[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 ^{o}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 ^{o}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, 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.

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.

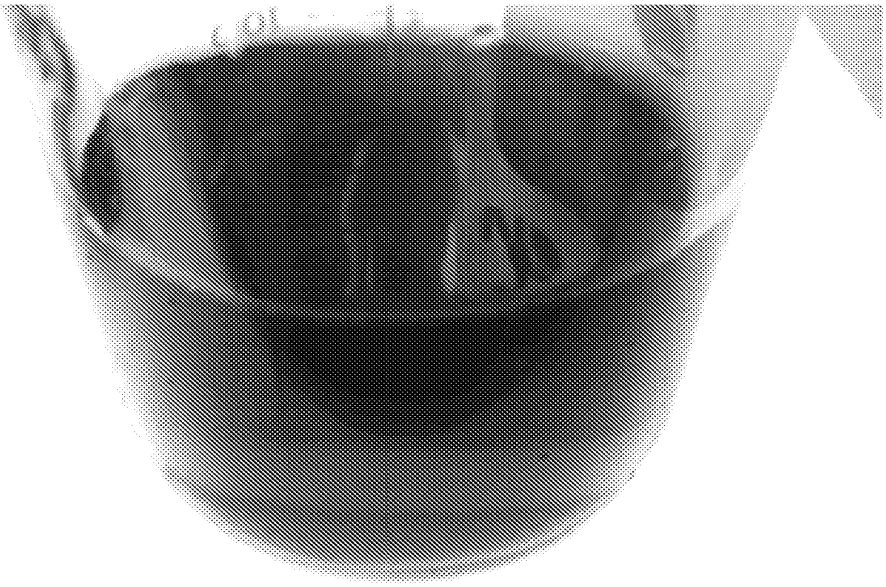


Figure 3.

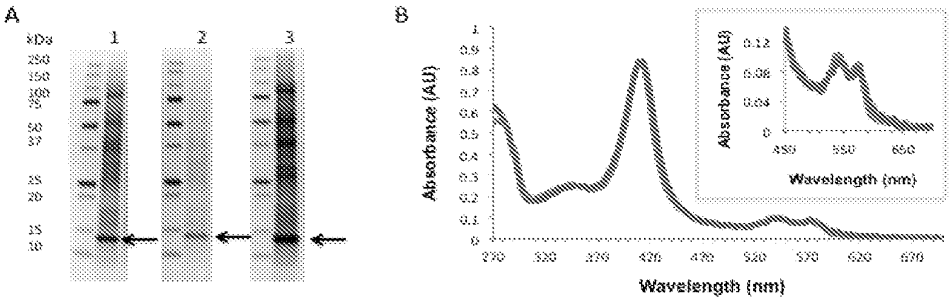


Figure 4

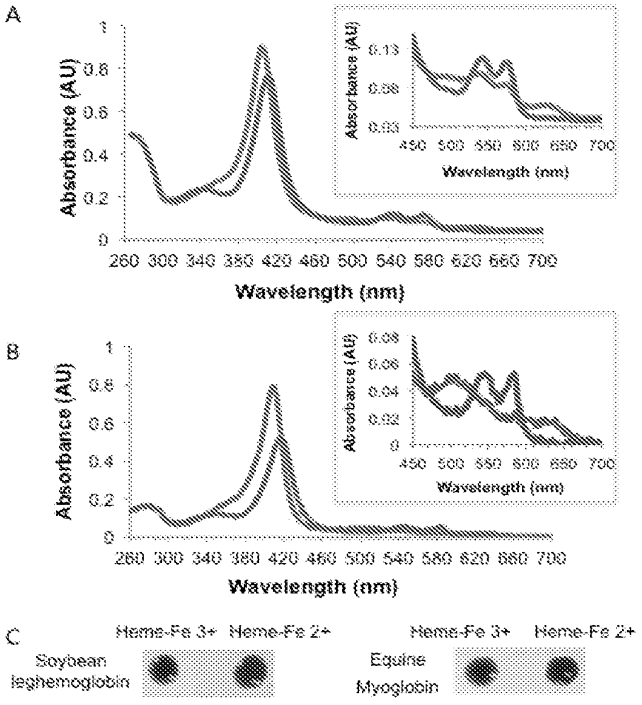


Figure 5

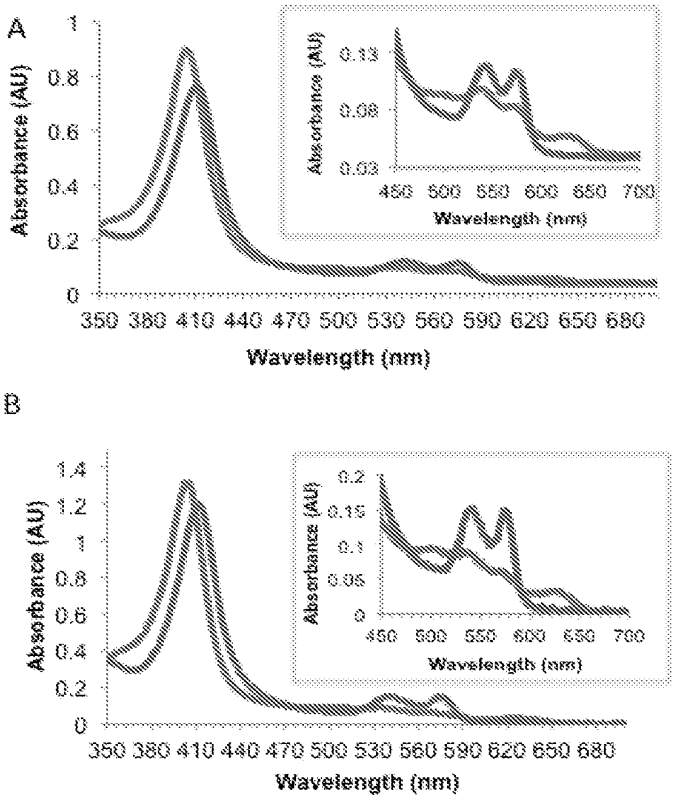


Figure 6

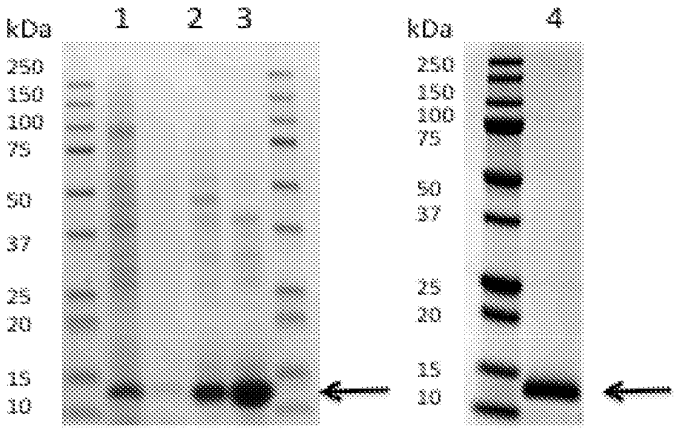


Figure 7

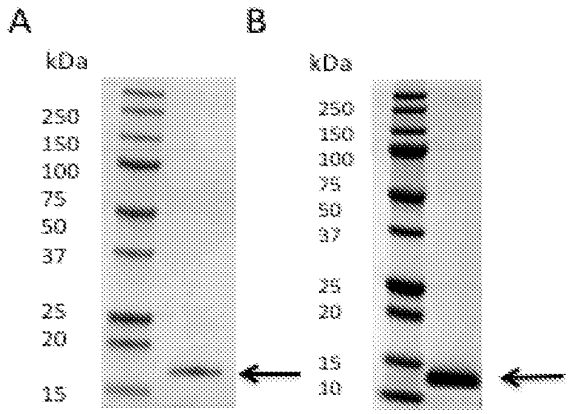

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Figure 8



Figure 9

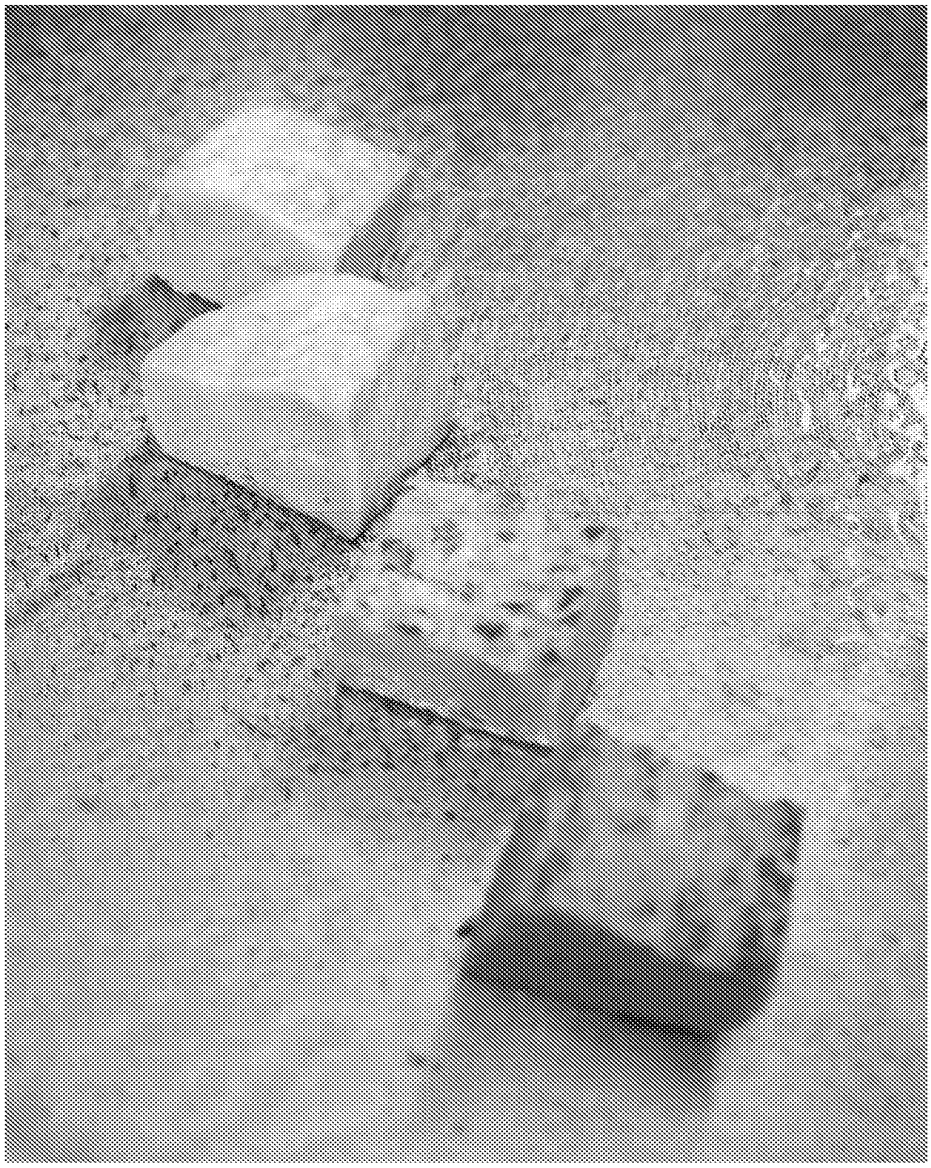


Figure 10



Figure 11

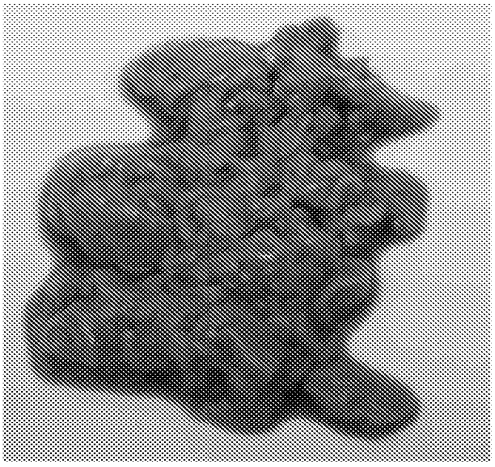


Figure 12

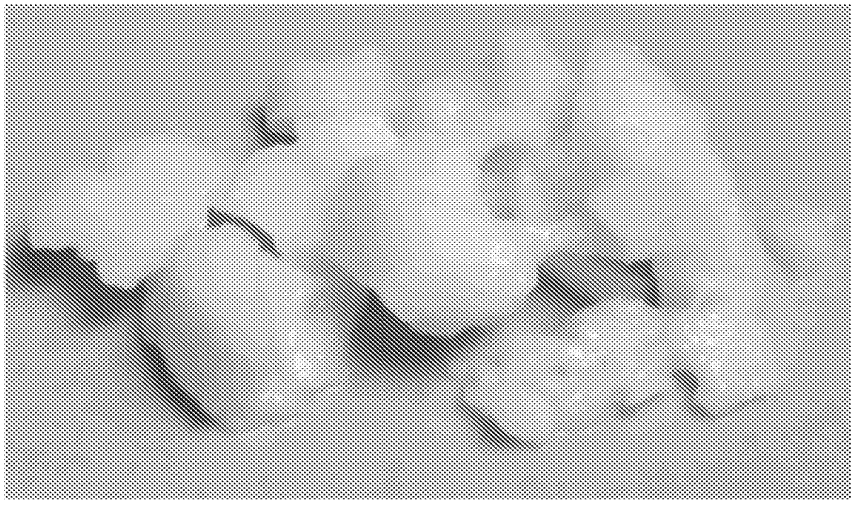


Figure 13

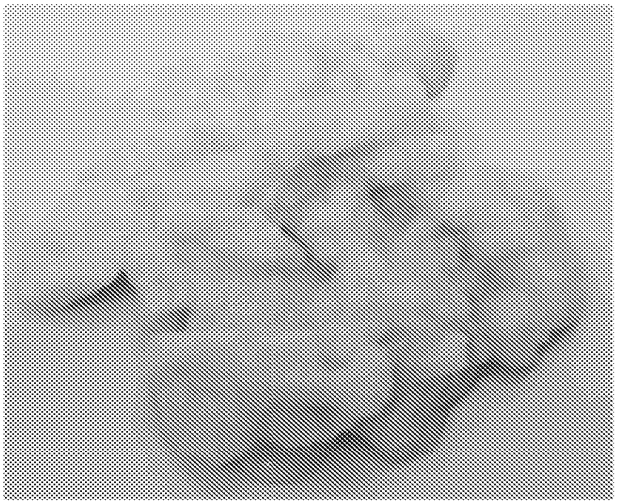


Figure 14



Figure 15

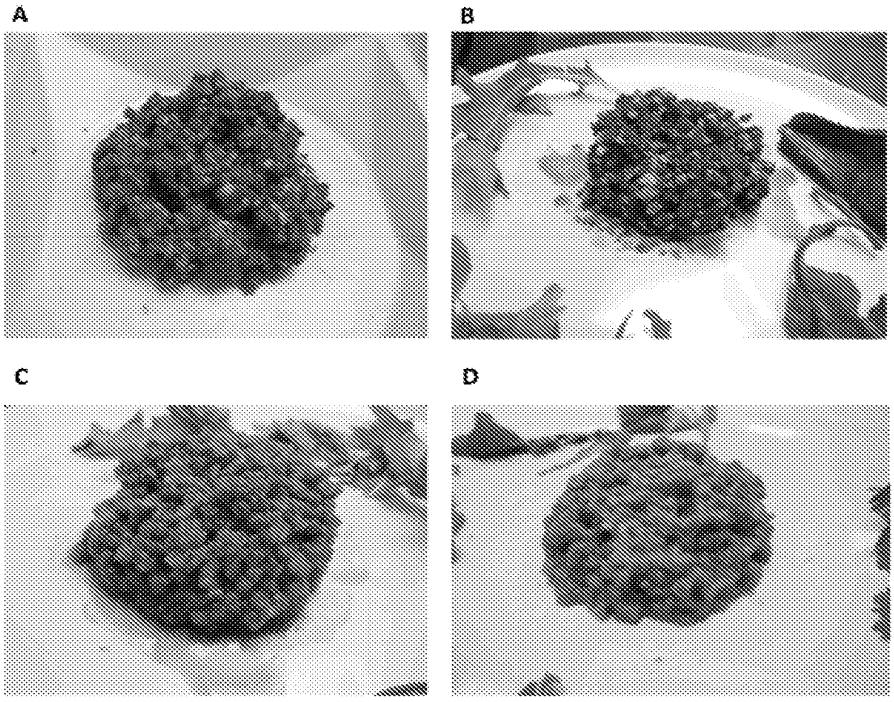


Figure 16

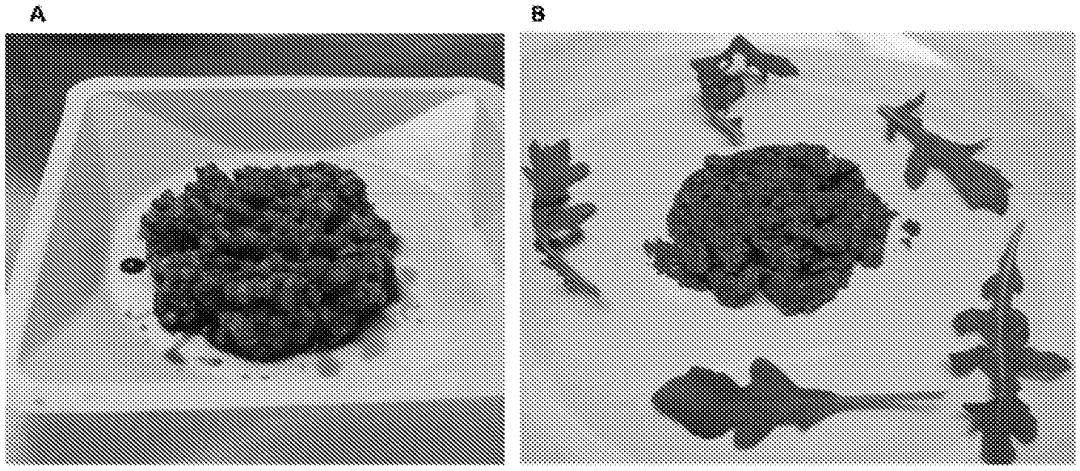


Figure 17

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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WO 2014/110532 A2

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

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

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

CROSS-REFERENCE TO RELATED APPLICATIONS

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

TECHNICAL FIELD

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

BACKGROUND

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

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

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

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

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

SUMMARY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DESCRIPTION OF THE DRAWINGS

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

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

DETAILED DESCRIPTION

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

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

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

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

TABLE 1

Flavor Precursor Molecules

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Modulating Flavor and/or Aroma Profiles

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

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

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

TABLE 2

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

yeasty	goaty
vegetable broth	

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

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

TABLE 3

Compounds Produced

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Food Products

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

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

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

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

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

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

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

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

Assessment of Food Products

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

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

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

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

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

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

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

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

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

EXAMPLES

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

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

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

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

TABLE 4

Replica Burger Ingredients

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

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

TABLE 5

Sensory evaluation of replica burger with Heme

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

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

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

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

TABLE 6

Improvement of overall taste with precursors added to LegH burgers

	with precursors	without precursors
Average	3.5	1.8
STDV	0.6	0.5

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

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

TABLE 7

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

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

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

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

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

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

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

TABLE 8

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

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

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

pyrrole	X	X
2-thiophenecarboxaldehyde		X
3-thiophenecarboxaldehyde		X

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Example 9 - Flavor compound production by different Heme-proteins

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

TABLE 9

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

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

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

2-pentyl-thiophene				x

Example 10 - Generation of Meat flavors from different lipids

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

Table 10

Legend showing components of each sample

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

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

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

TABLE 11

Aroma descriptions of each sample after it was cooked.

