of claim 2, but Tombs does not disclose wherein said beverage is an alcoholic beverage or a protein drink. Tsubaki discloses wherein said beverage is an alcoholic beverage (foods also include beverages, such as alcoholic beverages; paragraph [0044]) or a protein drink which may prepared from grains (beer, paragraph [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the method of including that protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the utilization of a plant protein in food that is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

As per claim 4, Tombs and Tsubaki, in combination, disclose the consumable product of claim 3, but Tombs does not disclose wherein said alcoholic beverage is a cream liquor. Tsubaki discloses wherein said alcoholic beverage is a cream liquor (foods also include beverages, such as alcoholic beverages including lactic acid beverages (cream beverages); paragraph [0044]; gramineous plant fats and oils, paragraph [0039]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the method of including that protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the use of a plant protein in that can be used in food which is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

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As per claim 5, Tombs and Tsubaki, in combination, disclose the consumable product of claim 4, but Tombs does not disclose said cream liquor further comprising a non-dairy lipid emulsion, wherein said cream liquor is free of animal products. Tsubaki discloses said cream liquor (foods also include beverages, such as alcoholic beverages including lactic acid beverages (cream beverages); paragraph [0044]; gramineous plant fats and oils, paragraph [0039]) further comprising a non-dairy lipid emulsion (fat (lipid) and oil composition containing beta-glucans extracted from a gramineous plant (non-dairy) can be used as a substitute in an emulsion; paragraph [0039]), wherein said cream liquor is free of animal products (fat (lipid) and oil composition containing β-glucans extracted from a gramineous plant can be used as a substitute (free of animal products) in an emulsion and can be alcoholic beverages including lactic acid beverages (cream beverages); paragraphs [0039], [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with including that protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the use of a plant protein that can be used in food which is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

As per claim 6, Tombs and Tsubaki, in combination, disclose the consumable product of claim 3, but Tombs does not disclose wherein said protein drink is a meal replacement beverage, a beer supplemented with said protein, or distilled alcoholic beverage supplemented with said protein. Tsubaki discloses wherein said protein drink is a meal replacement beverage, a beer supplemented with said protein, or distilled alcoholic beverage supplemented with said protein (fat (lipid) and oil composition containing β-glucans extracted from a gramineous plant can be used as a substitute (supplemented) in an emulsion and can be alcoholic beverages, including lactic acid beverages (cream beverages); paragraphs [0039], [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with integrating protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the utilization of the plant in food which is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), as a supplement, and a combination of these references would have resulted in a food product containing additional protein from a plant source.

As per claim 7, Tombs discloses the consumable product of claim 2, but Tombs does not disclose wherein said condiment is a mayonnaise *replica. Tsubaki discloses wherein said condiment is a mayonnaise replica (fat (lipid) and oil composition containing β-glucans extracted* from a gramineous plant can be used as a substitute for a part of, or the whole of (replica) food which can include mayonnaise; paragraphs [0039], [0045]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the method of including that protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the utilization of a plant protein as a fat or lipid substitute in food which is uniformly dispersible throughout a food and can be used in part of, or as whole of a fat or lipid in the food product (Tsubaki; paragraphs [0001], [0039]), and a combination of these references would have resulted in a food product containing a replacement protein from a plant source.

As per claims 9/3-9/7, Tombs and Tsubaki, in combination, disclose the consumable product of claims 3-7, and Tombs further discloses wherein said isolated and purified plant protein is at least 10 kDa in size (a plant protein valuable in food products (consumable), such as arachin (21 kDa protein), a purified plant protein, can be obtained (isolated); abstract; column 6, lines 20-21).

As per claim 52, Tombs discloses a method for making a consumable product comprising: a) preparing a solution comprising an isolated and purified plant protein (a plant protein valuable in food products (consumable) in its fluid form (solution), such as arachin, a purified plant protein, can be obtained (isolated); abstract; column 6, lines 20-21), wherein said isolated and purified plant protein has (i) a solubility in said solution of at least 25 at a temperature between about 2 degrees C and 32 degrees C, wherein said solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in said solution of at least 1 mg/ml at a temperature of between 90 degrees C and 110 degrees C, wherein said solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM (mesophases (proteins) of the invention comprise undenatured plant protein in a dissolved state (soluble), dissolved in aqueous solution of 0.1 N (0.1 N = 0.1 M = 100 mM) NaCl (sodium chloride) and specifically 500 g rape protein is extracted in 5 L water (at least 1 mg/ml) adjusted to pH 7.1 and the temperature required to set the mesophase is generally set by heating at 90 degrees C to 100 degrees C; column 1, lines 64-65; column 3, lines 12-13; column 8, lines 56-57; column 19, lines 32-34). Tombs does not disclose adding said solution to a beverage. Tsubaki discloses adding said solution to a beverage (foods also include beverages, such as alcoholic beverages; paragraph [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the method of including that protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the use of a plant protein that can be used in food which is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

As per claim 54/52, Tombs and Tsubaki, in combination, disclose the method of claim 52, but Tombs does not disclose wherein said beverage is clear. Tsubaki discloses wherein said beverage is clear (foods also include beverages, such as alcoholic beverages (many alcoholic beverages are clear); paragraph [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of extracting a plant protein, as previously disclosed by Tombs, with the method of including the protein in a beverage, as previously disclosed by Tsubaki, as the previous disclosure by Tombs includes the use of a plant protein in a food product, Tsubaki discloses the use of a plant protein that can be utilized in food which is uniformly dispersible throughout a food (Tsubaki; paragraph [0001]), and a combination of these references would have resulted in a food product containing additional protein from a plant source.

