

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

~~(54) METHOD~~ METHODS AND COMPOSITIONS FOR AFFECTING
THE
FLAVOR AND AROMA PROFILE OF CONSUMABLES

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Related U.S. Application Data

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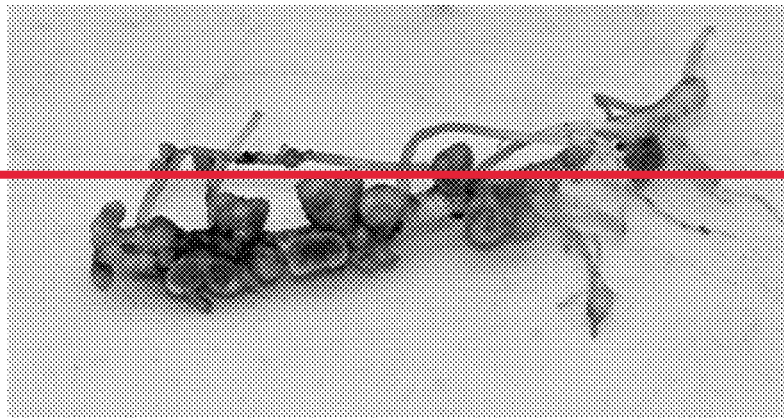


Figure 1

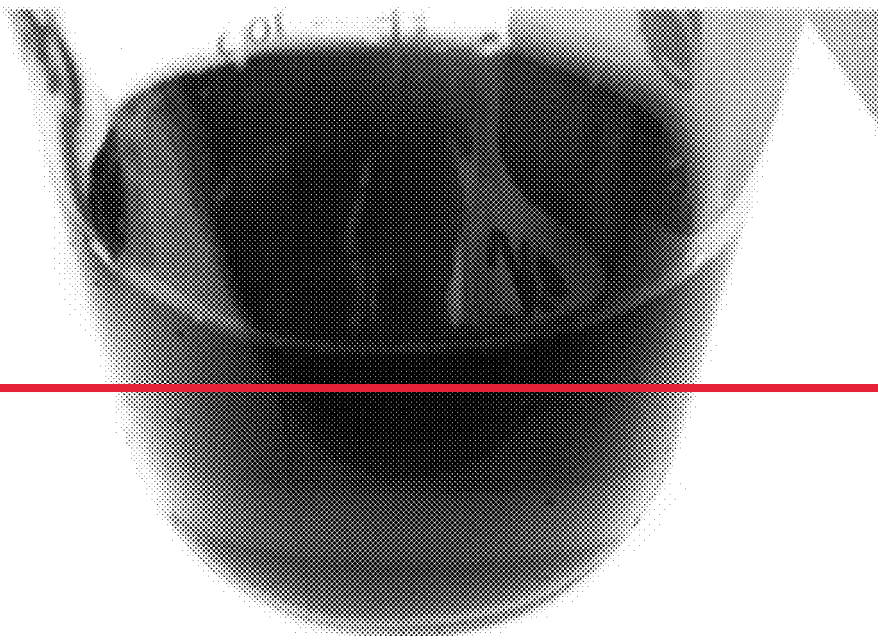


Figure 2

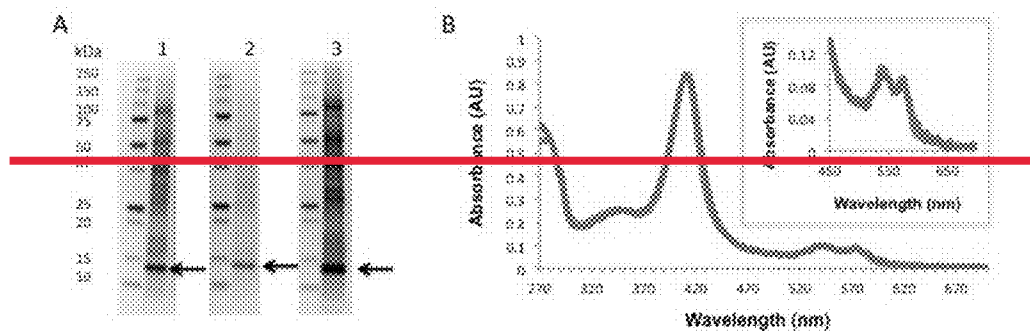


Figure 3

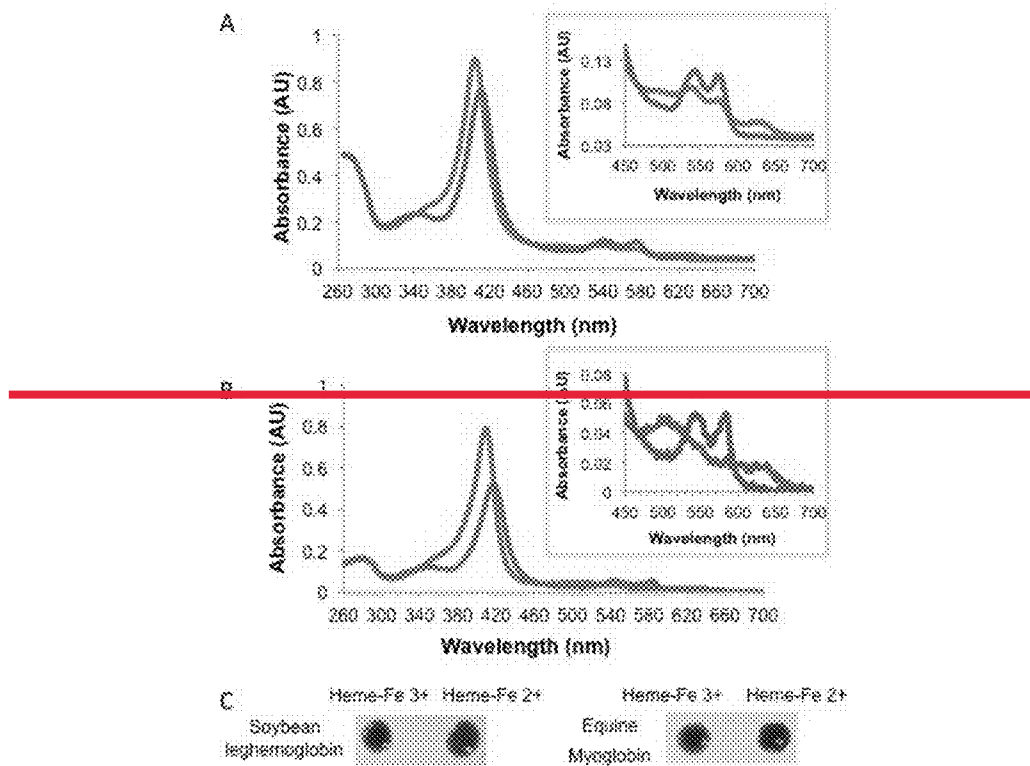


Figure 4

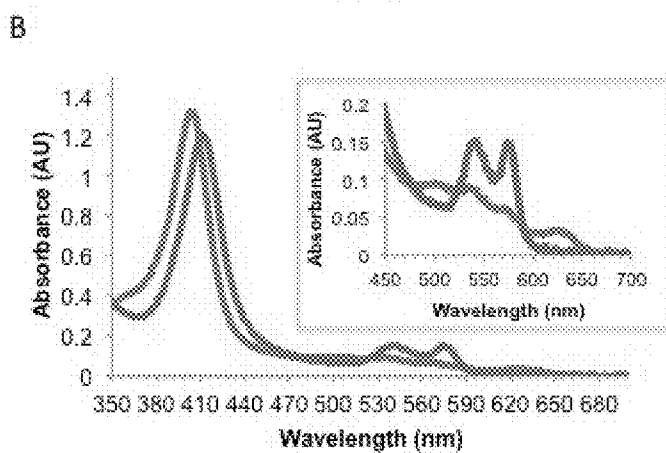
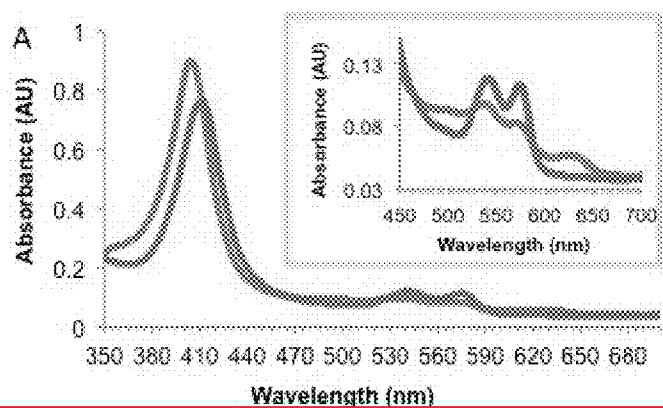