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

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

Table 12

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Example 11 - Creation of beefy flavors using complex precursor mixtures

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

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

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

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

Table 13

Chemical entities added to the Magic Mix

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

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

TABLE 14

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

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

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

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

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

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

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

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

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

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

TABLE 15

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

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

Preparation of Flavor Reaction Containing Ferrous Chlorin or Leghemoglobin

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

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

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

TABLE 16

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

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

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

Example 13 - Flavor creation by immobilized Hemin

Preparation of hemin linked CM Sepharose.

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

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

Flavor reaction

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

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

TABLE 17

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

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

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

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

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

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

Table 18

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

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

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

OTHER EMBODIMENTS

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

WHAT IS CLAIMED IS:

1. A food product comprising:

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

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

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

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

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

5. A food product comprising:

a) a heme-containing protein; and

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

FIGURE 1

SEQ ID NO:1 *Vigna radiata*

MTTTLERGFTEEQEALVVKSWNVMKKNSGELGLKFFLKIFEIAPSAQKLFSFLRDSTVP
LEQNPKLKPHAVSVFVMTCDSAVQLRKAGKVTVRESNLKKLGATHFRTGVANEHFEVTK
FALLETIKEAVPEMWSPAMKNAWGEAYDQLVDAIKYEMKPPSS

SEQ ID NO:2 *Methylacidiphilum infernorum*

MIDQKEKELIKESWKRIEPNKNEIGLLFYANLFKEEPTVSVLFQNPISSQSRKLMQVLG
ILVQGIDNLEGLIPTLQDLGRRHKQYGVVDSHYPLVGDCLLKSIQEYLGQGFTEEAKAA
WTKVYGIAAQVMTAE

SEQ ID NO:3 *Aquifex aeolicus*

MLSEETIRVIKSTVPLLKEHGTEITARMYELLFSKYPKTKELFAGASEEQPKKLANAII
AYATYIDRLEELDNAISTIARSHVRRNVKPEHYPLVKECLLQAIEEVLNPGEEVLKAWE
EAYDFLAKTLITLEKKLYSQP

SEQ ID NO:4 *Glycine max*

MGAFTEKQEALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLSNGVDPSNPKL
TGHAEKLFGLVRDSAGQLKANGTVVADAALGSIHAQKAITDPQFVVVKEALLKTIKEAV
GDKWSDELSSAWEVAYDELAAAIKKAF

SEQ ID NO:5 *Hordeum vulgare*

MSAAEGAVVFSEEKEALVLKSWAIMKKDSANLGLRFFLKIFEIAPSARQMFPFLRDSDV
PLETNPKLKTHAVSVFVMTCEAAAQLRKAGKITVRETTLKRLGGTHLKYGVADGHFEVT
RFALLETIKEALPADMWGPEMRNAWGEAYDQLVAAIKQEMKPAE

Magnaporthe oryzae, (SEQ ID NO:6)

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   1 mdgavrldwt gldltgheih dgvpiasrvq vmvsfplfkd qhiimsskes psrksstigq
  61 strngscqad tqkgqlppvg ekpkpvkenp mkklkemsqr plptqhgdgt yptekkltgi
 121 gedlkhirgy dvktllamvk sklkgeklkd dktmlmervm qlvarlptes kkraeltdsl
 181 inelwesldh pplnylgpeh syrtpdgsyn hpfnpqlgaa gsryarsvip tvtppgalpd
 241 pglifdsimg rtpnsyrkhp nnvssilwyw atiiihdifw tdprdintnk sssyldlapl
 301 ygnsqemqds irtfkdgrmk pdcyadkrla gmppgvsvll imfnrfhnhv aenlalineg
 361 grfnkpsdll egeareaawk kydndlfqva rlvtsglyin itlvdyvrni vnlnrvdttw
 421 tldprqdaga hvgtadgaer gtgnavsaef nlcyrwhsci sekdskfvea qfqnifgkpa
 481 sevrpdemwk gfakmeqntp adpgqrtfgg fkrgpdgkfd dddlvrcise avedvagafg
 541 arnvpqamkv vetmgiiqgr kwnvaglnef rkhfhlkpys tfedinsdpg vaealrrlyd
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 601 hpdnvelypg lvaeedkqpm vpgvgiapty tisrvvlsda vclvrgdrfy ttdftprnlt
 661 nwgykevdyd lsvnhgcvfy klfirafpnh fkqnsvyahy pmvvpsenkr ilealgradl
 721 fdfeapkyip prvnitsygg aeyiletqek ykvtwheglg flmgegglkf mlsgddplha
 781 qqrkcmaaql ykdgwteavk afyagmmeel lvsksyflgn nkhrhvdiir dvgnmvhvhf
 841 asqvfglplk taknptgvft eqemygilaa ifttiffdld psksfplrtk trevcqklak
 901 lveanvklin kipwsrgmfv gkpakdepls iygktmikgl kahglsdydi awshvvptsg
 961 amvpnqaqvf aqavdyylsp agmhyipeih mvalqpstpe tdalllgyam egirlagtfg
1021 syreaavddv vkedngrqvp vkagdrvfvs fvdaardpkh fpdpevvnpr rpakkyihyg
1081 vgphaclgrd asqiaitemf rclfrrrnvr rvpgpqgelk kvprpggfyv ymredwgglf
1141 pfpvtmrvmw dde
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Fusarium oxysporum (SEQ ID NO:7)

```
   1 mkgsatlafa lvqfsaasql vwpskwdeve dllymqggfn krgfadalrt cefgsnvpgt
  61 qntaewlrta fhdaithdak agtggldasi ywessrpenp gkafnntfgf fsgfhnprat
 121 asdltalgtv lavgacngpr ipfragrida ykagpagvpe pstnlkdtfa aftkagftke
 181 emtamvacgh aiggvhsvdf peivgikadp nndtnvpfqk dvssfhngiv teylagtskn
 241 plvasknatf hsdkrifdnd katmkklstk agfnsmcadi ltrmidtvpk svqltpvlea
 301 ydvrpyitel slnnknkihf tgsvrvritn nirdnndlai nliyvgrdgk kvtvptqqvt
 361 fqggtsfgag evfanfefdt tmdakngitk ffiqevkpst katvthdnqk tggykvddtv
 421 lyqlqqscav leklpnaplv vtamvrdara kdaltlrvah kkpvkgsivp rfqtaitnfk
 481 atgkkssgyt gfqaktmfee qstyfdivlg gspasgvqfl tsqampsqcs
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Fusarium graminearum (SEQ ID NO:8)

```
   1 masatrqfar aatratrngf aiaprqvirq qgrryyssep aqksssawiw ltgaavagga
  61 gyyfygnsas satakvfnps kedyqkvyne iaarleekdd yddgsygpvl vrlawhasgt
 121 ydketgtggs ngatmrfape sdhganagla aardflqpvk ekfpwitysd lwilagvcai
 181 qemlgpaipy rpgrsdrdvs gctpdgrlpd askrqdhlrg ifgrmgfndq eivalsgaha
 241 lgrchtdrsg ysgpwtfspt vltndyfrll veekwqwkkw ngpaqyedks tkslmmlpsd
 301 ialiedkkfk pwvekyakdn daffkdfsnv vlrlfelgvp faqgtenqrw tfkpthqe
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SEQ ID NO: 9 *Chlamydomonas eugametos*

MSLFAKLGGREAVEAAVDKFYNKIVADPTVSTYFSNTDMKVQRSKQFAFLAYALGGASE
WKGKDMRTAHKDLVPHLSDVHFQAVARHLSDTLTELGVPPEDITDAMAVVASTRTEVLN
MPQQ

SEQ ID NO:10 *Tetrahymena pyriformis*

MNKPQTIYEKLGGENAMKAAVPLFYKKVLADERVKHFFKNTDMDHQTKQQTDFLTMLLG
GPNHYKGKNMTEAHKGMNLQNLHFDAIIENLAATLKELGVTDAVINEAAKVIEHTRKDM
LGK

SEQ ID NO:11 *Paramecium caudatum*

MSLFEQLGGQAAVQAVTAQFYANIQADATVATFFNGIDMPNQTNKTAAFLCAALGGPNA
WTGRNLKEVHANMGVSNAQFTTVIGHLRSALTGAGVAAALVEQTVAVAETVRGDVVTV

SEQ ID NO:12 *Aspergillus niger*

MPLTPEQIKIIKATVPVLQEYGTKITTAFYMNMSTVHPELNAVFNTANQVKGHQARALA
GALFAYASHIDDLGALGPAVELICNKHASLYIQADEYKIVGKYLLEAMKEVLGDACTDD
ILDAWGAAYWALADIMINREAALYKQSQG

SEQ ID NO:13 *Zea mays*

MALAEADDGAVVFGEEQEALVLKSWAVMKKDAANLGLRFFLKVFEIAPSAEQMFSFLRD
SDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRETTLKRLGATHLRYGVADGHF
EVTGFALLETIKEALPADMWSLEMKKAWAEAYSQLVAAIKREMKPDA

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

MALVEGNNGVSGGAVSFSEEQEALVLKSWAIMKKDSANIGLRFFLKIFEVAPSASQMFS
FLRNSDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRDTTLKRLGATHFKYGVG
DAHFEVTRFALLETIKEAVPVDMWSPAMKSAWSEAYNQLVAAIKQEMKPAE

SEQ ID NO:15 *Arabidopsis thaliana*

MESEGKIVFTEEQEALVVKSWSVMKKNSAELGLKLFIKIFEIAPTTKKMFSFLRDSPIP
AEQNPKLKPHAMSVFVMCCESAVQLRKTGKVTVRETTLKRLGASHSKYGVVDEHFEVAK
YALLETIKEAVPEMWSPEMKVAWGQAYDHLVAAIKAEMNLSN

SEQ ID NO:16 *Pisum sativum*

MGFTDKQEALVNSSWESFKQNLSGNSILFYTIILEKAPAAKGLFSFLKDTAGVEDSPKL
QAHAEQVFGLVRDSAAQLRTKGEVVLGNATLGAIHVQRGVTDPHFVVVKEALLQTIKKA
SGNNWSEELNTAWEVAYDGLATAIKKAMT

SEQ ID NO:17 *Vigna unguiculata*

MVAFSDKQEALVNGAYEAFKANIPKYSVVFYTTILEKAPAAKNLFSFLANGVDATNPKL
TGHAEKLFGLVRDSAAQLRASGGVVADAALGAVHSQKAVNDAQFVVVKEALVKTLKEAV
GDKWSDELGTAVELAYDELAAAIKKAY

SEQ ID NO:18 Bos taurus

MGLSDGEWQLVLNAWGKVEADVAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGNTVLTALGGILKKKGHHEAEVKHLAESHANKHKIPVKYLEFISDAIIHVLHA
KHPSDFGADAQAAMSKALELFRNDMAAQYKVLGFHG

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

SEQ ID NO: 20 *Equus caballus*

MGLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHVLHS
KH PGDFGADAQGAMTKALELFRNDIAAKYKELGFQG

SEQ ID NO: 21 Nicotiana benthamiana

MSSFTEEQEALVVKSWDSMKKNAGEWGLKLFLKIFEIAPSAKKLFSFLKDSNVPL
EQNAKLKPHSKSVFVMTCEAAVQLRKAGKVVVRDSTLKKLGATHFKYGVADE
HFEVTKFALLETIKEAVPEMWSVDMKNAWGEAFDQLVNAIKTEMK

SEQ ID NO: 22 Bacillus subtilis

MGQSFNAPYEAIGEELLSQLVDTFYERVASHPLLKPIFPSDLTETARKQKQFLTQY
LGGPPLYTEEHGHPMLRARHLPFPITNERADAWLSCMKDAMDHVGLEGEIREFL
FGRLELTARHMVNQTEAEDRSS

SEQ ID NO: 23 Corynebacterium glutamicum

MTTSENFYDSVGGEETFSLIVHRFYEQVPNDDILGPMYPPDDFEGAEQRLKMFLS
QYWGGPKDYQEQRGHPRLRMRHVNYPIGVTAAERWLQLMSNALDGVDLTAEQ
REAIWEHMVRAADMLINSNPDPHA

SEQ ID NO: 24 Synechocystis PCC6803

MSTLYEKLGGTTAVDLAVDKFYERVLQDDRIKHFFADVDMAKQRAHQKAFLTY
AFGGTDKYDGRYMREAHKELVENHGLNGEHFDAVAEDLLATLKEMGVPEDLIA
EVAAVAGAPAHKRDVLNQ

SEQ ID NO: 25 Synechococcus sp. PCC 7335

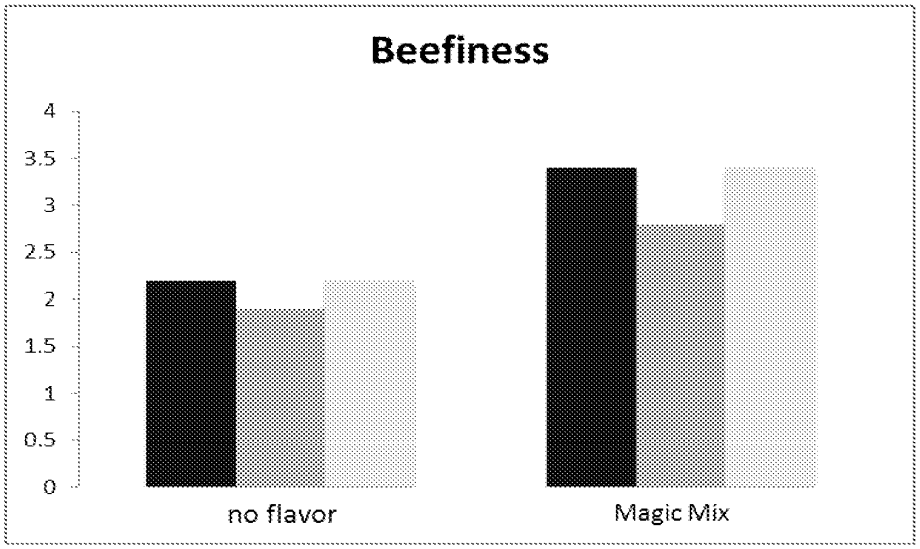
MDVALLEKSFEQISPRAIEFSASFYQNLFHHHPELKPLFAETSQTIQEKKLIFSLAAI
IENLRNPDILQPALKSLGARHAEVGTIKSHYPLVGQALIETFAEYLAADWTEQLA
TAWVEAYDVIASTMIEGADNPAAYLEPELTFYEWLDLYGEESPKVRNAIATLTH
FHYGEDPQDVQRDSRG

SEQ ID NO: 26 Nostoc commune

MSTLYDNIGGQPAIEQVVDELHKRIATDSLLAPVFAGTDMVKQRNHLVAFLAQIF
EGPKQYGGRPMDKTHAGLNLQQPHFDAIAKHLGERMAVRGVSAENTKAALDR
VTNMKGAILNK

SEQ ID NO: 27 Bacillus megaterium
MREKIHSPYELLGGEHTISKLVDAFYTRVGQHPELAPIFPDNLTETARKQKQFLTQ
YLGGPSLYTEEHGHPMLRARHLPFEITPSRAKAWLTCMHEAMDEINLEGPERDE
LYHRLILTAQHMINSPEQTDEKGFSH

FIGURE 2



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Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

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

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

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/11347

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A23D 7/005, 9/013; A23L 1/30 (2014.01)
USPC - 426/92, 641, 647
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): A23D 7/00, 59/013, 9/00, 9/06, 7/02; A23L 1/30, 1/226, 1/226, 1/217, 1/235 (2014.01)
USPC: 426/613, 506, 535, 808, 537, 92, 540, 96, 644, 643, 641, 647, 250, 646

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; PubMed; ProQuest; flavor, taste, precursor, iron, 'heterocycline ring,' heme, phenylacetaldehyde, food, consumable, beef, chicken, pork, lamb, turkey

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 2009/0274817 A1 (YAMAGUCHI, S et al.); November 05, 2009: paragraphs [0024], [0029], [0035], [0040], [0045], [0053], [0078]; Table 9	1-10, 35-37, 39 ----- 28-34
Y	US 3829582 A (GUADAGNI, DG et al.); August 13, 1974; abstract; column 1, lines 30-35; Claims 1-3	28-34
Y	US 5443852 A (SHAHIDI, F et al.); August 22, 1995; column 8, lines 10-25; column 8, lines 50-60; column 42, lines 5-15	29-33

☐ 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
09 June 2014 (09.06.2014)	03 JUL 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

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/11347

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:

-***-Please See Supplemental Page-***-

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.:

Groups I+: Claims 1-10, 28-37, 39, phenylacetaldehyde (flavor compound, not the precursor molecule)

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/US14/11347

-***-Continued from Box III -***-

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 1-10, 28-37, 39, and Tables 3 , 8, 9, 12, 14, 16, and 17 (flavor compounds) are directed toward a food product comprising: a) a highly conjugated heterocyclic ring complexed to iron ion; and b) one or more flavor precursor molecules; and a method for producing a flavor compound, said method comprising: a) combining a highly conjugated heterocyclic ring complexed to iron ion with one or more flavor precursor molecules to form a mixture, and b) heating the mixture to form one or more flavor compounds; a food product comprising: a) a heme-containing protein; and b) one or more flavor precursor molecules; a method for producing a flavor compound, said method comprising: a) combining a heme-containing protein, cysteine, and a sugar, and one or more optional flavor precursor molecules to form a mixture, and b) heating the mixture to form one or more flavor compounds; and a method for imparting a meat-like flavor to a food product comprising contacting said food product with a flavoring composition, said flavoring composition comprising i) a heme-containing protein; and ii) one or more flavor precursor molecules, wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product.

Group II: Claim 40 is directed toward a method for producing a flavor compound, said method comprising: a) incubating a heme-moiety bound to a solid support with one or more flavor precursor molecules and b) heating the heme-moiety bound to said solid support and one or more flavor precursors to form one or more flavor compounds.

The food product, the methods for producing a flavor compound, and the method for imparting a meat-like flavor to a food product will be searched to the extent that they encompass the first item in Table 3, phenylacetaldehyde (flavor compound). It is believed that Claims 1-10, 28-37 and 39 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass the first item in Table 3, phenylacetaldehyde (flavor compound). Applicants must indicate, if applicable, the claims which encompass this first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" groups will result in only the first claimed invention to be searched/examined. An Exemplary Election would be: the second item in Table 3, 1-octen-3-one (flavor compound).

The inventions listed as Groups I+-II 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 a method for imparting a meat-like flavor to a food product comprising contacting said food product with a flavoring composition, wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product, which are not present in Group II, the special technical features of Group II including a heme-moiety bound to a solid support.