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Claims 19-21, 22/20 and 22/21 lack an inventive step under PCT Article 33(3) as being obvious over Zhang in view of Tsubaki.

As per claim 19, Zhang discloses the consumable product of claim 17, but Zhang does not disclose said product further comprising a plant derived lipid or a microbial-derived lipid. Tsubaki discloses a product further comprising a plant derived lipid or a microbial-derived lipid (fat and oil compositions in which the Beta glucans extracted from a gramineous plant are not particularly limited and include lipid of dry microbial cells; paragraph [0029]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of the inclusion of a coacervate comprising one or more isolated and purified proteins, as previously disclosed by Zhang, with the plant derived lipid, as previously disclosed by Tsubaki, as the previous disclosure by Zhang includes the use of the coacervate to enhance food products, Tsubaki discloses the use of a plant protein that can be used as a fat or lipid substitute in food, which is uniformly dispersible throughout the food and can be used in part of, or as whole of a fat or lipid in the food product (Tsubaki; paragraphs [0001], [0039]), and a combination of these references would have resulted in an enhanced food product containing plant protein.

As per claim 20, Zhang and Tsubaki, in combination, disclose the consumable product of claim 19, but Zhang does not disclose wherein said plant derived lipid or microbial-derived lipid comprises lecithin and/or an oil. Tsubaki discloses a plant derived lipid or microbial-derived lipid comprises lecithin and/or an oil (fat and oil compositions in which the β-glucans extracted from a gramineous plant are not particularly limited and include lipid of dry microbial cells, and lecithin can be added; paragraphs [0029], [0038]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of the inclusion of a coacervate comprising one or more isolated and purified proteins, as previously disclosed by Zhang, with the plant derived lipid, as previously disclosed by Tsubaki, as the previous disclosure by Zhang includes use of the coacervate for enhancing food products, Tsubaki discloses the use of a plant protein as a fat or lipid substitute, in food which is uniformly dispersible throughout a food and can be used in part of, or as whole of a fat or lipid in the food product and can include lecithin (Tsubaki; paragraphs [0001], [0038], [0039]), and a combination of these references would have resulted in an enhanced food product containing plant protein.

As per claim 21, Zhang and Tsubaki, in combination, disclose the consumable product of claim 20, but Zhang does not disclose said product comprising up to about 1% lecithin by weight. Tsubaki discloses a product comprising up to about 1% lecithin by weight (fat and oil compositions in which the β-glucans extracted from a gramineous plant are not particularly limited, and include lipid of dry microbial cells and lecithin can be added in general amounts of 0.01 to 15%; paragraphs [0029], [0038]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of the inclusion of a coacervate comprising one or more isolated and purified proteins, as previously disclosed by Zhang, with the plant derived lipid, as previously disclosed by Tsubaki, as the previous disclosure by Zhang includes use of the coacervate to enhance food products, Tsubaki discloses the use of a plant protein that can be used as a fat or lipid substitute in food which is uniformly dispersible throughout a food and can be used in part of, or as whole of a fat or lipid in the food product, and can include lecithin (Tsubaki; paragraphs [0001], [0038], [0039]), and a combination of these references would have resulted in an enhanced food product containing plant protein.

As per claims 22/20 and 22/21, Zhang and Tsubaki, in combination, disclose the consumable product of claims 20 and 21, but Zhang does not disclose wherein said product comprises lecithin and said oil. Tsubaki discloses wherein said product comprises lecithin and said oil (fat and oil compositions in which the β-glucans extracted from a gramineous plant are not particularly limited and include lipid of dry microbial cells and lecithin can be added in general amounts of 0.01 to 15%; paragraphs [0029], [0038]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of the inclusion of a coacervate comprising one or more isolated and purified proteins, as previously disclosed by Zhang, with the plant derived lipid, as previously disclosed by Tsubaki, as the previous disclosure by Zhang includes use of the coacervate for enhancing food products, Tsubaki discloses the use of a plant protein that can be used as a fat or lipid substitute in food, which is uniformly dispersible throughout a food and can be used in part of, or as whole of a fat or lipid in the food product, and can include lecithin (Tsubaki; paragraphs [0001], [0038], [0039]), and a combination of these references would have resulted in an enhanced food product containing plant protein.

Claim 18 lacks an inventive step under PCT Article 33(3) as being obvious over Zhang in view of Tombs.

As per claim 18, Zhang discloses the consumable product of claim 17, but Zhang does not disclose wherein said consumable product is a meat replica. Tombs discloses a consumable product that is a dried meat substitute formed wholly from vegetable protein using unset mesophase binder (it is possible to produce a dried meat substitute formed wholly from vegetable protein using unset mesophase binder; column 14, lines 3-5). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have combined the method of the inclusion of a coacervate comprising one or more isolated and purified proteins, as previously disclosed by Zhang, with method of extracting a plant protein, as previously disclosed by Tombs, as the previous disclosure by Zhang discloses an aqueous dispersion of complex coacervates in stable form suitable for use in foods (Zhang; paragraph [0004]), Tombs discloses the use of a plant protein in a food product, such as a meat replica (Tombs; abstract; column 6, lines 20-21), and a combination of these references would have resulted in a superior food product containing plant protein in a meat replica.

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