Figure 5

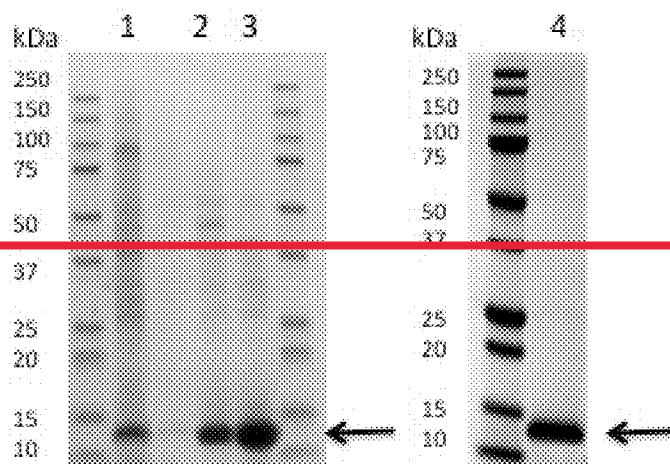


Figure 6

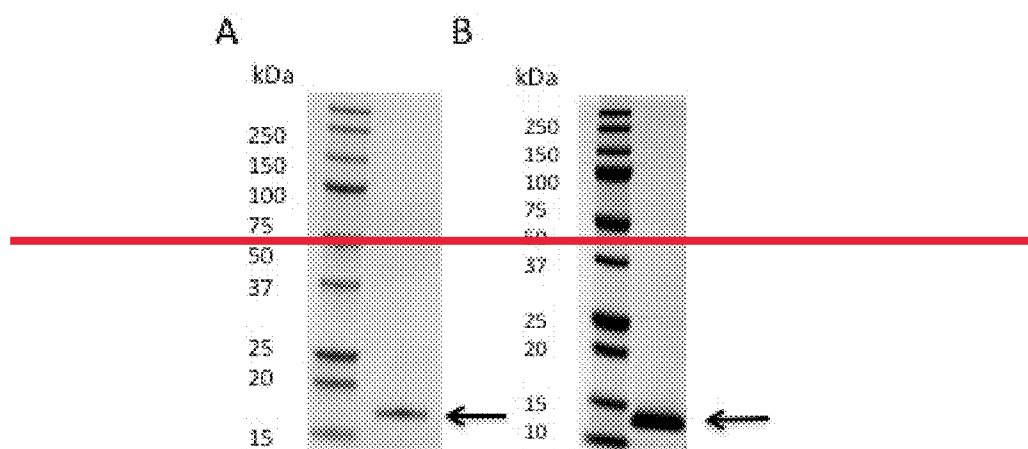


Figure 7