Groups I+-II share the technical features including a method for producing a flavor compound, said method comprising: a) combining a heme-moiety with one or more flavor precursor molecules selected from a group including a sugar and cysteine; and b) heating the heme-moiety and one or more flavor precursors to form one or more flavor compounds selected from a group including phenylacetaldehyde. Groups I+ share the technical features including a food product comprising: a) a highly conjugated heterocyclic ring complexed to iron ion; and b) one or more flavor precursor molecules; and a method for producing a flavor compound, said method comprising: a) combining a highly conjugated heterocyclic ring complexed to iron ion with one or more flavor precursor molecules to form a mixture, and b) heating the mixture to form one or more flavor compounds; a food product comprising: a) a heme-containing protein; and b) one or more flavor precursor molecules; a method for producing a flavor compound, said method comprising: a) combining a heme-containing protein, cysteine, and a sugar, and one or more optional flavor precursor molecules to form a mixture, and b) heating the mixture to form one or more flavor compounds; and a method for imparting a meat-like flavor to a food product comprising contacting said food product with a flavoring composition, said flavoring composition comprising i) a heme-containing protein; and ii) one or more flavor precursor molecules, wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product.

-***-Continued Within the Next Supplemental Box-***-

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/11347

-***-Continued from Previous Supplemental Box-***-

However, these shared technical features are previously disclosed by US 2009/0274817 A1 to Yamaguchi, et al. (hereinafter 'Yamaguchi') in view of the publication entitled 'The Chemistry Of Beef Flavor: Executive Summary' by Brewer, and the publication entitled 'Prediction Of Beef Flavor By Precursor And Volatile Compounds' by Brooks, et al. (hereinafter 'Brooks'). Yamaguchi discloses a method for producing a flavor compound (creating a body taste-improver; paragraphs [0023]-[0027]), said method comprising: a) combining a heme-moiety with one or more flavor precursor molecules (the body-taste improver combines heme iron with another component (flavor precursor molecule); paragraphs [0024]-[0027], [0029]); and b) heating the heme-moiety and one or more flavor precursors (heating the body taste improver, comprising heme iron with another flavor component, enhances the taste enhancing effect; paragraphs [0041], [0043], [0053]); a food product (a food with an improved body taste; paragraph [0053]) comprising: a) a highly conjugated heterocyclic ring complexed to iron ion (a body taste-increasing component consists of heme iron; paragraphs [0027], [0029]); and b) one or more flavor precursor molecules (the body-taste improver combines heme iron with another component (flavor precursor molecule); paragraphs [0024]-[0027], [0029]); and a method for producing a flavor compound (a method of creating a body taste-improver; paragraphs [0023]-[0027]), said method comprising: a) combining a highly conjugated heterocyclic ring complexed to iron ion with one or more flavor precursor molecules (the body-taste improver combines heme iron with another component (flavor precursor molecule); paragraphs [0024]-[0027], [0029]) to form a mixture (a composition comprising various body taste improving components (a mixture); paragraph [0024]), and b) heating the mixture (heating the body taste improver, comprising heme iron with another flavor component, enhances the taste enhancing effect; paragraphs [0041], [0043], [0053]). Yamaguchi does not disclose flavor precursor molecules selected from a group including a sugar and cysteine; forming one or more flavor compounds selected from the group including phenylacetaldehyde; a food product comprising: a) a heme-containing protein; and b) one or more flavor precursor molecules selected from a group including a sugar and cysteine; a method comprising: a) combining a heme-containing protein, cysteine, and a sugar; or a method for imparting a meat-like flavor to a food product comprising contacting said food product with a flavoring composition, said flavoring composition comprising i) a heme-containing protein; and ii) one or more flavor precursor molecules selected from a group including a sugar, wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product. Brewer discloses flavor precursor molecules selected from a group including a sugar (precursor compounds include sugars such as glucose, fructose or ribose; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1) and cysteine (precursor compounds include amino acids such as cysteine; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1); a food product (beef (food product); page 2, left column, first paragraph to right column, first paragraph) comprising: a) a heme-containing protein (beef contains heme protein which can contribute to its flavor; page 10, left column, second paragraph; page 11, right column, first paragraph); and b) one or more flavor precursor molecules selected from a group including a sugar (precursor compounds include sugars, such as glucose, fructose or ribose; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1) and cysteine (precursor compounds include amino acids such as cysteine; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1); a method comprising: a) combining a heme-containing protein (beef contains heme protein which can contribute to its flavor; page 10, left column, second paragraph; page 11, right column, first paragraph), cysteine (precursor compounds include amino acids, such as cysteine; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1), and a sugar (precursor compounds include sugars, such as glucose, fructose or ribose; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1), and one or more optional flavor precursor molecules to form a mixture (optional language not analyzed); and a method for imparting a meat-like flavor to a food product (developing a beef flavor in beef (food product); page 2, left column, first paragraph to right column, first paragraph) comprising contacting said food product with a flavoring composition (precursor compounds including flavor-active volatiles embedded in a matrix (composition); page 2, left column, first paragraph), said flavoring composition comprising i) a heme-containing protein (beef contains heme protein which can contribute to its flavor; page 10, left column, second paragraph; page 11, right column, first paragraph); and ii) one or more flavor precursor molecules selected from a group including a sugar (precursor compounds include sugars such as glucose, fructose or ribose; page 2, left column, first paragraph to right column, first paragraph; page 2, table 1) and cysteine (precursor compounds include amino acids such as cysteine; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1), wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product (beef flavor is developed by heating the beef; page 2, left column, first paragraph to right column, first paragraph). Brooks discloses a flavor compound selected from the group including phenylacetaldehyde (phenylacetaldehyde is a volatile flavor compound present in cooked steak; page 4, Table 8). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the methods for producing a flavor compound and the food product, as previously disclosed by Yamaguchi, for including the use of the flavor precursor molecules and the method for imparting a meat-like flavor to a food product, as previously disclosed by Brewer, as the previous disclosure by Yamaguchi includes the use of compounds which when heated oxidize and enhance the flavor of foods, including meats (Yamaguchi; paragraphs [0022], [0041], [0043], [0053]), Brewer discloses flavor precursor molecules which, when heated, impart various flavors to beef (Brewer; page 2, left column, first paragraph to right column, first paragraph; page 2, Table 1), and a combination of these references would have resulted in an improved flavor enhancer for imparting meat flavors to foods when cooked. Additionally, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the methods for producing a flavor compound and the food product, as previously disclosed by Yamaguchi, for including forming flavor compounds, such as phenylacetaldehyde, as previously disclosed by Brooks, as the previous disclosure by Yamaguchi includes the use of volatile compounds which, when heated, oxidize and enhance the flavor of foods, including meats (Yamaguchi; paragraphs [0022], [0041], [0043], [0044], [0053]), Brooks discloses volatile flavor compounds present in cooked steaks, including phenylacetaldehyde, which is present in higher concentrations in Prime quality grade steaks (Brooks; page 4, Table 8), and a combination of these references would have resulted in an improved method for imparting meat flavors to food, based on the formation of desirable volatile compounds, when the food is cooked.

Since none of the special technical features of the Groups I-II+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by Yamaguchi, Brewer and Brooks references, unity of invention is lacking.

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(54) **Title**: METHODS AND COMPOSITIONS FOR CONSUMABLES

(57) **Abstract**: Methods and compositions for the production of non-meat consumable products are described herein. A meat substitute is described which is constructed from a muscle analog, a fat analog, and a connective tissue analog.

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METHODS AND COMPOSITIONS FOR CONSUMABLES

CROSS-REFERENCE TO RELATED APPLICATIONS

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

TECHNICAL FIELD

This invention relates to consumable products and more particularly, to non-animal based replicas of animal-based food products, that can be produced, in some embodiments, by breaking down non-animal materials into their constituent parts and reassembling those parts into the consumables.

BACKGROUND

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

The consumption of meat has a profound negative impact on human health. The health benefits of a vegetarian diet are well established. If the human population would shift to a more vegetarian diet, there would be a decrease in health care costs.

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

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

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

Food typically has its origin in a photosynthetic organism, typically from plants. Some food is obtained directly from plants; but even animals that are used as food sources are raised by feeding them food derived from plants. Edible fungi and bacteria are used to transform materials from plants or animals into other food products, mushrooms, bread, yogurt and the like.

In most cases, the plant or animal is fractionated into a variety of different portions, depending upon the purpose of the food. Often, certain portions of the plant, such as the seeds or fruits, are more highly prized by humans than others and these are selected for human consumption whilst other less desirable portions, such as the stalks of grasses, are typically used for feeding animals.

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

While many foods can be eaten raw, many also undergo some form of preparation for reasons of safety, palatability, texture, or flavor. At the simplest level, this may involve washing, cutting, trimming, or adding other foods or ingredients. It may also involve mixing, heating or cooling or fermentation and individual foods may be combined with other food products to achieve the desired mix of properties.

In recent years, attempts have been made to bring scientific rigor to the process of food preparation, under the fields of food science and molecular gastronomy. Food science broadly studies the safety, microbiology, preservation, chemistry, engineering and physics of food preparation, whereas molecular gastronomy focuses on the use of scientific tools such as liquid nitrogen, emulsifying agents such as soy lecithin and gelling agents such as calcium alginates to transform food products into unexpected forms.

However, the raw material is typically an entire organism (plant or animal) or an isolated tissue such as a steak, the fruiting body of a fungus, or the seed of a plant. In some cases, the isolated tissue is modified before food preparation, such as making flour or isolating oils and bulk proteins from seeds.

Despite that fact that all of these items comprise a mixture of proteins, carbohydrates, fats, vitamins and minerals, the physical arrangement of these materials in the original plant or animal determines the use to which the plant or animal tissue will be put. Disclosed herein are improved methods and composition for the production of consumables.

SUMMARY

Provided herein are consumable products and methods of making the same. The consumables can be non-animal based consumable goods, e.g., containing mainly plant or entirely-plant based proteins and/or fats, and can be in the form of a beverage (e.g., an alcoholic beverage such as cream liquor, or a protein drink), a protein supplement, a baked good (e.g., a bread or a cookie), a condiment (e.g., a mayonnaise, a mustard), a meat product, or a meat substitute product (e.g., a ground beef product). For example, the protein drink can be a meal replacement beverage, a beer supplemented with the

protein, or a distilled alcoholic beverage (e.g., vodka or rum) supplemented with the protein. The condiment can be mayonnaise. The meat product can be a pate, a sausage, or a meat substitute that can include a muscle replica, plant-based adipose and/or connective tissue. Coacervates that include one or more proteins can be used to help bind the ingredients to each other in the consumable products (e.g., a ground beef product).

Accordingly, provided herein is a consumable product comprising an isolated and purified plant protein, wherein the isolated and purified plant protein has (i) a solubility in a solution of at least 25 g/L at a temperature between about 2°C and about 32°C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in a solution of at least 1 mg/ml at a temperature of between 90°C and 110°C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM. In some embodiments, the consumable product is a beverage, a protein supplement, a baked good, a condiment, a meat product, or a meat substitute product. In some embodiments, the beverage is an alcoholic beverage or a protein drink. In some embodiments, the alcoholic beverage is a cream liquor. The cream liquor can further include a non-dairy lipid emulsion, where the cream liquor is free of animal products. In some embodiments, the protein drink is a meal replacement beverage, a beer supplemented with said protein, or a distilled alcoholic beverage supplemented with the protein. A condiment can be a mayonnaise replica. In some embodiments, the meat product can be a pate, a sausage replica, or a meat substitute. In some embodiments, the isolated and purified plant protein is at least 10 kDa in size. In some embodiments, the isolated and purified plant protein is not fully denatured. In some cases, the isolated and purified plant protein is not derived from soy. In some embodiments, the isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin.

In some embodiments, the isolated and purified plant protein comprises a dehydrin, a hydrophilin, an intrinsically disordered protein, or a protein identified based on its ability to stay soluble after boiling at a pH and a salt concentration comparable to a food. In some embodiments, the consumable product further comprises a plant derived

lipid or a microbial-derived lipid. In some embodiments, the consumable product further includes a second isolated and purified protein, and/or a seasoning agent, a flavoring agent, an emulsifier, a gelling agent, a sugar, or a fiber.

The disclosure also provides a consumable product comprising a coacervate comprising one or more isolated and purified proteins. In some embodiments, the consumable product is a meat replica. In some embodiments, the consumable product further includes a plant derived lipid or a microbial-derived lipid. The plant derived lipid or microbial-derived lipid can comprise lecithin and/or an oil. The product can include up to about 1% lecithin by weight. The product can include lecithin and the oil. In some embodiments, the oil is canola oil, palm oil, or cocoa butter. The product can include about 1% to about 9% of the oil. The one or more isolated and purified proteins can comprise plant proteins. The one or more plant proteins can comprise one or more pea proteins, chickpea proteins, lentil proteins, lupine proteins, other legume proteins, or mixtures thereof. In some embodiments, the one or more pea proteins are legumins, vicilin, a convicilin, or a mixture thereof.

The disclosure also provides a meat replica comprising a muscle replica, a connective tissue replica, an adipose tissue replica, and a coacervate comprising one or more isolated and purified proteins. The coacervate further can comprise a plant-derived lipid or microbial-derived lipid. The plant derived lipid or microbial-derived lipid can be lecithin and/or an oil. The meat replica can be a ground beef replica.

Also provided is a consumable product comprising a comprising a cold set gel comprising one or more isolated and purified proteins from a non-animal source and a salt. In some embodiments, the isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin. In some embodiments, the isolated and purified plant protein comprises a dehydrin, a hydrophilin, or an intrinsically disordered protein. In some embodiments, the cold set gel further comprises a plant derived lipid or microbial derived lipid. In some embodiments, the plant derived lipid or microbial derived lipid is lecithin and/or oil.

The disclosure further provides an adipose tissue replica comprising one or more isolated plant proteins, one or more plant or algal derived oils, and optionally a

phospholipid. In some embodiments, the phospholipid is lecithin. In some embodiments, the plant based oils are selected from the group consisting of corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, and combinations thereof. In some embodiments, the fat release temperature of the adipose tissue replica is between 23°C to 33°C, 34°C to 44°C, 45°C to 55°C, 56°C to 66°C, 67°C to 77°C, 78°C to 88°C, 89°C to 99°C, 100°C to 110°C, 111°C to 121°C, 122°C to 132°C, 133°C to 143°C, 144°C to 154°C, 155°C to 165°C, 166°C to 167°C, 168°C to 169°C, 170°C to 180°C, 181°C to 191°C, 192°C to 202°C, 203°C to 213°C, 214°C to 224°C, 225°C to 235°C, 236°C to 246°C, 247°C to 257°C, 258°C to 268°C, 269°C to 279°C, 280°C to 290°C, or 291°C to 301°C. In some embodiments, the percent fat release of the adipose tissue replica is 0 to 10%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, 50% to 60%, 60% to 70%, 70% to 80%, 80% to 90%, or 90% to 100% upon cooking. In some embodiments, the isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin.

In some embodiments, the adipose tissue replica comprises from about 40% to about 90% of the oil. In some embodiments, the adipose tissue replica comprises from about 1% to about 6% of the isolated and purified plant protein. In some embodiments, the adipose tissue replica comprises from about 0.05 to about 2% of the phospholipid. In some embodiments, the firmness of the adipose tissue replica is similar to that of beef adipose tissue.

Also provided is a consumable product comprising a heme-containing protein and (i) carbon monoxide and/or (ii) a nitrite, wherein the consumable product does not comprise meat. In some embodiments, the heme-containing protein accounts for at least 0.01% of the composition. In some embodiments, the consumable product further comprises one or more ammonium, sodium, potassium, or calcium salts. In some embodiments, the isolated and purified proteins are crosslinked.

Further provided is a consumable product comprising a gelled emulsion, wherein the gelled emulsion comprises:

a) an isolated and purified protein;

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

c) a second lipid that when not in the consumable product is liquid at the selected temperature range; wherein the melting temperature of the mixture of the first and second lipids is similar to the melting temperature of lipids found in meat, and wherein the first and second lipids are plant derived lipids or microbial derived lipids.

The disclosure also provides a method for making a consumable product comprising:

a) preparing a solution comprising an isolated and purified plant protein, wherein the isolated and purified plant protein has (i) a solubility in the solution of at least 25 at a temperature between about 2°C and 32°C, wherein the solution has a pH between 3 and 8, and has a sodium chloride content of 0 to 300 mM or (ii) a solubility in the solution of at least 1 mg/ml at a temperature of between 90°C and 110°C, wherein the solution has a pH between 5 and 8 and has a sodium chloride content of 0 to 300 mM; and

b) adding the solution to a beverage.

In some embodiments, the solution comprises two or more isolated and purified plant proteins. In some embodiments, the beverage is clear. In some embodiments, the isolated and purified plant protein is at a concentration of at least 1% by weight in the solution. In some embodiments, the isolated and purified plant protein is selected from the group consisting of RuBisCo, a moong globulin, a soy globulin, a pea globulin, a pea albumin, a prolamin, a lentil protein, a dehydrin, a hydrophilin, and an intrinsically disordered protein. In some embodiments, the isolated and purified plant protein is lyophilized prior to making said solution. In some embodiments, the beverage has an improved mouthfeel compared to a corresponding beverage without the isolated and purified protein.

Also provided is a method for extending the shelf-life of a meat-free consumable product, the method comprising adding a heme-containing protein to the consumable product, wherein the heme containing protein oxidizes more slowly than myoglobin under equivalent storage conditions. In some embodiments, the heme-containing protein comprises an amino acid sequence with at least 70% homology to an amino acid sequence set forth in any one of SEQ ID NOs: 1-27.

Further provided is a method for making a meat replica comprising a cold set gel, wherein the method includes:

a) denaturing a solution comprising at least one isolated and purified protein from a non-animal source under conditions wherein the isolated and purified protein does not precipitate out of the solution;

b) optionally adding any heat-labile components to the solution of denatured protein;

c) gelling the solution of denatured protein at about 4°C to about 25°C by increasing the ionic strength of the solution to form a cold set gel; and

d) incorporating the cold set gel into a meat replica.

In some embodiments, the gelling is induced using 5 to 100 mM sodium or calcium chloride. In some embodiments, the heat-labile components are proteins or lipids, or mixtures thereof. In some embodiments, the protein is a heme-containing protein. In some embodiments, the cold set gel is formed in a matrix comprising a freeze-aligned plant protein.

In some embodiments, the isolated and purified protein from a non-animal source is a plant protein. In some embodiments, the plant protein is selected from the group consisting of RuBisCo, a moong globulin, a soy globulin, a pea globulin, a pea albumin, a prolamin, a lentil protein, a dehydrin, a hydrophilin, and an intrinsically disordered protein.

Further provided is an adipose tissue replica, comprising

a) an isolated and purified non-animal protein;

b) a non-animal lipid; and

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

Also provided is a connective tissue replica comprising one or more isolated and purified proteins assembled into fibrous structures by a solution spinning process. In some embodiments, the fibrous structures are stabilized by a cross-linking agent.

Provided herein is a method for imparting a beef like flavor to a consumable product, comprising adding to the consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to the consumable composition.

Also provided is a method for making a poultry or a fish composition taste like beef, the method comprising adding a heme protein to the poultry or fish composition, respectively.

In some embodiments, the heme-containing protein has an amino acid sequence with at least 70% homology to any one of the amino acid sequences set forth in SEQ ID NOs: 1-27.

Further provided is a method of making a coacervate, the method comprising

a) acidifying a solution of one or more plant proteins to a pH between 3.5 and 5.5, wherein the solution comprises 100 mM or less of sodium chloride; and

b) isolating the coacervate from the solution. In some embodiments, the pH is between 4 and 5. In some embodiments, the plant proteins comprise one or more pea proteins, chickpea proteins, lentil proteins, lupine proteins, other legume proteins, or mixtures thereof. In some embodiments, the pea proteins comprise isolated and purified legumins, isolated and purified vicilins, isolated and purified convicilins, or combinations thereof. In some embodiments, the isolated and purified pea proteins comprise isolated and purified vicilins and isolated and purified convicilins. In some embodiments, the acidifying step is done in the presence of a plant derived lipid or microbial derived lipid. In some embodiments, the plant derived lipid or microbial derived lipid comprises oils and/or phospholipids.

Provided herein is a method of making an adipose tissue replica, the method comprising forming an emulsion comprising one or more isolated plant proteins, one or more plant or algal derived oils, and optionally a phospholipid. In some embodiments, when the phosoholipid is included, it is lecithin. In some embodiments, the plant based oils are selected from the group consisting of corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, and combinations thereof. In some embodiments, the fat release temperature of the adipose tissue replica is between 23°C to 33°C, 34°C to 44°C, 45°C to 55°C, 56°C to 66°C, 67°C to 77°C, 78°C to 88°C, 89°C to 99°C, 100°C to 110°C, 111°C to 121°C, 122°C to 132°C, 133°C to 143°C, 144°C to 154°C, 155°C to 165°C, 166°C to 167°C, 168°C to 169°C, 170°C to 180°C, 181°C to 191°C, 192°C to 202°C, 203°C to 213°C, 214°C to 224°C, 225°C to 235°C, 236°C to 246°C, 247°C to 257°C, 258°C to 268°C, 269°C to 279°C, 280°C to 290°C, or 291°C to 301°C. In some embodiments, the percent fat release of the adipose tissue replica is 0 to 10%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, 50% to 60%, 60% to 70%, 70% to 80%, 80% to 90%, or 90% to 100% upon cooking. In some embodiments, the isolated and purified plant protein comprises one or more of RuBisCo, moong 8S globulin, a pea globulin, a pea albumin, a lentil protein, zein, or an oleosin. In some embodiments, the emulsion comprises from about 40% to about 90% of the oil. In some embodiments, the emulsion comprises from about 1% to about 4% of the isolated and purified plant protein. In some embodiments, the adipose tissue replica comprises from about 0.05 to about 1% of the phospholipid. In some embodiments, the emulsion is formed by high-pressure homogenization, sonication, or hand homogenization.

Further provided is a method of minimizing undesirable odors or flavors in a composition comprising plant proteins, the method comprising contacting the composition with a ligand having affinity for one or more lipoxygenases.

Also provided is a method of minimizing undesirable odors or flavors in a composition comprising plant proteins, the method comprising contacting the

composition with activated carbon then removing the activated carbon from the composition.

Also provided is a method of minimizing undesirable odors or flavors in a composition comprising plant proteins, the method comprising adding a lipoxygenase inhibitor and/or an antioxidant to the composition.

The disclosure further provides a chocolate flavored spread comprising:

a) sugar

b) a chocolate flavoring, and

c) a cream fraction from a plant based milk.

Provided herein is a method for altering the texture of a consumable during or after cooking comprising incorporating within the consumable one or more plant proteins with a low denaturation temperature. In some embodiments, at least one of the one or more plant proteins is isolated and purified. In some embodiments, the one or more plant proteins are selected from the group consisting of rubisco, pea proteins, lentil proteins, or other legume proteins. In some embodiments, the pea proteins comprise pea albumin proteins. In some embodiments, the consumable becomes firmer during or after cooking.

Also provided is a tissue replica, which comprises a freeze-aligned non-animal protein. In some embodiments, the non-animal protein is a plant protein. In some embodiments the non-animal protein is isolated and purified. In some embodiments the tissue replica is amuscle tissue replica.

The disclosure also provides a meat replica which includes a tissue replica comprising a freeze-aligned non-animal protein.

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

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

DESCRIPTION OF THE DRAWING

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

FIG. 2A is a bar graph depicting the percent fat release based on the amount of lecithin.

FIG. 2B is a bar graph depicting the temperature of the fat release based on the amount of lecithin.

FIG. 2C is a bar graph depicting the firmness of adipose replicas based on the amount of lecithin.

FIG. 3 is a bar graph depicting the percent fat release of adipose replicas containing different oils (canola oil, cocoa butter, coconut oil, or rice bran oil).

FIG. 4 is a bar graph depicting the fat release temperature of adipose replicas containing different oils (canola oil, cocoa butter, coconut oil, or rice bran oil).

DETAILED DESCRIPTION

I. Consumables

Methods and compositions for producing consumables are described herein. In some cases, the consumables are non-animal based replicas of animal-based food products that can be produced by breaking down non-animal materials into their constituent parts and reassembling those parts into the consumables. In certain instances, the consumables are not intended to replicate an animal-based food and instead have their own unique characteristics desirable as a food. Additionally, the consumables may, in some instances, act as nutraceuticals or carriers for pharmaceutical compositions rather than serving a primary function as food.

The advantages of the consumables described herein can include, for example, the using less energy or water in the production of the consumable compared to similar food products, using no animals in the production of the consumable, making a healthier product, using raw materials that would otherwise be discarded, or allowing for the elimination (or lack of incorporation) of certain components (e.g., allergens) from the consumables. The consumables also may have a higher degree of production consistency, allowing for improved quality control of the products. Another advantage is that the consumables can be designed intentionally to have desirable characteristics for food preparation that are superior to traditional food products.

The consumables can be for animal consumption, including human consumption. The consumables can be food for domestic animals (e.g., dog food could be produced according to the present inventions) or wild animals (e.g., food for non-domesticated predatory animals).

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

The consumable can be approved by suitable regulatory authorities. For example, the consumable could be prepared to be suitable for the U.S. Food and Drug Administration. Methods of the invention can include steps necessary to satisfy regulatory agencies.

The consumables of the present invention can replicate, compete with, supplement or replace conventional food products (herein referred to as "food products"). Food products can be any foods which presently exist. The consumables of the invention can be made to replicate the food products, e.g., an equivalent meat product. The equivalent meat product can be a white meat or a dark meat. The equivalent meat product can be derived from any animal. Non-limiting examples of animals used to derive the equivalent meat product include farmed animals such as, e.g., cattle, sheep, pig, chicken, turkey, goose, duck, horse, dog or game animals (whether wild or farmed) such as, e.g., rabbit, deer, bison, buffalo, boar, snake, pheasant, quail, bear, elk, antelope,

pigeon, dove, grouse, fox, wild pig, goat, kangaroo, emu, alligator, crocodile, turtle, groundhog, marmot, possum, partridge, squirrel, raccoon, whale, seal, ostrich, capybara, nutria, guinea pig, rat, mice, vole, any variety of insect or other arthropod, or seafood such as, e.g., fish, crab, lobster, oyster, muscle, scallop, abalone, squid, octopus, sea urchin, tunicate and others.

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

A consumable (e.g., a meat substitute) can comprise one or more of a first composition comprising a muscle tissue replica, a second composition comprising an adipose tissue replica, and/or a third composition comprising a connective tissue replica, wherein the one or more compositions are combined in a manner that recapitulates the physical organization of meat. The present invention also provides distinct compositions for a muscle tissue replica (herein referred to as "muscle replica"), an adipose tissue replica (herein referred to as an "adipose replica" or "fat replica"), and a connective tissue replica (herein referred to as "connective tissue replica"). In some embodiments, these compositions are principally or entirely composed of ingredients derived from non-animal sources (e.g., 10% or less of the ingredients are from animal sources). In alternative embodiments, the muscle, fat, and/or connective tissue replica, or the meat substitute products comprising one or more of the replicas, are partially derived from animal sources but supplemented with ingredients derived from non-animal sources. In some embodiments, as much as 90% of the food product is derived from animal sources. In some embodiments about 75% of the food product is derived from animal sources. In some embodiments, about 50% of the food product is derived from animal sources. In some embodiments about 10% of the food product is derived from animal sources. In yet other alternative embodiments, the invention provides meat products substantially

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

Examples of other food products which the consumable can replicate or replace include: beverages (e.g., cream liquor or milk), protein drinks (e.g., RuBisCo can be used as a protein supplement in beer, distilled alcohol beverages such as vodka, fruit juices, meal replacement beverages, or water), pastes (e.g. Nutella™, cream, nacho cheese or mayonnaise replicas), pate, blood sausage, meat extenders, eggs, fish, sausage, tenders , spam or chilled foods (e.g., ice cream , yogurt, kefir, sour cream or butter replicas).

The consumables can be a meat replica. The consumables can be made to mimic the cut or appearance of meat. For instance, a consumable may be visually similar to or indistinguishable from ground beef or a particular cut of beef. In an example embodiment, the replicas are combined in a manner that approximates the physical organization of natural ground meat (e.g., ground beef, ground chicken, or ground turkey). In other embodiments, the replicas are combined in a manner that approximates different cuts of beef, such as, e.g., rib-eye, filet mignon, London broil, among others. Alternatively, the consumables can be a made with a unique look or appearance. For instance, the consumable could contain patterns (e.g., lettering or pictures) that are formed from the structure of the consumable. In some instances, the consumables look like traditional food products after they are prepared. For example, a consumable may be produced which is larger than a traditional cut of beef but which, after the consumable is sliced and cooked, appears the same as a traditional cooked meet. In some embodiments the consumable may resemble a traditional food product shape in two dimensions, but not

in a third. For example the consumable may resemble a cut of meat in two dimensions (for example when viewed from the top), but may be much longer (or thicker) than the traditional cut. In this example the composition can be cut repeatedly into traditionally meat shaped products.

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

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

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

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

The consumable may have health benefits compared to an animal product it replaces in the diet. For example it may have less cholesterol or lower levels of saturated fats than comparable meat products. The American Heart Association and the National Cholesterol Education Program recommends limiting cholesterol intake from food to 300 mg per day, which is equivalent to the consumption of 12 ounces of beef or two egg yolks. Consumables described herein that are indistinguishable to animal products such as ground beef, and having a reduced cholesterol content or no cholesterol, can help maintain a low cholesterol diet. In another example, a consumable described herein may contain no cholesterol, or higher levels of poly-unsaturated fatty acids compared to the animal product it replaces.

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

The consumable may have a smaller "carbon footprint" than the meat products they replace. For example the consumable may result in net greenhouse gas emissions of 1%, 5%, 10%, 25%, 50% or 75% of the greenhouse gas emissions attributable to the animal product it replaces. By way of example, according to the Environmental Working Group (2011) "meat eaters guide to Climate Change and Health," the production of beef causes the emission of 27 kg equivalents of carbon dioxide per kilogram of beef that is

consumed, and the production of lamb causes the emission of 39 kg equivalents of carbon dioxide per kilogram of beef that is consumed.

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

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

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

II. Properties of the Consumable

The consumables described herein are typically designed to replicate the experience of eating a food product, e.g., meat. The look, texture, and taste of the consumable can be such that it is similar to, or indistinguishable from, a food product, e.g., meat. The consumable can also be produced to have the desirable characteristics of

food products without incorporating other undesirable characteristics. For example, a consumable can be a replica steak that does not have gristle or other components not typically consumed in a predicate food product.

The invention provides, in certain embodiments, methods for determining the suitability for a consumable to qualify as a replica of a food product, for example, by determining whether an animal or human can distinguish the consumable from a predicate food product, e.g., a particular meat. One method to determine whether the consumable is comparable to a food product (e.g. meat) is to a) define the properties of meat and b) determine whether the consumable has similar properties.

Properties that can be tested or used to compare or describe a food product or the consumable include mechanical properties such as hardness, cohesiveness, brittleness, chewiness, gumminess, viscosity, elasticity, and adhesiveness. Properties of food products that can be tested also include geometric properties such as particle size and shape, and particle shape and orientation. The three dimensional organization of particles may also be tested. Additional properties can include moisture content and fat content. These properties can be described using terms such as "soft," "firm" or "hard" describe hardness; "crumbly," "crunchy," "brittle," "chewy," "tender," "tough," "short," "mealy," "pasty," or "gummy," to describe cohesiveness; "thin" or "viscous" to describe viscosity; "plastic" or "elastic" to describe elasticity; "sticky," "tacky" or "gooey" to describe adhesiveness; "gritty," "grainy" or "course" to describe particle shape and size; "fibrous," "cellular" or "crystalline" to describe particle shape and orientation, "dry," "moist," "wet," or "watery" to describe moisture content; or "oily" or "greasy" to describe fat content. Accordingly, in one embodiment, a group of people can be asked to rate a certain food product, for instance ground beef, according to properties which describe the food product. A consumable described herein can be rated by the same people to determine equivalence.

Flavor of the food product can also be assessed. Flavors can be rated according to similarity to food products, e.g., "eggy," "fishy," "buttery," "chocolaty," "fruity", "peppery," "baconlike," "creamy," "milky," "or "beefy." Flavors can be rated according to the seven basic tastes, i.e., sweet, sour, bitter, salty, umami (savory), pungent (or

piquant), and metallic. Flavors can be described according to the similarity to an experience caused by a chemical, e.g., diacetyl (buttery), 3-hydroxy-2 butanone (buttery), nona-2E-enal (fatty), 1-octene-3-ol (mushroom), hexanoic acid (sweaty), 4-hydroxy-5-methyl furanone (HMF, meaty), pyrazines (nutty), bis(2-methyl-3-furyl) disulfide (roast meat), decanone (musty/fruity), isoamyl acetate (banana), benzaldehyde (bitter almond), cinnamic aldehyde (cinnamon), ethyl propionate (fruity), methyl anthranilate (grape), limonene (orange), ethyl decadienoate (pear), allyl hexanoate (pineapple), ethyl maltol (sugar, cotton candy), ethylvanillin (vanilla), butanoic acid (rancid), 12-methyltridecanal (beefy), or methyl salicylate (wintergreen). These ratings can be used as an indication of the properties of the food product. The consumables of the present invention can then be compared to the food product to determine how similar the consumable is to the food product. In some instances the properties of the consumables are then altered to make the consumable more similar to the food product. Accordingly, in some embodiments, the consumable is rated similar to a food product according to human evaluation. In some embodiments the consumable is indistinguishable from real meat to a human.

The consumables can be made to eliminate properties associated with the source of the components of the consumables. For example a consumable can be made from components obtained from beans but can be made to lack a "beany" flavor or texture. One way this can be achieved is by breaking down the component source materials into isolated and purified components and not using components that cause undesired characteristic properties of the source. In addition, as described herein, off-flavors or aromas (e.g., undesirable flavors or aromas) in isolated and/or purified components can be minimized by deodorizing with activated charcoal or by removing enzymes such as lipoxygenases (LOX), which can be present in trace amounts and which can convert unsaturated triacylglycerides (such as linoleic acid or linolenic acid) into smaller and more volatile molecules. LOX are naturally present in legumes such as peas, soybeans, and peanuts, as well as rice, potatoes, and olives. When legume flours are fractionated into separate protein fractions, LOX can act as undesirable "time-bombs" that can cause undesirable flavors or aromas on aging or storage. As shown in Example 34, compositions containing plant proteins (e.g., from ground plant seeds) can be subjected to

purification to remove LOX using, for example, an affinity resin that binds to LOX and removes it from the protein sample. The affinity resin can be linoleic acid, linolenic acid, stearic acid, oleic acid, propyl gallate, or epigalloccatechin gallate attached to a solid support such as a bead or resin. See, e.g., WO2013138793. In addition, depending on the protein component, certain combinations of antioxidants and/or LOX inhibitors can be used as effective agents to minimize off-flavor or off-odor generation in protein solutions, especially in the presence of fats and oils. Such compounds can include, for example, one or more of β-carotene, α-tocopherol, caffeic acid, propyl gallate, or epigallocatechin gallate. These may be included during purification of the proteins or during subsequent food processing steps to mitigate generation of off-flavors or off-odors in protein-based foods.

In some compositions, subjects asked to identify the consumable identify it as a form of a food product, or as a particular food product, e.g., a subject will identify the consumable as meat. For example, in some compositions a human will identify the consumable as having properties equivalent to meat. In some embodiments one or more properties of the consumable are equivalent to the corresponding properties of meat according to a human's perception. Such properties include the properties that can be tested. In some embodiments a human identifies a consumable of the present invention as more meat like than any meat substitutes found in the art.

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

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

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

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

Consumables of the invention can also have similar physical characteristics as food products, e.g., traditional meat. In one embodiment, the force required to pierce a 1 inch thick structure (e.g., a patty) made of a consumable of the invention with a fixed diameter steel rod is not significantly different than the force required to pierce a 1 inch thick similar food product structure (e.g., a ground beef patty) with a similar fixed diameter steel rod. Accordingly, the invention provides for consumables with similar physical strength characteristics to meat. In another embodiment, the force required to tear a sample of the invention with a cross-sectional area of 100 mm^2 is not significantly different than the force required to tear a sample of animal tissue (muscle, fat or connective tissue) with a cross-sectional area 100 mm^2 measured the same way. Force can be measured using, for example, TA.XT Plus Texture Analyzer (Textrue Technologies Corp.). Accordingly, the invention provides for consumables with similar physical strength characteristics to meat.

Consumables described herein can have a similar cook loss characteristic as a food product, e.g., meat. For example a consumable can have a similar fat and protein

content to ground beef and have the same reduction in size when cooked as real ground beef. Similarities in size loss profiles can be achieved for various compositions of consumables described herein matched to various meats. The cook loss characteristics of the consumable also can be engineered to be superior to food products. For example a consumable can be produced that has less loss during cooking but achieves similar tastes and texture qualities as the cooked products. One way this is achieved is by altering the proportions of lipids based on melting temperatures in the consumable composition. Another way this is achieved is by altering the protein composition of the consumable by controlling the concentration of protein or by the mechanism by which the tissue replica is formed.

In some embodiments, the consumable is compared to an animal based food product (e.g., meat) based upon olfactometer readings. In various embodiments the olfactometer can be used to assess odor concentration and odor thresholds, or odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of appreciation, or relative intensity of odors. In some embodiments, the olfactometer allows the training and automatic evaluation of expert panels. So in some embodiments the consumable is a product that causes similar or identical olfactometer readings. In some embodiments the differences are sufficiently small to be below the detection threshold of human perception.

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

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

Shelf life is the length of time that consumables are given before they are considered unsuitable for sale, use, or consumption. Generally, it is important to maintain a meat product at about 2°C as the shelf life decreases with exposure to higher temperatures.

The shelf life of meat is determined through research into the meat products' sensory cues over time (odor, visual appearance of the package, color, taste and texture), and through laboratory analysis under controlled conditions to determine how long a product remains safe, wholesome and enjoyable. Ground beef is being used as an example, but similar conditions would apply to steaks, chops and roasts from other meat types. Beef in its natural state is dark bluish-purple. However, oxygen can permeate into the meat and cause a chemical reaction with the myoglobin in meat, leading to a red color. Ongoing exposure to oxygen causes oxidation of myoglobin and causes red meat to become brown and develop "off" flavors. To control this oxidation, there has been significant research into different methods of storing and displaying meat products to increase the shelf life of meat products. These include the use of vacuum packing,

modified atmosphere packing (high oxygen), modified atmosphere packaging (low oxygen with carbon monoxide), and/or High Pressure Pasteurization (HPP).

The main determinant of the color of meat is the concentration of iron carrying proteins in the meat. In the skeletal muscle component of meat products, one of the main iron-carrying proteins is myoglobin. It is estimated that the white meat of chicken has under 0.05% myoglobin; pork and veal have 0.1-0.3% myoglobin; young beef has 0.4-1.0% myoglobin; and old beef has 1.5-2.0% myoglobin. Normally, myoglobin in meat exists in three states: Oxymyoglobin (Fe^{2+}) (oxygenated = bright red); myoglobin (Fe^{2+}) (non-oxygenated = purplish/magenta); and metmyoglobin (Fe^{3+}) (oxidized = brown). The transition of oxymyoglobin to metmyoglobin in the presence of oxygen is thought to be the cause of the color change of ground meat from red to brown. Meat shelf life extenders have been developed to extend the lifetime of the red color of meat products including but not limited to carbon monoxide, nitrites, sodium metabisulfite, Bombal, vitamin E, rosemary extract, green tea extract, catechins and other anti-oxidants.

However an intrinsically more stable heme protein such a hemoglobin isolated from *Aquifex aeolicus* (SEQ ID NO:3) or *Methylacidiphilum infernorum* (SEQ ID NO: 2) will oxidize more slowly than a mesophilic hemoglobin such as myoglobin. The heme proteins described herein (see, e.g., FIG. 1) also may have the lifetime of the reduced heme-Fe^{2+} state extended by meat shelf life extenders such as carbon monoxide and sodium nitrite. Heme proteins may be selected for the desired color retention properties. For example for low temperature *sous-vide* cooking, a relatively unstable heme protein such as one from *Hordeum vulgare* may provide a brown product that appears cooked under conditions wherein myoglobin would retain its red, uncooked appearance. In some embodiments the heme protein may be selected to have increased stability where for example the meat replica may retain an attractive medium rare appearance despite being thoroughly cooked for food safety.

The main determinant of rancidity and production of off flavors or off odors is the oxidation of components of the consumable, including but not limited to, the fats. For example, oxidation of unsaturated fatty acids is a known cause of rancid odors. In some embodiments, meat replicas have extended shelf life because the makeup of the chemical

properties of the meat replica are controlled such that the taste, texture, smell, and chemical properties do not react with oxygen to create off flavors or off odors. In some embodiments the meat replicas are less sensitive to oxidation due to the presence of higher degree of unsaturated fatty acids than present in beef. In some embodiments the meat replica contains no unsaturated fatty acids. In other embodiments the meat replica contains higher levels of anti-oxidants such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases than are present in meat. In other embodiments, off flavor or off odor generating components such as lipoxygenase are not present.

In some embodiments, a consumable described herein shows increased stability under commercial packaging conditions. In some embodiments, the improved shelf life is improved by using components with increased oxidative stability such as lipids with reduced levels of unsaturated fatty acids, and/or by using a more stable heme protein such a hemoglobin isolated from *Aquifex aeolicus* (SEQ ID NO:3) or *Methylacidiphilum infernorum* (SEQ ID NO: 2). In some embodiments, the improved shelf life is due to the combination of components used in the consumable. In some embodiments, the consumable is designed specifically for the desired packaging method.

III. Composition of the Consumables

A consumable described herein includes one or more isolated and purified proteins. "Isolated and purified protein" refers to a preparation in which the cumulative abundance by mass of protein components other than the specified protein, which can be a single monomeric or multimeric protein species, is reduced by a factor of 2 or more, 3 or more, 5 or more, 10 or more, 20 or more, 50 or more, 100 or more or 1000 or more relative to the source material from which the specified protein was isolated. For clarity, the isolated and purified protein is described as isolated and purified relative to its starting material (e.g., plants or other non-animal sources). In some embodiments, the term "isolated and purified" can indicate that the preparation of the protein is at least 60% pure, e.g., greater than 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% pure. The fact that a consumable may comprise materials in addition to the isolated and purified protein

does not change the isolated and purified nature of the protein as this definition typically applies to the protein before addition to the composition.

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

A consumable described herein may be substantially or entirely composed of ingredients derived from non-animal sources, e.g., plant, fungal, or microbial based sources. The plant sources can be organically grown sources. Proteins can be extracted from the source material (e.g., extracted from animal tissue, or plant, fungal, algal, or bacterial biomass, or from the culture supernatant for secreted proteins) or from a combination of source materials (e.g., multiple plant species). The consumables also can be made from a combination of plant based and animal based sources. For instance, the consumable may be a ground beef product supplemented with plant based products of the invention.

A. SOURCES OF COMPONENTS OF THE CONSUMABLE

As described above, isolated and purified proteins can be derived from non-animal sources such as plants, algae, fungi (e.g., yeast or filamentous fungi), bacteria, or Archaea. In some embodiments, the isolated and purified proteins can be obtained from genetically modified organisms such as genetically modified bacteria or yeast. In some embodiments, the isolated and purified proteins are chemically synthesized or obtained via *in vitro* synthesis.

In some embodiments, the one or more isolated and purified proteins are derived from plant sources. The isolated and purified proteins can be isolated from a single plant source or, alternatively, multiple plant sources can serve as the starting material for the isolation and purification of proteins. As described herein, isolated and purified plant proteins are soluble in solution. The solution can comprise EDTA (0 – 0.1M), NaCl (0-1M), KCl (0-1M), $NaSO_4$ (0 – 0.2M), potassium phosphate (0-1M), sodium citrate (0-1M), sodium carbonate (0-1M), sucrose (0-50%), Urea (0-2M) or any combination

thereof. The solution can have a pH of 3 to 11. In some embodiments, plant proteins can have a solubility in a solution of >25 g/L (e.g., at least 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, or 225 g/L) at a temperature between about 2°C and about 32°C (e.g., between 3°C and 8°C, 10°C and 25°C, or 18°C and 25°C), wherein the solution has a pH between 3 and 8 (e.g., pH of 3-6, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, or 8), and has a sodium chloride content of 0 to 300 mM (e.g., 50, 100, 150, 200, 250, or 300 mM). In some embodiments, the isolated and purified proteins are soluble in solution at greater than 10, 15, 20, 25, 50, 100, 150, 200, or 250g/L.

One of skill in the art will understand that proteins that can be isolated from any organism in the plant kingdom may be used to produce the consumables described herein. Non-limiting examples of plant sources include grain crops such as, e.g., maize, oats, rice, wheat, barley, rye, millet, sorghum, buckwheat, amaranth, quinoa, triticale (a wheat rye hybrid), teff (*Eragrostis tef*); oilseed crops including cottonseed, sunflower seed, safflower seed, *Crambe*, *Camelina*, mustard, rapeseed (*Brassica napus*); *Acacia*, or plants from the legume family, such as, e.g., clover, *Stylosanthes*, *Sesbania*, vetch (*Vicia*), *Arachis*, *Indigofera*, *Leucaena*, *Cyamopsis*, peas such as cowpeas, english peas, yellow peas, or green peas, or beans such as, e.g., soybeans, fava beans, lima beans, kidney beans, garbanzo beans, mung beans, pinto beans, lentils, lupins, mesquite, carob, soy, and peanuts (*Arachis hypogaea*); leafy greens such as, e.g., lettuce, spinach, kale, collard greens, turnip greens, chard, mustard greens, dandelion greens, broccoli, or cabbage; or green matter not ordinarily consumed by humans, including biomass crops such as switchgrass (*Panicum virgatum*), *Miscanthus*, *Arundo donax*, energy cane, *Sorghum*, or other grasses, alfalfa, corn stover, kelp or other seaweeds, green matter ordinarily discarded from harvested plants, sugar cane leaves, leaves of trees, root crops such as cassava, sweet potato, potato, carrots, beets, or turnips; or coconut.

Protein can be isolated from any portion of the plant, including the roots, stems, leaves, flowers, or seeds. For example, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) can be isolated from, for example, alfalfa, carrot tops, corn stover, sugar cane leaves, soybean leaves, switchgrass, *Miscanthus*, energy cane, *Arundo donax*, seaweed, kelp, algae or mustard greens.

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

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

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

The one or more isolated and purified proteins can include highly soluble proteins such as dehydrins, hydrophilins, natively unfolded proteins (also referred to as intrinsically disordered proteins), or other proteins of the late-embryogenesis abundant (LEA) family. LEA proteins have been found in animals, plants and microorganisms and are thought to act as osmoprotectants and stress response proteins. See, e.g., Battaglia, *et al., Plant Physiol.*, 148:6-24 (2008). Such proteins also are heat stable. Such LEA protiens can have a solubility in a solution of at least 1 g/L (e.g., 2, 4, 6, 8, 10, 15, 20, 25, 50, 100, 150, 200, or 250g/L) at a temperature of between 90°C and 110°C (e.g., between 95°C and 105°C, 95°C, or 100°C), wherein the solution has a pH between 5 and 8 (e.g., pH of 5, 5.5., 6, 6.5, 7, 7.5, or 8) and has a sodium chloride content of 0 to 300 mM (e.g., 50, 100, 150, 200, 250, or 300 mM). In some cases, the LEA proteins may be isolated by heating a protein extract to 90°C-110°C (e.g., 95°C or 100°) and, after centrifugation or filtration of insoluble material, concentrating the LEA protein fraction by, for example, ultrafiltration. In some cases, isoionic pH precipitation, trichloroacetic acid precipitation, and/or ammonium sulfate precipitation steps can be done before or after the heating step to additionally remove non-LEA proteins. Heating the solution to 90°C-110°C denatures most proteins, allows the majority of the proteins to be removed from solution.

B. PROTEINS

Without being bound by theory, it is believed that by isolating and purifying non-animal proteins (e.g., plant proteins), consumables can be made with greater consistency and greater control over the properties of the consumable. In some embodiments, about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component of the consumable is comprised of one or more isolated and purified proteins. The isolated and purified protein may be greater than 60%, 70%, 80%, 85%, 90%, 95%, 99% or 100% pure.

The isolated and purified proteins can be isolated from one or more other components of a non-animal source. For example, a protein fraction may be isolated from an isolate of a plant. The isolated proteins may in some cases be purified, wherein a certain kind of protein is separated from other components found in the non-animal source. Proteins can be separated on the basis of their molecular weight, for example, by size exclusion chromatography, ultrafiltration through membranes, or density centrifugation. In some embodiments, the proteins can be separated based on their surface charge, for example, by isoelectric precipitation, anion exchange chromatography, or cation exchange chromatography. Proteins also can be separated on the basis of their solubility, for example, by ammonium sulfate precipitation, isoelectric precipitation, surfactants, detergents or solvent extraction. Proteins also can be separated by their affinity to another molecule, using, for example, hydrophobic interaction chromatography, reactive dyes, or hydroxyapatite. Affinity chromatography also can include using antibodies having specific binding affinity for the protein of interest, nickel NTA for His-tagged recombinant proteins, lectins to bind to sugar moieties on a glycoprotein, or other molecules which specifically binds the protein of interest.

Isolating proteins allows for the elimination of unwanted material. In some embodiments, an isolated and purified protein is a protein that has been substantially separated from unwanted material (e.g., nucleic acids such as RNA and DNA, lipid membranes, phospholipids, fats, oils, carbohydrates such as starch, cellulose, and

glucans, phenolic compounds, polyphenolic compounds, aromatic compounds, or pigments) in the seeds, leaves, stems, or other portion of the plant.

The isolated and purified proteins also can be recombinantly produced using polypeptide expression techniques (e.g., heterologous expression techniques using bacterial cells, insect cells, fungal cells such as yeast cells, plant cells, or mammalian cells). In some cases, standard polypeptide synthesis techniques (e.g., liquid-phase polypeptide synthesis techniques or solid-phase polypeptide synthesis techniques) can be used to produce proteins synthetically. In some cases, cell-free translation techniques can be used to produce proteins synthetically.

The protein or proteins incorporated into the consumable can serve a nutritional function. In some instance, the protein also serves to alter the properties of the consumable, e.g., the flavor, color, odor, and/or texture of the consumable. For example, a meat substitute product can comprise a protein indicator that indicates cooking progression from a raw state to a cooked state, wherein the meat substitute product is derived from non-animal sources.

Examples of proteins that can be isolated and purified, and used in the consumables described herein include ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose bisphosphate aldolase, phosphofructokinases, triose phosphate isomerases, phosphoglycerate kinases, phosphoglycerate mutases, enolases, pyruvate kinases, proteases, lipases, amylases, glycoproteins, lectins, mucins, glyceraldehyde-3-phosphate dehydrogenases, pyruvate decarboxylases, actins, translation elongation factors, histones, ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo), ribulose-1,5-bisphosphate carboxylase oxygenase activase (RuBisCo activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, extensins, triticeae gluten, collagens, zein, kafirin, avenin, dehydrins, hydrophilins, late embyogenesis abundant proteins, natively unfolded proteins, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin, globulin, pea globulins, and pea albumins.

In some embodiments, an isolated and purified protein can be a protein that interacts with lipids and helps to stabilize lipids in a structure, a protein that binds lipids and helps crosslink lipid structures, or a protein that binds lipids and helps crosslink lipid structures and non-lipid interacting proteins. Without wishing to be bound by a particular theory, using such proteins in a consumable described herein may improve the integration of lipids and/or fat replicas with other components of the meat substitute product, resulting in improved mouthfeel and texture of the final product. A non-limiting example of a lipid-interacting plant protein includes proteins in the oleosin family. Oleosins are lipid-interacting proteins that are found in oil bodies of plants. Other non-limiting examples of plant proteins that can interact with lipids and stabilize emulsions include seed storage proteins from Great Northern beans, albumins from peas, globulins from peas, 8S globulins from moong bean, 8S globulins from Kidney bean, prolamin and lipid transfer proteins.

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

more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption

Non-limiting examples of heme-containing polypeptides can include an androglobin, a cytoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a leghemoglobin, a flavohemoglobin, Hell's gate globin I, a myoglobin, an erythrocruorin, a beta hemoglobin, an alpha hemoglobin, a protoglobin, a cyanoglobin, a cytoglobin, a histoglobin, a neuroglobins, a chlorocruorin, a truncated hemoglobin (e.g., HbN or HbO), a truncated 2/2 globin, a hemoglobin 3 (e.g., Glb3), a cytochrome, or a peroxidase.

Heme-containing proteins that can be used in the consumables described herein can be from mammals (e.g., farms animals such as cows, goats, sheep, horses, pigs, ox, or rabbits), birds, plants, algae, fungi (e.g., yeast or filamentous fungi), ciliates, or bacteria. For example, a heme-containing protein can be from a mammal such as a farm animal (e.g., a cow, goat, sheep, pig, ox, or rabbit) or a bird such as a turkey or chicken. Heme-containing proteins can be from a plant such as *Nicotiana tabacum* or *Nicotiana sylvestris* (tobacco); *Zea mays* (corn), *Arabidopsis thaliana*, a legume such as *Glycine max* (soybean), *Cicer arietinum* (garbanzo or chick pea), *Pisum sativum* (pea) varieties such as garden peas or sugar snap peas, *Phaseolus vulgaris* varieties of common beans such as green beans, black beans, navy beans, northern beans, or pinto beans, *Vigna unguiculata* varieties (cow peas), *Vigna radiata* (Mung beans), *Lupinus albus* (lupin), or *Medicago sativa* (alfalfa); *Brassica napus* (canola); *Triticum sps.* (wheat, including wheat berries, and spelt); *Gossypium hirsutum* (cotton); *Oryza sativa* (rice); *Zizania sps*. (wild rice); *Helianthus annuus* (sunflower); *Beta vulgaris* (sugarbeet); *Pennisetum glaucum* (pearl millet); *Chenopodium sp.* (quinoa); *Sesamum sp.* (sesame); *Linum usitatissimum* (flax); or *Hordeum vulgare* (barley). Heme-containing proteins can be isolated from fungi such as *Saccharomyces cerevisiae*, *Pichia pastoris*, *Magnaporthe oryzae*, *Fusarium graminearum*, or *Fusarium oxysporum*. Heme-containing proteins can be isolated from bacteria such as *Escherichia coli*, *Bacillus subtilis, Bacillus megaterium, Synechocistis sp., Aquifex aeolicus, Methylacidiphilum infernorum*, or thermophilic bacteria (e.g, that grow at temperatures greater than 45°C) such as *Thermophilus*. Heme-containing proteins can be isolated from algae such as *Chlamydomonas eugametos*. Heme-

containing proteins can be isolated from protozoans such as *Paramecium caudatum* or *Tetrahymena pyriformis*. In some embodiments, the bacterial hemoglobins are selected from the group consisting of *Aquifex aeolicus*, *Thermobifida fusca*, *Methylacidiphilum infernorum* (Hells Gate), *Synechocystis SP,* or *Bacillus subtilis*. The sequences and structure of numerous heme-containing proteins are known. See for example, Reedy, *et al.*, *Nucleic Acids Research*, 2008, Vol. 36, Database issue D307–D313 and the Heme Protein Database available on the world wide web at http://hemeprotein.info/heme.php.

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

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

caballus (SEQ ID NO:20) myoglobin, a heme-protein from *Nicotiana benthamiana* (SEQ ID NO:21), *Bacillus subtilis* (SEQ ID NO:22), *Corynebacterium glutamicum* (SEQ ID NO:23), Synechocystis PCC6803 (SEQ ID NO:24), Synechococcus sp. PCC 7335 (SEQ ID NO:25), *Nostoc commune* (SEQ ID NO:26), or *Bacillus megaterium* (SEQ ID NO:27). See FIG. 1.

The percent identity between two amino acid sequences can be determined as follows. First, the amino acid sequences are aligned using the BLAST 2 Sequences (Bl2seq) program from the stand-alone version of BLASTZ containing BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from Fish & Richardson's web site (e.g., www.fr.com/blast/) or the U.S. government's National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov). Instructions explaining how to use the Bl2seq program can be found in the readme file accompanying BLASTZ. Bl2seq performs a comparison between two amino acid sequences using the BLASTP algorithm. To compare two amino acid sequences, the options of Bl2seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two amino acid sequences: C:\Bl2seq –i c:\seq1.txt –j c:\seq2.txt –p blastp –o c:\output.txt. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology, then the designated output file will not present aligned sequences. Similar procedures can be following for nucleic acid sequences except that blastn is used.

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

78.17, 78.18, and 78.19 is rounded up to 78.2. It also is noted that the length value will always be an integer.

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

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

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

In some embodiments, heme-containing proteins are isolated and purified from other components of the source material (e.g., other animal, plant, fungal, algal, or bacterial proteins) using techniques described above. As used herein, the term "isolated and purified" indicates that the preparation of heme-containing protein is at least 60% pure, e.g., greater than 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% pure.

Heme-containing proteins also can be recombinantly produced using polypeptide expression techniques (e.g., heterologous expression techniques using bacterial cells, insect cells, algal cells, fungal cells such as yeast cells, plant cells, or mammalian cells). For example, the heme-containing protein can be expressed in *E. coli* cells. The heme-containing proteins can be tagged with a heterologous amino acid sequence such as FLAG, polyhistidine (e.g., hexahistidine, HIS tag), hemagluttinin (HA), glutathione-S-

transferase (GST), or maltose-binding protein (MBP) to aid in purifying the protein. In some embodiments, a recombinant heme containing protein including a HIS-tag and a protease (e.g., TEV) site to allow cleavage of the HIS-tag, can be expressed in *E.coli* and purified using His-tag affinity chromatography (Talon resin, CloneTech). In some cases, standard polypeptide synthesis techniques (e.g., liquid-phase polypeptide synthesis techniques or solid-phase polypeptide synthesis techniques) can be used to produce heme-containing proteins synthetically. In some cases, cell-free translation techniques can be used to produce heme-containing proteins synthetically..

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

The proteins used in the consumable can be altered (e.g., hydrolyzed, cleaved, crosslinked, denatured, polymerized, extruded, electrospun, spray dried or lyophilized, or derivatized or chemically modified). For example, the proteins can be modified by covalent attaching sugars, lipids, cofactors, peptides, or other chemical groups including phosphate, acetate, methyl, and other natural or unnatural molecule. For example, the peptide backbones of the proteins can be cleaved by exposure to acid or proteases or other means. For example, the proteins can be denatured, i.e., their secondary, tertiary, or quaternary structure can be altered, by exposure to heat or cold, changes in pH, exposure to denaturing agents such as detergents, urea, or other chaotropic agents, or mechanical stress including shear. The alignment of proteins in a solution, colloid, or a solid assembly can be controlled to affect the mechanical properties including tensile strength, elasticity, deformability, hardness, or hydrophobicity.

Proteins also can be assembled into fibers that can form a matrix for a structure for the compositions. A 3-dimensional matrix of protein fibers can, for example, contain chemicals that promote formation of inter-molecular disulfide cross-links (mixed glutathione, dithiothreitol (DTT), beta-mercaptoethanol (BME)). In some embodiments, the chemicals are proteins (thioredoxin, glutaredoxin). In some embodiments, the proteins are enzymes (disulfide isomerase). In some embodiments, fibers are crosslinked

by chemical cross-linkers with two reactive groups selected from the group consisting of N-hydroxysuccinimide (NHS) esters, imidoesters, aryl fluorides, aldehydes, maleimides, pyridyldithiols, haloacetyls, aryl azides, diazirines, carbodiimides, hydrazides and isocyanates.

In some embodiments, coacervates comprising one or more plant proteins can be formed and used, for example, as binding agents in meat or other replicas. Coacervation is the process during which a homogeneous solution of charged polymers undergoes a phase separation to result in a polymer-rich dense phase (the 'coacervate') and a solvent-rich phase (supernatant). Protein-polysaccharide coacervates have been used in the development of biomaterials. See, for example, Boral and Bohidar (2010) *Journal of Physical Chemistry B*. Vol 114 (37): 12027-35; and Liu *et al.*, (2010) *Journal of Agricultural and Food Chemistry*, Vol 58:552-556. Formation of such coacervates is driven by associative interactions between oppositely charged polymers. However, as described herein, coacervates can be formed using proteins (e.g., plant proteins comprise one or more pea proteins, chickpea proteins, lentil proteins, lupine proteins, other legume proteins, or mixtures thereof). In general, a coacervate can be formed by acidifying a low ionic strength solution (e.g., a buffered solution at or below100 mM sodium chloride) comprising one or more isolated and purified plant proteins such as pea legumins or vicilins (e.g., a vicilin fraction comprising convicilins), a combination of both vicilins and legumins, or unfractionated pea proteins to a pH of 3.5 to 5.5. (e.g., pH 4 to 5). Under these conditions, the proteins separate out of solution and the mixture can be centrifuged to cleanly separate out the coacervate. This coavervate, unlike a precipitate, is a viscous material that can be stretched by pulling and that melts on heating. The process can be carried out in the presence of oils (up to 70%, e.g., palm or other oil), to form a creamy material. By varying the composition of the solution (ratio of vicilin: legumin, type and amount of oil used), the binding properties of the coacervate can be tuned as desired. In some embodiments, one or more gums (e.g., acacia gum or xanthan gum) can be used to form a coacervate. Coacervates can be used as binding agents in beef patty replicas to bind and hold together the adipose-, muscle- and connective tissue replicas.

Binding materials with different adhesive and cooking characteristics can be prepared by combining wheat gluten (0-20%) and pea protein fractions (0-50%) in the presence of a plasticizer such as glycerol (0-30%) or polyethylene glycol. Leghemoglobin or other heme-containing protein can be added to the mixture if necessary. Upon mixing to remove any clumps, the material may be incorporated into beef patty replicas.

In some embodiments, proteins can be subjected to freeze alignment to texturize the proteins without extrusion. The method involves slow freezing of protein comprising materials to allow for formation of ice crystals. When cooled from one side, ice crystals form preferentially in a direction perpendicular to the cooled side. After freezing, the ice can be removed from the material in a freeze-dryer, leaving behind material with several layers. The structure can then be stabilized by heating under pressurized, moist conditions to produce a material that can be used in meat replicas. Freeze-alignment of soy proteins has been described by Lugay and Kim (1981) (see Freeze alignment: A novel method for protein texturization. Page 177-187, Chapter 8 in: D.W. Stanley, E.D. Murray and D.H. Lees eds. 1981. Utilization of Protein Resources. Westport, CT: Food & Nutrition Press, Inc). The freeze-aligned proteins can be subjected to further processing (by soaking in solutions comprising beef flavors and/or leghemoglobin) and used in combination with adipose- and connective-tissue replicas to form beef replicas. The replicas may also be used as structures around which cold-set gels (comprising, for example, pea proteins and myoglobin) or crosslinked gels (comprising, for example, pea proteins and leghemoglobin) can be formed prior to their combination with adipose- and connective-tissues.

C. LIPIDS

Consumables described herein can include a lipid component. Lipids can be isolated and/or purified and can be in the form of triglycerides, monoglycerides, diglycerides, free fatty acids, sphingosides, glycolipids, phospholipids, or oils, or assemblies of such lipids (e.g., membranes, lecithin, lysolecithin, or fat droplets containing a small amount of lipid in a bulk water phase). In some embodiments, the lipid sources are oils obtained from non-animal sources (e.g., oils obtained from plants,

algae, fungi such as yeast or filamentous fungi, seaweed, bacteria, or Archae), including genetically engineered bacteria, algae, archaea or fungi. Non-limiting examples of plant oils include corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, or rice bran oil; or margarine. The oils can be hydrogenated (e.g., a hydrogenated vegetable oil) or non-hydrogenated.

In some embodiments, the lipid can be triglycerides, monoglycerides, diglycerides, free fatty acids, sphingosides, glycolipids, lecithin, lysolecithin, phospholipids such as phosphatidic acids, lysophosphatidic acids, phosphatidyl cholines, phosphatidyl inositols, phosphatidyl ethanolamines, or phosphatidyl serines; sphingolipids such as sphingomyelins or ceramides; sterols such as stigmasterol, sitosterol, campesterol, brassicasterol, sitostanol, campestanol, ergosterol, zymosterol, fecosterol, dinosterol, lanosterol, cholesterol, or episterol; lipid amides, such as N-palmitoyl proline, N-stearoyl glycine, N-palmitoyl glycine, N-arachidonoyl glycine, N-palmitoyl taurine, N-arachidonoyl histidine, or anandamide; free fatty acids such as palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid (C18:2), eicosanoic acid (C22:0), arachidonic acid (C20:4), eicosapentanoic acid (C20:5), docosapentaenoic acid (C22:5), docosahexanoic acid (C22:6), erucic acid (C22:1), conjugated linoleic acid, linolenic acid (C18:3), oleic acid (C18:1), elaidic acid (trans isomer of oleic acid), trans-vaccenic acid (C18:1 *trans* 11), or conjugated oleic acid; or esters of such fatty acids, including monoacylglyceride esters, diacylglyceride esters, and triacylglyceride esters of such fatty acids.

The lipids can comprise phospholipids, lipid amides, sterols or neutral lipids. The phospholipids can comprise a plurality of amphipathic molecules comprising fatty acids (e.g., see above), glycerol and polar groups. In some embodiments, the polar groups are, for example, choline, ethanolamine, serine, phosphate, glycerol-3-phosphate, inositol or inositol phosphates. In some embodiments, the lipids are, for example, sphingolipids,

ceramides, sphingomyelins, cerebrosides, gangliosides, ether lipids, plasmalogens or pegylated lipids.

In some embodiments, the lipids used in the consumable are the cream fraction created from seeds, nuts, and legumes, including but not limited to sunflower seeds, safflower seeds, sesame seeds, rape seeds, almonds, macadamia, grapefruit, lemon, orange, watermelon, pumpkin, cocoa, coconut, mango, butternut squash, cashews, brazilnuts, chestnuts, hazelnuts, peanuts, pecans, walnuts and pistachios. As used herein, the term "cream fraction" can refer to an isolated emulsion comprising lipids, proteins and water.

To obtain a cream fraction from seeds, nuts, or legumes, one or more of the following steps can be performed. Seeds, nuts or legumes can be blended from 1 minute up to 30 minutes. For example, the seeds, nuts, or legumes can be blended by increasing the speed gradually to maximum speed over 4 minutes, then blending at maximum speed for 1 minute. The seeds, nuts or legumes can be blended in water or solutions that contain all or some of the following: EDTA (0 – 0.1M), NaCl (0-1M), KCl (0-1M), $NaSO_4$ (0 – 0.2M), potassium phosphate (0-1M), sodium citrate (0-1M), sodium carbonate (0-1M), and/or sucrose (0-50%), from pH of 3 to 11 to obtain a slurry. The slurry can be heated to 20°C to 50°C and centrifuged to obtain the cream fraction (the top layer, also referred to as the "cream"). Further purification of the cream fraction may be achieved by washing the cream fraction with 0.1M to 2M urea solution before re-isolating the cream fraction by centrifugation. The residual liquid (referred to as the "skim" layer) that is a solution comprising proteins in water can also be used.

The "cream" can be used as is, or subjected to further purification steps. For example, washing and heating can remove color and flavor molecules (e.g., unwanted molecules), or unwanted grainy particles to improve the mouth feel and creaminess. In particular, washing with a high pH buffer (pH >9) can remove bitter tasting compounds and improve mouth feel, washing with urea can remove storage proteins, washing below pH 9, followed by washing with a pH above pH 9 can remove unwanted color molecules, and/or washing with salts can decrease taste compounds. Heating can increase the removal of grainy particles, color and flavor compounds. For example, the cream

fraction can be heated from 0-24 hours, at temperatures ranging from 25°C to 80°C. In some embodiments, the resulting creamy fraction comprises seed storage proteins. In some embodiments, the seed storage proteins are substantially removed from the resulting creamy fraction.

D. FIBER

Fiber can be isolated and/or purified for inclusion in the consumables described herein. Fiber can refer to non-starch polysaccharides such as arabinoxylans, cellulose, and other plant components such as resistant starch, resistant dextrins, inulin, lignin, waxes, chitins, pectins, beta-glucans, and oligosaccharides from any plant source.

Fibers can refer to extruded and solution spun proteins as described herein.

E. SUGARS

In some embodiments, the consumable also can comprise sugars. For example the consumable can comprise: monosaccharides, including but not limited to glucose (dextrose), fructose (levulose), galactose, mannose, arabinose, xylose (D- or L-xylose), and ribose, disaccharides including but not limited to sucrose, lactose, melibiose, trehalose, cellobiose, or maltose, sugar alcohols such as arabitol, mannitol, dulcitol, or sorbitol, sugar acids such as galacturonate, glucuronate, or gluconate, oligosaccharides and polysaccharides such as glucans, starches such as corn starch, potato starch, pectins such as apple pectin or orange pectin, raffinose, stachyose, or dextrans; plant cell wall degradation products such as salicin, and/or sugar derivatives such as N-acetylglucosamine.

F. GEL FORMATION

The components of the composition can be formed into a gel. In some embodiments, gels comprise protein, where the protein is derived from non-animal source (e.g., a plant source or other non-animal source such as a genetically modified yeast or bacteria). Gels can be formed using a variety of methods. The protein

concentration, enzyme concentration, pH, and/or process temperature will affect the rate of gel formation and quality of the final tissue replica.

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

In some embodiments, a gel can be formed by subjecting the composition containing the protein and any other components (e.g., a lipid) to high pressure processing.

In some embodiments, gels can be produced by adjusting the pH of the solution. For example, the pH of a concentrated protein solution can be adjusted to near the isolectric pH of the main protein component by adding hydrochloric acid or other acid, or sodium hydroxide or other base.

In some embodiments, gels can be produced by soaking protein powders in solutions. For example, protein powder can be soaked with at least 1%, 5%, 10%, 20% (wt/v) or more of a concentrated sodium hydroxide solution. In other examples, protein powder can be soaked in mixed water/ethanol solutions.

In some embodiments, a cold set gel is formed to avoid denaturing or the breakdown of any heat-labile components (e.g., oxidizing the iron in a heme moiety or generating undesirable flavors). See, Ju and Kilara A. (1998) *J. Food Science*, Vol 63(2): 288-292; and Maltais *et al.*, (2005) *J. Food Science*, Vol 70 (1): C67-C73) for general methodologies for forming cold set gels. In general, cold set gels are formed by first heat denaturing a protein solution below its minimum gelling concentration (dependent on pH and type of protein, typically <8% (w/v) at pH 6-9 for globular plant proteins such as pea proteins). The protein solution can heated to a temperature above the denaturation temperature of the protein under conditions where it does not precipitate out of solution (e.g., 0-500 mM sodium chloride, pH 6-9). The solution can be cooled back to room temperature or below, and any heat-labile components (e.g., heme-containing proteins

and/or oils) can be mixed in when the solution is sufficiently cool, but before gelling.. Gelation can be induced by adding sodium chloride or calcium chloride (e.g., 5 to 100 mM), and the solution can be incubated at or below room temperature to allow for gel formation (typically minutes-hours). The resulting gel can be used as-is in meat replicas or processed further (e.g., stabilized) before incorporation in meat replicas.

In some embodiments, gels can comprise or be produced (e.g., stabilized by) at least in part by a cross-linking enzyme. The cross-linking enzyme can be, for example, a transglutaminase, a tyrosinase, a lipoxygenase, a protein disulfide reductase, a protein disulfide isomerase, a sulfhydryl oxidase, a peroxidase, a hexose oxidase, a lysyl oxidase, or an amine oxidase.

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

Gels can be stabilized by chemical crosslinking by chemical cross-linkers with two reactive groups selected from the group consisting of N-hydroxysuccinimide (NHS) esters, imidoesters, aryl fluorides, aldehydes, maleimides, pyridyldithiols, haloacetyls, aryl azides, diazirines, carbodiimides, hydrazides and isocyanates.

In some embodiments, gels can be stabilized by the addition of starches and gums.

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

G. MUSCLE REPLICAS

A large number of meat products comprise a high proportion of skeletal muscle. Accordingly, the present invention provides a composition, which can be derived from non-animal sources which replicates or approximates key features of animal skeletal muscle. A composition derived from non-animal sources, which replicates or approximates animal skeletal muscle can be used as a component of a consumable, for

example, a meat replica. Such a composition will be labeled herein as "muscle replica." In some embodiments, the muscle replica and/or meat substitute product comprising the muscle replica are partially derived from animal sources. In some embodiments, the muscle replica and/or meat substitute product comprising the muscle replica are entirely derived from non-animal sources.

The muscle tissue replica can comprise a protein content, wherein the protein content comprises one or more isolated and purified proteins, wherein the muscle tissue replica approximates the taste, texture, or color of an equivalent muscle tissue derived from an animal source.

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

The connective tissue component of skeletal muscle substantially contributes to texture, mouth feel and cooking behavior of meat products. Connective tissue is composed of protein (collagen, elastin) fibers in the range of 0.1 – 20 microns. In some embodiments, a mixture of fibers of diameters of <1-10 microns, and 10-300 microns is produced to replicate the fiber composition of animal connective tissue. In some embodiments, the 3-dimensional matrix of fibers is stabilized by protein crosslinks to replicate the tensile strength of animal connective tissue. In some embodiments, the 3-dimensional matrix of fibers contains an isolated, purified cross-linking enzyme. The cross-linking enzyme can be, for example, a transglutaminase, a tyrosinase, a lipoxygenase, a protein disulfide reductase, a protein disulfide isomerase, a sulfhydryl oxidase, a peroxidase, a hexose oxidase, a lysyl oxidase, or an amine oxidase.

Some proteins (e.g., 8S globulin from Moong bean seeds, or the albumin or globulin fraction of pea seeds) have favorable properties for constructing meat replicas because of their ability to form gels with textures similar to animal muscle or adipose

tissue. See also the proteins identified in Section III A and B. The proteins may be artificially designed to emulate physical properties of animal muscle tissue.

In some embodiments, one or more isolated and purified proteins accounts for about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component by weight of the meat replica. In some embodiments, one or more isolated and purified proteins accounts for about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein content of a consumable.

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

Additional components of animal muscle tissue include sodium, potassium, calcium, magnesium, and other metal ions, lactic acid and other organic acids, free amino acids, peptides, nucleotides, and sulfur compounds. Thus, in some embodiments, a muscle replica can include sodium, potassium, calcium, magnesium, other metal ions such as iron, zinc, copper, nickel, lithium, or selenium, lactic acid, and other organic acids such as fatty acids, free amino acids, peptides, nucleotides and sulfur compounds glutathione, beta mercaptoethanol, or dithiothreitol. In some embodiments, the concentration of sodium, potassium, calcium, magnesium, other metal ions, lactic acid, other organic acids, free amino acids, peptides, nucleotides and/or sulfur compounds in

the muscle replica or consumable are within 10% of the concentrations found in a muscle or meat being replicated.

The invention also provides methods for making a muscle replica. In some embodiments, the method includes forming the composition into asymmetric fibers prior to incorporation into the consumable. In some embodiments, these fibers replicate muscle fibers. In some embodiments the fibers are spun fibers. In other embodiments the fibers are extruded fibers. Accordingly, the present invention provides for methods for producing asymmetric or spun protein fibers. In some embodiments, the fibers are formed by extrusion of the protein component through an extruder. Methods of extrusion are well known in the art, and are described, for example, in U.S. Patent No. 6,379,738, U.S. Patent No. 3,693,533, and U.S. Patent Publication No. 20120093994, which are herein incorporated by reference. These methods can be applied to making the compositions provided herein.

Extrusion can be conducted using, for example, a Leistritz Nano-16 twin-screw co-rotating extruder (American Leistritz Extruder Corp. USA, Sommerville, NJ). Active cooling of the barrel section can be used to limit denaturation of proteins. Active cooling of the die section can be used to limit expansion of the extruded product and excessive moisture loss. Protein feed and liquid are added separately: protein is fed by a volumetric plunger feeder or a continuous auger-type feeder, and liquid can be added into the barrel through a high pressure liquid injection system. Die nozzles with various inner diameters and channel length can be used for precise control of extrudiate pressure, cooling rate and product expansion. In some examples, extrusion parameters were: screw speed 100-200 rpm, die diameter 3 mm, die length 15 cm, product temperature at the end of the die of 50°C, feed rate of 2 g/min, and water-flow rate of 3 g/min. Product temperature at the die during extrusion is measured by a thermocouple.

Spun fibers can be produced by preparing a high viscosity protein "dope" by adding sodium hydroxide to concentrated protein solutions or to precipitated proteins, and forcing the solution with a plunger-type device (in some examples, a syringe with a syringe pump) through a small steel capillary (in some examples, 27 gauge hypodermic needle) into a coagulating bath. In some examples, the bath is filled with a concentrated

acid solution (e.g. 3 M hydrochloric acid). In some examples, the bath is filled with a buffer solution at a pH approximately equal to the isoionic point of the protein. Coagulating protein solution jet forms a fiber that collects at the bottom of the bath.

Bundles of spun fibers can be produced by forcing protein "dope" through spinnerets with many small holes. In some examples, spinnerets are stainless steel plates with approximately 25,000 holes per cm^2, with diameter of each hole approximately 200 microns. In some embodiments muscle tissue replica is produced by immersing the 3-dimensional matrix of fibers (connective tissue replica) in solutions of proteins and creating protein gels incorporating 3-dimensional matrix of fibers.

H. FAT REPLICAS

Animal fat is important for the experience of eating cooked meat and are important for some of the nutritional value of meat. Accordingly, the present invention provides compositions derived from non-animal sources, which recapitulate key features of animal fat, including the texture and/or flavor, by using components that mimic the chemical composition and physical properties of, for example, ground beef. In another aspect, the present invention provides a meat substitute product that comprises a composition derived from non-animal sources, which recapitulates animal fat. Such a composition will be labeled herein as an "adipose replica" or a "fat replica." In some embodiments, the adipose replica and/or meat substitute product comprising the adipose replica are partially derived from animal sources. The consumable can also include adipose replicas that recapitulate key features of non-animal fats, including texture, flavor, firmness, percent fat release, and/or temperature of fat release. The fat content of the consumable can be at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, 80%, 90%, or 95% fat.

Ground beef is typically prepared by mixing lean beef with adipose (fat) that is cut from steaks, with the adipose tissue added to 16-30% (Cox 1993). Without adipose, meat passed through a grinder is tough, crumbly, and dries out quickly. Fat is added to lean beef so that fat released during cooking provides a liquid surface to aid in cooking, and to generate key beef flavors, which largely are products of the fatty acids.

Engineering an adipose tissue replica that plays the same key roles in texture and flavor of the plant-based ground beef is an important driver for texture and flavor.

The adipose tissue replicas described here have a great health benefit over the beef adipose tissue as the fatty acid composition can be controlled such that the amount of saturated fats can be decreased. Additionally, the plant-based adipose replicas are cholesterol free. The plant-based adipose replicas can contain a lower percent total fat, and still have the same amount of fat being released or retained for the desired cooking properties, flavor, and texture.

As described herein, adipose replicas comprising emulsions of plant-derived lipids and one or more isolated and purified proteins can be produced in which the composition (e.g., fatty acid composition), cooking characteristics (e.g., fat release temperature or percent fat release), and physical properties (e.g., firmness) can be controlled, allowing the plant based composition to mimic animal based adipose. The adipose tissue replica includes (1) a vegetable oil containing triacylglycerides of fatty acids; (2) one or more isolated and purified proteins from non-animal sources (e.g., a plant protein); and (3) a phospholipid such as lecithin. The proteins can be plant or microbial proteins as described above (e.g., RuBisCo, an oleosin, an albumin, a globulin, or other seed storage protein). See, also the proteins described in Sections III A and B. The vegetable oils can be any of the oils described herein. See, e.g., Section III C.

The fat replica can be a gelled emulsion. In some embodiments, the gel is a soft, elastic gel comprising proteins and optionally carbohydrates. The gelled emulsion can comprise a protein solution comprising multiple proteins, e.g., 1-5 or 1-3 isolated and purified proteins, wherein the protein solution accounts for 1-30% of the volume of the emulsion. The gelled emulsion can comprise a fat droplet, wherein the fat droplet accounts for 70-99% of the volume of the emulsion. The gelled emulsion can comprise an isolated, purified cross-linking enzyme, wherein the cross-linking enzyme accounts for 0.0005% to 0.5% of the emulsion weight by volume, 0.5-2.5% of the emulsion weight by volume, or 0.001% or lower of the emulsion weight by volume. The emulsion of fat droplets in the protein solution can be stabilized by forming the emulsion into a gel by the cross-linking enzyme, e.g., a transglutaminase, by gelling proteins via heating and

cooling protein solutions, by forming a cold-set gel, by formation of a coacervate, or by combinations of these techniques as described for coacervates in section C and gel formation in section F.

In some embodiments, the fat replica comprises cross-linking enzymes that catalyze reactions leading to covalent crosslinks between proteins. Cross-linking enzymes can be used to create or stabilize the desired structure and texture of the adipose tissue replica, to mimic the desired texture of an equivalent desired animal fat. In some embodiments, the cross-linking enzymes are isolated and purified from a non-animal source, examples and embodiments of which are described herein. In some embodiments, the fat replica comprises at least 0.0001%, at least 0.001%, at least 0.01%, at least 0.1%, or at least1% (wt/vol) of a cross-linking enzyme. The cross-linking enzyme can be selected from, for example, transglutaminases, tyrosinases, lipoxygenases, protein disulfide reductases, protein disulfide isomerases, sulfhydryl oxidases, peroxidases, hexose oxidases, lysyl oxidases, and amine oxidases. In some embodiments, the cross-linking enzyme is transglutaminase, a lysyl oxidase (e.g., a *Pichia pastoris* lysyl oxidase), or other amine oxidase.

The fat replica can comprise a gel with droplets of fat suspended therein. The fat droplets used in some embodiments of the present invention can be from a variety of sources. In some embodiments, the sources are non-animal sources (e.g., plant sources). See, e.g., the examples provided in Section III C. In some embodiments, the fat droplets are derived from animal products (e.g., butter, cream, lard, and/or suet). In some embodiments, fat droplets are derived from pulp or seed oil. In other embodiments, the source may be algae, yeasts, oleaginous yeasts such as *Yarrowia lipolytica*, or mold. For instance, in one embodiment, triglycerides derived from *Mortierella isabellina* can be used. In some embodiments, the fat droplets contain synthetic or partially synthetic lipids

In some embodiments, the fat droplets are stabilized by addition of surfactants, including but not limited to phospholipids, lecithins, and lipid membranes. The lipid membranes may be derived from algae, fungi or plants. In some embodiments the surfactants comprise less than 5% of the fat replica. The fat droplets can in some examples range from 100 nm to 150 μm in diameter. The diameter of these stabilized

droplets may be obtained by homogenization, high-pressure homogenization, extrusion or sonication.

In some embodiments, plant oils are modified to resemble animal fats. The plant oils can be modified with flavoring or other agents such as heme proteins, amino acids, organic acids, lipids, alcohols, aldehydes, ketones, lactones, furans, sugars, or other flavor precursor, to recapitulate the taste and smell of meat during and after cooking. Accordingly, some aspects of the invention involve methods for testing the qualitative similarity between the cooking properties of animal fat and the cooking properties of plant oils in the consumable.

In some embodiments, additional polysaccharides can be added to a fat replica, including flax seed polysaccharides and xanthan gum.

The creation of a plant-based adipose replica requires stabilization of the oil in water emulsions. Typically animal adipose tissue contains ~95% fat, and is stabilized by the phospholipid bilayer and associated proteins. Adipose replicas described herein can be created with up to 95% fat in some instances, with 80% fat under many conditions, or with lower amounts of fat (e.g., 50% or less) while mimicking the properties of animal fat. Achieving a high percent fat is controlled by the stabilization of the emulsion.

The composition (e.g., fatty acid composition), cooking characteristics (e.g., fat release temperature or percent fat release), and physical properties (e.g., firmness) can be manipulated by controlling the type and amount of fat, the amount of protein, the type and amount of lecithin, the presence of additives, and the method of gelling.

In some embodiments, the protein component comprises about 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, or 20%, 25%, or more of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises about 0.1-5% or about 0.5-10% or more of the fat replica by dry weight or total weight. In some embodiments, the protein component is 0.5 to 3.5% or 1 to 3% of the fat replica by dry weight or total weight. In some embodiments, the protein component comprises a solution containing one or more isolated, purified proteins. The type of protein can affect the stability of the emulsion, RuBisCo and pea albumins allow for fat replicas to made a greater than 90%

fat. Addition of polysaccharides including flax seed and xanthan gum aid in emulsifying the mixture, allowing for an increase in fat content.

The type and amount of fat can be controlled by choosing the source of the fat and its lipid composition. In general, oils with higher amounts of saturated fatty acids are better able to be emulsified at lower protein concentrations, while oils with more unsaturated fatty acids require higher protein concentrations to be emulsified. Protein is required to stabilize the emulsion, and an increase in protein content increases the stability. If the amount of protein added is too little to emulsify the amount of fat, the mixture will separate into layers.

Lecithin also is a modulator of emulsion, and can either stabilize or disrupt the emulation depending on the amount of protein present and type of oil used. For example, lecithin may disrupt the protein/fat matrix to make a less stable emulsion, but can be added at low levels to modulate other physical properties. Emulsions made from oils with higher amounts of unsaturated fats can be de-stabilized by a high amount of lecithin (1%), such that the emulsion does not solidify. Emulsions made from oils with higher amounts of saturated fats can solidify at high amounts of lecithin (1%), but are very soft.

As described herein adipose replicas can be prepared that can range from very soft to very firm. The composition and amount of the fat controls the firmness of the replica. Firmer oils, which contain more long-chained saturated fats, make firmer gels. Oils that produce softer gels typically contain more unsaturated fatty acids or short-chained saturated fatty acids. In general, the firmness of the gel increases as total percent fat increases, as long as the emulsion is held and does not separate. The amount of protein also contributes to the firmness of the replica. In general, an increase in protein concentration increases replica firmness. The amount of lecithin is a modulator of replica firmness. Higher amounts of lecithin (1%) are much softer than lower amounts of lecithin (0.05%) when gels are formed with high percent protein (3%). When protein is decreased (1.8%), all gels are softer, and there is little difference in firmness between low level of lecithin (0.05%) and high (1%) if emulsion is held.

Addition of polysaccharides to replicas including but not limited to xanthan gum and flax seed paste can increase the firmness of adipose-replica gels.

When an adipose replica is cooked, fat leaks from the structured replica as it is cooked. Often there is fat that remains in the cooked product; it is important to achieve the balance between fat released to aid in cooking and fat retained for texture and taste. The percent fat released (per total fat) can be determined by measuring the amount of fat released upon cooking to completion. Percent fat released is reported as the weight of fat released per the total fat of the replica. For example, the percent fat release of an adipose tissue replica described herein can be 0 to 10%, 10% to 20%, 20% to 30%, 30% to 40%, 40% to 50%, 50% to 60%, 60% to 70%, 70% to 80%, 80% to 90%, or 90% to 100% upon cooking. Adipose replicas typically release 0- 90% fat under standard cooking conditions. In comparison, beef adipose tissue typically releases 40-55% fat under equivalent conditions.

While vegetable oils have set melting temperatures, the range of temperatures over which adipose replicas can be made to release fat is wide. Fat release temperature is the temperature at which fat is visibly released from the replica at the cooking surface. As described herein, the fat release temperature of the adipose replica can be tailored based on the type and amount of fat, the amount of protein, the type and amount of lecithin, the presence of additives, the method of emulsification, and the method of gelling. The resulting adipose replicas can have a fat release temperature of between 23°C to 33°C, 34°C to 44°C, 45°C to 55°C, 56°C to 66°C, 67°C to 77°C, 78°C to 88°C, 89°C to 99°C, 100°C to 110°C, 111°C to 121°C, 122°C to 132°C, 133°C to 143°C, 144°C to 154°C, 155°C to 165°C, 166°C to 167°C, 168°C to 169°C, 170°C to 180°C, 181°C to 191°C, 192°C to 202°C, 203°C to 213°C, 214°C to 224°C, 225°C to 235°C, 236°C to 246°C, 247°C to 257°C, 258°C to 268°C, 269°C to 279°C, 280°C to 290°C, or 291°C to 301°C. Beef fat was measured to release fat at 100-150°C.

Emulsification also is a factor in controlling the temperature of fat release: Once fats are incorporated into a replica with protein, or protein and lecithin, the temperature at which the fat is released increases significantly above the temperature at which the fat alone melts.

Fatty acid composition also is a factor in the fat release temperature and percent fat release. Vegetable oils that contain a higher proportion of unsaturated fatty acids have

low melting temperatures and many are liquid at room temperature. Vegetable oils that contain a higher proportion of saturated fatty acids have a higher melting temperature, and are solid at room temperature. Replicas with a greater amount of unsaturated fats have a higher temperature of fat leakage than the same replica made with more saturated fatty acids. Gels made from 75% oils with higher amounts of unsaturated fatty acids, a high protein content (3%), and minimal lecithin content (0.05%), where the mixture was emulsified by a hand-held homogenizer and gelled using the heat-cool method, can be heated to 200°C with little or no fat release. Replicas containing oils with more long-chained saturated fats typically have more fat release at high protein content, but release less percent total fat compared to replicas containing oils with more short chained fats and at low percent protein. Gels made from oils with a higher proportion of short-chained saturated fatty acids, high protein content (3%), and minimal lecithin content (0.05%), can be heated to 200°C with little fat release.

The percent of fat release as an adipose replica is cooked is also is a function of the amount of protein and the amount of lecithin. Typically, the adipose replica contains 1-3% protein by mass. Increasing protein content leads to increasing temperatures of fat release, and reduces the fraction of fat released. Increasing the lecithin content to 1% can decrease the fat release temperature to 60-115°C, and increase the fraction of fat released (e.g., 25-30%). The source or composition of the lecithin used can modulate the amount of fat release and the temperature threshold for fat release. Without being bound to a particular mechanism, it is thought that lecithin destabilizes the emulsion by disrupting the protein-protein interactions. In one embodiment, at a high protein concentration of 3%, increasing the lecithin content to 1% decreased the fat release temperature to 55-60°C, and increased the percent fat leaked to 60-65%.

The method of making the emulsion also is a factor in determining the amount of fat release. Emulsification forms a homogenous mixture of the fat held in a matrix of proteins and lecithin. Methods of emulsification can include high-pressure homogenization, sonication, or hand homogenization. The alternative methods result in characteristic differences in the size of the oil droplets in the emulsion, which influences the stability of the resulting emulsions and the maximum fat concentration at which

stable emulsions can be formed.

The method of gelling the replica also is a factor in determining the amount of fat release. While adipose replicas can be formed without forming gels, gelation results in a firmer and more stable emulsion. Methods of gelling are described above, and can include, for example, the addition of a crosslinking enzyme such as a transglutaminase (TG), or subjecting the emulsion to a heat/cool cycle. For example, either treatment with TG or the heat/cool method can convert an emulsion, as described above, into a gel. Furthermore, gelled emulsions formed by crosslinking catalyzed by TG typically release fat at a temperature higher than that at which emulsions gelled by the heat/cool technique do so. Gels formed by crosslinking with a TG also typically release less fat than do gels formed by the heat-cool technique.

In some embodiments, a fat replica can be made with a protein content <1.5% and a minimal lecithin content (0.05%) and have a fat release temperature of 45-65°C, and a high amount of fat released (e.g., 70-90%). These gels are at the higher end of percent fat released.

In some embodiments, a fat replica can be made with a lower protein content (<1.5%) and a high lecithin (>1%), and have a lower fat release temperature (e.g., 30-50°C, e.g., 30 to 45°C), and with an intermediate percent fat leaked (45-65%). Thus, in gels formed from oils with short-chained fatty acids or long-chained fatty acids at low protein concentrations, lecithin may play a role in stabilizing the emulsion.

In some embodiments, >2% rubisco or pea albumin can be used to produce adipose replicas with greater than 70% fat. In some embodiments, gels formed with > 3% isolated and purified protein can result in adipose replicas with greater than 70% fat.

In some embodiments, adipose replicas made from oils with a higher proportion of long-chained saturated fatty acids, a protein content of 3%, and a minimal lecithin content (0.05%), can release fat at a temperature similar to that at which beef fat does so (50-100°C), and can release a low to intermediate level of fat (15-45%).

In some embodiments, an adipose replica with a higher protein concentration (> 3%), and a lecithin content > 1% can have a fat release temperature of 50-70°C, and a higher amount of fat release (50-80%). At high protein and low lecithin concentration,

gels with higher saturated fatty acids typically leak about 10% more fat than do corresponding gels formed with unsaturated fats.

In some embodiments, protease treatment of the protein constituents before gel formation can lead to an increase in fat release.

In some embodiments, a adipose tissue replica matrix stabilized by crosslinking enzymes releases more fat than a adipose tissue replica matrix stabilized by heat/cool protein denaturation. In one embodiment, an adipose tissue matrix comprised of moong bean 8S protein and canola oil, or an equal mixture of coconut, cocoa, olive, and palm oils, retains more mass when formed upon heat/cool denaturation than when formed by cross-linking with an enzyme. In one embodiment, an adipose tissue matrix formed by heat/cool denaturation of a preformed protein-oil emulsion containing Rubisco and cocoa butter, has a higher melting temperature than an adipose replica of similar composition stabilized by a cross-linking enzyme.

In some embodiments, adipose tissue replicas constructed from 1.4% wt/v moong bean 8S protein with 90% v/v canola oil and 0.45% wt/v soybean lecithin, can be homogenized in the presence of variable concentrations of sunflower oleosins. Concentration of oleosins can be varied from 1:10 to $1:10^6$ molar ratio of oleosin:triglyceride. An increase in mass retention after cooking is observed as the concentration of oleosins in the adipose tissue replica increase.

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

In one embodiment, the firmness of adipose replica formed by stabilizing protein oil emulsion can be modified by varying amount of protein in the adipose replica. In one embodiment, adipose tissue replicas made from Rubisco and 70% sunflower oil are softer

at lower concentrations, such as 1%, of RuBisCo than at higher concentrations of Rubisco, such as 3%, in adipose tissue replicas.

In another aspect, the invention provides methods for making a fat replica. The fat can be isolated and homogenized. For example an organic solvent mixture can be used to help solubilize a lipid in a gel and then removed to provide the final gel. At this point, the lipid can be frozen, lyophilized, or stored. So in one aspect, the invention provides for a method for isolating and storing a lipid, which has been selected to have characteristics similar to animal fat. The lipid film or cake can then be hydrated. The hydration can utilize agitation or temperature changes. The hydration can occur in a precursor solution to a gel. After hydration the lipid suspension can be sonicated, homogenized, high-pressure homogenized or extruded to further alter the properties of the lipid in the solution.

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

In some embodiments, the tensile strength of the fat replica mimics the tensile strength of adipose tissue. The tensile strength of the gelled emulsions can be increased by incorporation of fibers. The fibers may be derived from non-animal sources including, but not limited to, watermelon, jack fruit, squash, coconut, green hair algae, corn and/or cotton. In some embodiments, the fibers are derived from self-polymerization of proteins, e.g., oleosins and prolamins. In some embodiments the fibers

are derived from electrospun or extruded proteins. The fibers may form a three dimensional mesh or strands wherein each fiber may be less than 1 mm in diameter.

The adipose replica can be an emulsion comprising a solution of one or more proteins and one or more fats suspended therein as droplets. Slowly adding the oil phase to the aqueous phase can provide a more robust emulsion and prevents occasional failures to emulsify. Adding lecithin can, in some circumstances, destabilize a protein-stabilized emulsion, allowing for increased fat leakage when the replica is cooked. In some embodiments, the emulsion is stabilized by one or more cross-linking enzymes into a gel. In some embodiments, the emulsion is stabilized by a matrix formed by proteins induced to gel by a heat-cool technique or a cold-set-gel technique. Heating a protein-stabilized emulsion can heat denature the proteins leading to an increase in the firmness of the adipose replica. Heating to a sufficient temperature also can reduce the viability of natural microflora by at least 100x. In some embodiments, the emulsion is stabilized by gelled protein matrix formed by a combination of one or more protein cross-linking enzymes and a heat/cool technique or a cold-set gel technique. After the emulsion has sufficiently cooled, but before gelling is complete, one or more optional ingredients can be added, such as a heme-containing protein (e.g., up to about 0.4% such as 0.15, 0.2., 0.25, 0.3, or 0.4%) to give the adipose a more natural looking pink color and/or one of more flavor compounds such as amino acids, sugars, thiamine, or phospholipids to provide an improved flavor to the final product.

The one or more proteins in solution can comprise isolated and purified proteins, e.g. a purified pea albumin enriched fraction, a purified pea globulin enriched fraction, a purified Moong bean 8S globulin enriched fraction, and/or a Rubisco enriched fraction. In other embodiments, the one or more fats are derived from plant-derived oils (a rice bran oil or canola oil). See, e.g., Section III C. In some cases the composition comprises a cross-linking enzyme such as a transglutaminase, lysyl oxidase, or other amine oxidase. Thus, in some embodiments, an adipose tissue replica can be made by isolating and purifying one or more proteins; preparing a solution comprising one or more proteins; emulsifying one or more fats in the solution; and stabilizing the solution into a gelled emulsification with one or more cross-linking reagents.

In some embodiments, the fat replica is a high fat emulsion comprising a protein solution of purified pea albumin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel.

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

The fat can be isolated from plant tissues and emulsified. The emulsification can utilize high-speed blending, homogenization, high pressure homogenization, sonication, shearing, agitation or temperature changes. The lipid suspension can be sonicated or extruded to further alter the properties of the lipid in the solution. At this point, in some embodiments other components of the consumable are added to the solution followed by a gelling agent. In some embodiments crosslinking agents (e.g. transglutaminase or lysyl oxidase) are added to bind the components of the consumable. In other embodiments the gelling agent is added and the lipid/gel suspension is later combined with additional components of the consumable.

CONTROL OF MELTING POINT BY CONTROL OF FAT COMPOSITION

The process of cooking meat is integral to the experience of using and enjoying meat. One important property of meat is that as the meat is heated, fats are released from the meat, which lubricates the cooking surface and increases heat transfer and is a component of the visual, aural and olfactory experience of cooking meat. The amount of fat that is released rather than retained during cooking varies with cooking temperature and contributes to the visual, aural and olfactory experience of cooking meat.

The composition and ratio of fatty acids in triglycerides and phospholipids, along with the ratio of phospholipid headgroups, contribute to the generation of distinct flavor profiles of cooked meat. For example, increased levels of phosphatidylcholine and phosphatidylethanolamine in fat provide a more intense beef flavor. As discussed above, the flavor of meat replicas can be modified by varying the ratios and type of different oils, and phospholipids that comprise the meat replica. For example, the flavor of the cooked meat replica can be controlled by varying the amount of phospholipids, sterols

and lipids (e.g., 0.2-1% wt/wt). In one embodiment, the flavor of the cooked meat replica can be controlled by varying the ratio of different phospholipid headgroups.

In some embodiments, the phospholipids comprise a plurality of amphipathic molecules comprising fatty acids, glycerol and polar groups. See, e.g., Section III C for examples of fatty acids, phospholipids, polar groups, and sterols associated with phospholipids. See also section III C for examples of useful plant oils.

In different cuts of meat, the fat has different properties, ranging from the structurally important nature of fat in bacon to the soft melting behavior of the marbling fat in Wagyu beef.

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

In some embodiments, the fat release temperature of a fat replicas can be controlled by mixing different ratios of vegetable oils containing triacylglycerides and phospholipids (e.g., lecithin). The melting point of fats is governed by the chemical composition of fatty acids. In general, fats comprising saturated fatty acids (e.g., C10:0, C12:0, C14:0, C16:0, C18:0, C20:0, C22:0) are solid at refrigeration temperatures (e.g., about 1°C to about 5°C and at room temperature (e.g., about 20°C to 25°C). By controlling the fat release temperature upon cooking in a adipose tissue replica, the firmness of a adipose tissue replica during refrigeration (e.g., about 1.5°C to about 4°C) and at ambient temperature (e.g., about 20°C to 25°C) can be controlled. Fats comprising monounsaturated fatty acids (e.g., C16:1 or C18:1) are generally solid at refrigeration

temperatures and liquid at room temperatures. Fats comprising polyunsaturated fatty acids (e.g., C18:2, C18:3, C20:5, or C22:6) are generally liquid at refrigeration temperatures and at room temperatures. For example, virgin coconut oil melts at about 24°C while hydrogenated coconut oil melts at 36-40°C.

For example, adipose tissue replicas containing triglycerides and phospholipids that are liquid at room temperature (about 20°C to 25°C) will be softer than adipose tissue replicas containing triglycerides and phospholipids that are solid at refrigeration temperatures.

Adipose tissue replicas can contain oils from a single or multiple sources that are liquid at both refrigeration and ambient room temperatures (e.g., canola oil, sunflower oil, and/or hazelnut oil). In one embodiment, a adipose tissue replica contains oils from single or multiple sources that are solid at refrigeration temperature, but liquid at room temperature (e.g., olive oil, palm oil, and/or rice brain oil). In one embodiment, a adipose tissue replica contains oils from single or multiple sources that are solid at room temperature but liquid at mouth-temperatures (about 37°C) (e.g., palm kernel oil, coconut oil, and/or cocoa butter. In one embodiment, a adipose tissue replica contains oils from single or multiple sources that are solid at mouth temperature (about 37°C) (e.g., oil from mango butter).

In one embodiment, a adipose tissue replica includes triglycerides and phospholipids with a high ratio of saturated fatty acids, and is firmer than a adipose tissue replica containing a higher ratio of monounsaturated and polyunsaturated triglycerides and lipids. For example, a adipose tissue replica containing sunflower oil is softer than a adipose tissue replica containing cocoa butter. Adipose tissue replicas can be formed with 0%, 0.18%, 1.6%, or 2.4% wt/v Rubisco with 70%, 80%, or 90% v/v sunflower or cocoa butter. Each adipose tissue replica that contained cocoa butter was firmer than the replicas that were formed with sunflower oil.

In one embodiment, a adipose tissue replica made as a stable emulsion of moong bean 8S protein with sunflower oil is softer than a adipose tissue replica made as a stable emulsion of moong bean 8S protein and cocoa butter. Adipose tissue replicas formed with 2%, 1%, or 0.5% wt/v moong bean 8S protein with 70%, 80%, or 90% v/v

sunflower or cocoa butters. Each adipose tissue replica that contained cocoa butter was firmer than the replicas that were formed with sunflower oil.

In one embodiment, a adipose tissue replica made as a stable emulsion of moong bean 8S protein with canola oil is softer than a adipose tissue replica made as a stable emulsion of moong bean 8S protein with an equal mixture of coconut, cocoa, olive, and palm oils. Adipose tissue replicas can be formed with 1.4% wt/v moong bean 8S protein with 50%, 70%, or 90% v/v sunflower or a mixture of oils. Each adipose tissue replica that contained a mixture of oils was firmer than the replicas that were formed with sunflower oil.

In one embodiment, an adipose tissue replica made as a stable emulsion of soy proteins with sunflower oil is softer than a adipose tissue replica made as a stable emulsion of soy proteins and cocoa butter. Adipose tissue replicas were formed with 0.6%, 1.6%, or 2.6% wt/v Soy with 50%, 70%, 80%, or 90% v/v sunflower or mixture of oils. Each adipose tissue replica that contained a mixture of oils was firmer than the replicas that were formed with sunflower oil.

In some embodiments, the adipose tissue replicas comprising 0%, 0.18%, 1.6%, and 2.4% wt/v Rubisco with 70%, 80%, and 90% v/v cocoa butter are solid at room temperature but melt at about mouth temperature. In some embodiments, the adipose tissue replicas comprising 0.6%, 1.6%, and 2.6% wt/v soy with 50%, 70%, 80%, and 90% v/v cocoa butter are solid at room temperature but melt at about mouth temperature. In some embodiments, the adipose tissue replicas comprising 1.4% wt/v moong bean 8S protein with 50%, 70%, and 90% v/v of an equal mixture of coconut, cocoa, olive, and palm oil is solid at room temperature but melts at about mouth temperature. In one embodiment, the melting temperature of adipose tissue replicas will be similar to beef fat. In some embodiments the fat replicas comprise oils with a 1:1 ratio of saturated to unsaturated fatty acids. In some embodiments, the adipose tissue replica contains equal amounts of cocoa and mango butters. In some embodiments, the adipose tissue replica contains equal amounts of coconut oil, cocoa butter, olive oil and palm oil.

In one embodiment, a adipose tissue replica that comprises triglycerides and phospholipids will contain a ratio of fatty acids similar to that found in beef (C14:0 0-5%

wt/wt, C16:0 0-25%, C18:0 0-20%, C18:1 0-60%, C18:2 0-25%, C18:3, 0-5%, C20:4 0-2%, and C20:6 0-2%). For example the adipose tissue replica may comprise equal proportions of olive oil, cocoa butter, coconut oil and mango butter. In another example, the adipose tissue replica may comprise equal proportions of olive oil and rice brain oil.

In one embodiment, the melting temperature of adipose tissue replicas will be similar to Wagyu beef fat. In some embodiments the fat replicas comprise oils with a 1:2 ratio of saturated to unsaturated fatty acids (e.g., for example 1 part coconut oil to 2 parts sunflower oil). In some embodiments, the adipose tissue replica contains equal amounts of olive oil, rice bran oil, cocoa butter and mango butter.

I. CONNECTIVE TISSUE REPLICA

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

Animal connective tissue can generally be divided into fascia-type and cartilage-type tissue. Fascia-type tissue is highly fibrous, resistant against extension (has high elastic modulus), and has a high protein content, a moderate water content (ca. 50%), and low-to-no fat and polysaccharide content. Accordingly, the present invention provides a connective tissue replica that recapitulates key features of fascia type tissue. In some embodiments, the connective tissue replica comprises about 50% protein by total weight, about 50% by liquid weight, and has a low fat and polysaccharide component.

The fibrous nature of fascia type connective tissue is largely comprised of collagen fibers. Collagen fibers are observed to be cord or tape shaped species, 1-20 microns wide. These fibers consist of closely packed thin collagen fibrils 30 to 100

nanometers thick. These fibrils also associate into elastic and reticular fibrous networks with individual fibers that may be 200 nanometers thick.

In one embodiment, the fascia-type connective tissue replica consists of a fibrous or fibrous-like structure which can consist of proteins. In some embodiments, the protein content is derived from non-animal source (e.g., a plant source, algae, bacteria, or fungi, see e.g., Sections IIIA and B). In some embodiments, the isolated proteins account for 50%, 60%, 70%, 80%, or 90% or more of the protein content by weight. In some embodiments, multiple isolated proteins are isolated and purified separately and account for the total protein content.

In fascia-type connective tissue, the prolamin family of proteins, individually or combinations thereof, demonstrates suitability for the protein component because they are highly abundant, similar in global amino acid composition to collagen (high fraction of proline and alanine), and amenable to processing into films. In some embodiments, the prolamin family proteins are selected from the group consisting of zein (found in corn), hordein from barley, gliadin from wheat, secalin, extensins from rye, kafirin from sorghum, or avenin from oats. In some embodiments, the one or more isolated and purified proteins is zein. In some embodiments, other proteins can be used to supplement prolamins in order to achieve targets specifications for physicochemical and nutritional properties. See, the list in Sections III A and B, including any major seed storage proteins, animal-derived or recombinant collagen, or extensins (hydroxyproline-rich glycoproteins abundant in cell walls e.g. *Arabidopsis thaliana,* monomers of which are "collagen-like" rod-like flexible molecules).

The proteins can be freeze-dried and milled and combined with one or more other ingredients (e.g., wheat gluten, fiber such as bamboo fiber, or soy protein isolate).

The fibrous or fibrous-like structures can be formed by extrusion. In some embodiments extrusion are conducted using Leistritz Nano-16 twin-screw co-rotating extruder (American Leistritz Extruder Corp. USA, Sommerville, NJ). Active heating and cooling of the barrel section is used to optimize the mechanical properties, extent of puffing and water content of fibers. For example, water content can be adjusted to about 50% to make a hard connective tissue replica. Protein feed and liquid are added

separately: protein is feed by a volumetric plunger feeder, and liquid is added into the barrel through a high pressure liquid injection system. In some examples, extrusion parameters were: screw speed 200 rpm, product temperature at the die of 120°C, feed rate of 2.3 g/min, and water-flow rate of 0.7 g/min. Product temperature at the die during extrusion is measured by a thermocouple.

The fibrous or fibrous-like structures can be formed by extrusion through filament and multi-filament dies to produce fibrous structures. In some embodiments, dies incorporating multiple different orifice sizes in the range 10-300 microns can be used to create mixed fibrous tissue replicas with precise control over dimensions and compositions of fibers. Fibers of different sizes can be incorporated into the compositions to control the properties of the compositions.

Electrospinning can be used to create fibers in the <1-10 micron range. In some embodiments, electrospinning is used to create fibers in the <1-10 micron diameter range. For example, a concentrated solution of moong bean globulin (140 mg/ml) containing 400 mM sodium chloride can be mixed with solution of poly(vinyl alcohol) or poly(ethylene oxide) (9% w/v) to obtain mixed solutions with 22.5 mg/ml moong bean globulin and 6.75% w/v of the respective polymer. The resulting solution is slowly (for example, at 3 μl/min) pumped, using a syringe pump, from a 5 ml syringe through a Teflon tube and a blunted 21gauge needle. The needle is connected to a positive terminal of a high voltage supply (for example, Spellman CZE 30 kV) and fixed 20-30 cm from a collection electrode. Collection electrode is an aluminum drum (ca. 12 cm long, 5 cm in diameter) that is wrapped in aluminum foil. The drum is attached to a spindle that is rotated by an IKA RW20 motor at about 600 rpm. The spindle is connected to a ground terminal of the high voltage supply. Portein/polymer fiber accumulate on foil and, after electrospinning is completed, are removed from foil and added to tissue replicas.

The dimension and composition of the fibers produced by the methods of the invention has an effect upon the taste, texture, and mechanical properties of the tissue replicas. Tissues comprising between 1 and 50% of the fibers in the <1-10 micron range, and between 10 and 50% of fibers in the 10-300 micron range most closely approach animal connective tissues in terms of taste, mouthfeel and mechanical properties.

Cartilage-type tissue is macroscopically homogenous, resistant against compression, has higher water content (up to 80%), lower protein (collagen) content, and higher polysaccharide (proteoglycans) contents (ca. 10% each). Compositionally, cartilage-type connective tissue replicas are similar to fascia-type tissue replicas with the relative ratios of each adjusted to more closely mimic 'meat' connective tissue. During extrusion, water content can be adjusted to about 60% to make a soft connective tissue replica.

Methods for forming cartilage-type connective tissue are similar to those for fascia-type connective tissue, but methods that produce isotropic non-fibrous gels are preferred.

A connective tissue replica can be made by isolating and purifying one or more proteins; and precipitating the one or more proteins, wherein the precipitating results in the one or more proteins forming physical structures approximating the physical organization of connective tissue. The precipitating can comprise solubilizing the one or more proteins in a first solution; and extruding the first solution into a second solution, wherein the one or more proteins is insoluble in the second solution, wherein the extruding induces precipitation of the one or more proteins.

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

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

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

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

J. OMISSIONS FROM THE COMPOSITIONS

Because the consumable can be put together from defined ingredients, which may themselves be isolated and purified, it is possible to produce consumables that do not contain certain components. This, in some cases, allows for the production of consumables that are lacking ingredients that may be not desirable to consumers (e.g., proteins that some humans are allergic to can be omitted or additives). In some embodiments, the consumable contains no animal products. In some embodiments the consumable contains no or less than 1% wheat gluten. In some embodiments the consumable contains no methylcellulose. In some embodiments the consumable contains no carrageenan. In some embodiments the consumable contains no caramel color. In some embodiments the consumable contains no Konjac flour. In some embodiments the consumable contains no gum arabic (also known as acacia gum). In some embodiments the consumable contains no wheat gluten. In some embodiments the consumable contains no soy protein isolate. In some embodiments the consumable contains no tofu. In some

embodiments the consumable contains less than 5% carbohydrates. In some embodiments the consumable contains less than 1% cellulose. In some embodiments the consumable contains less than 5% cellulose. In some embodiments the consumable contains less than 5% insoluble carbohydrates. In some embodiments the consumable contains less than 1% insoluble carbohydrates. In some embodiments the consumable contains no artificial color. In some embodiments the consumable contains no artificial flavorings.

In some embodiments the consumable contains one or more of the following characteristics: no animal products; no methylcellulose; no carrageenan; no Konjac flour; no gum arabic; less than 1% wheat gluten; no wheat gluten; no tofu; about 5% carbohydrates; less than 5% cellulose; less than 5% insoluble carbohydrates; less than 1% insoluble carbohydrates; no edible colorants such as caramel color, paprika, cinnamon, beet color, carrot oil, tomato lycopene extract, raspberry powder, carmine, cochineal extract, annatto, turmeric, saffron, F.D&C Red No.3, Yellow number 5, Yellow No 6., Green No.3, Blue No.2, Blue No. 1, Violet No. 1,FD&C Red No. 40 – Allura Red AC, and/or E129 (red shade); and/or no artificial flavorings. In some embodiments the consumable contains no soy protein isolate. In other embodiments, the consumable contains no soy protein or protein concentrate.

In some embodiments, the muscle tissue replica additionally contains less than 10%, less than 5%, less than 1%, or less than 0.1% wheat gluten. In some embodiments, the muscle tissue replica contains no wheat gluten.

IV. Combinations of the components

A. MEAT REPLICAS

A meat substitute product (alternatively a meat replica) can comprise compositions described herein. For example a meat replica can comprise a muscle replica; a fat tissue replica; and a connective tissue replica (or a sub-combination thereof). The muscle replica, adipose tissue replica, and/or connective tissue replica can be assembled in a manner that approximates the physical organization of meat. In some

embodiments, a binding agent such as a coacervate is used to help binding the replicas to each other.

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

In some embodiments, a meat substitute composition comprises between 10-30% protein, between 5-80% water, and between 5-70% fat, wherein the composition includes one or more isolated and purified proteins. Such a meat substitute can include no animal protein. In some embodiments, the meat substitute compositions comprise a transglutaminase.

In some embodiments, a meat substitute product includes a muscle replica, an adipose tissue replica, and connective tissue replica, where the muscle replica accounts for 40-90% of the product by weight, the adipose tissue replica accounts for 1-60% of the