Figure 8

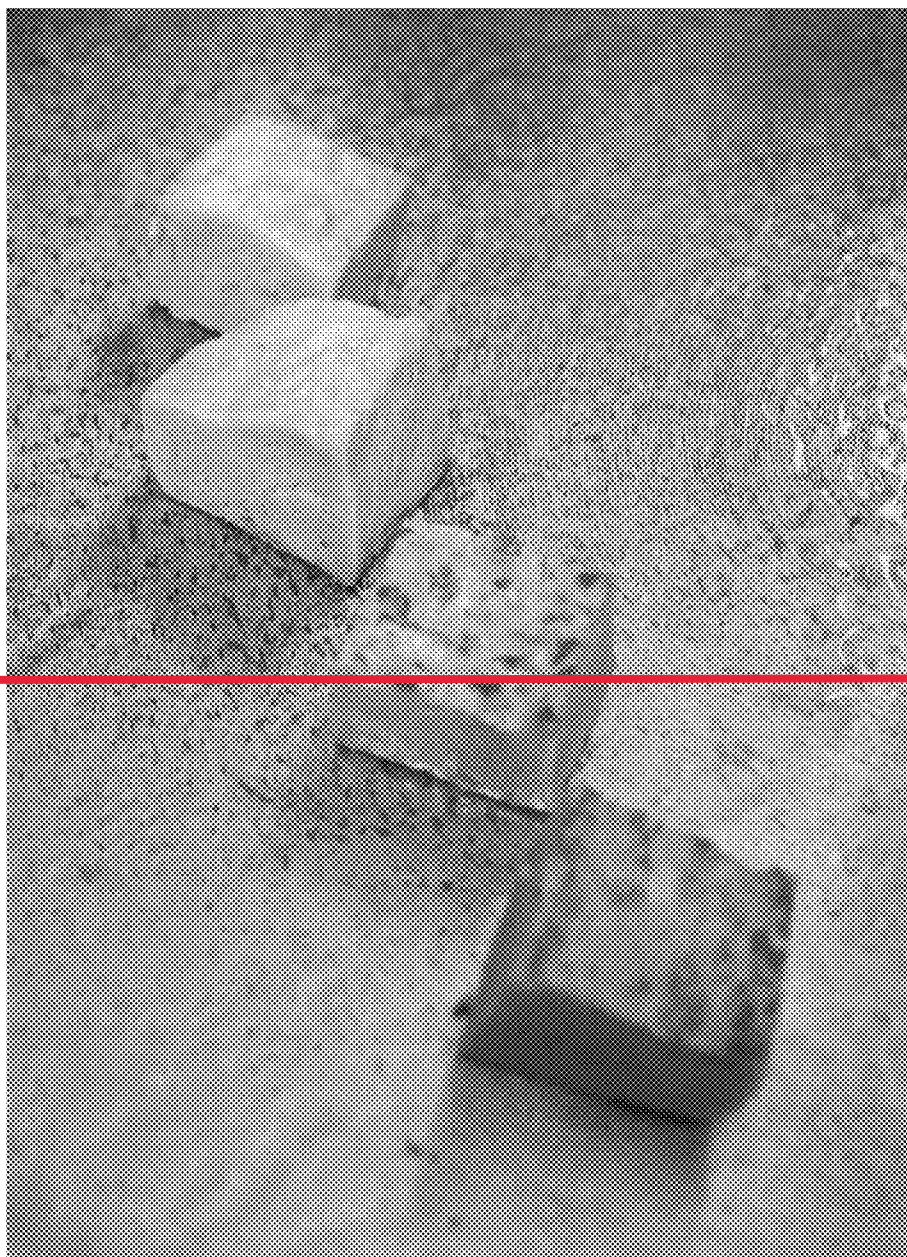


Figure 9

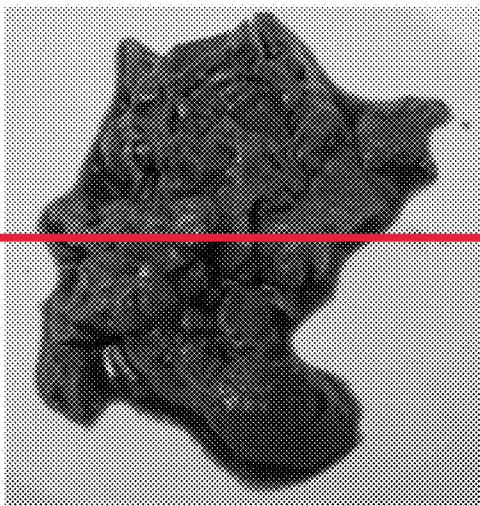


Figure 10

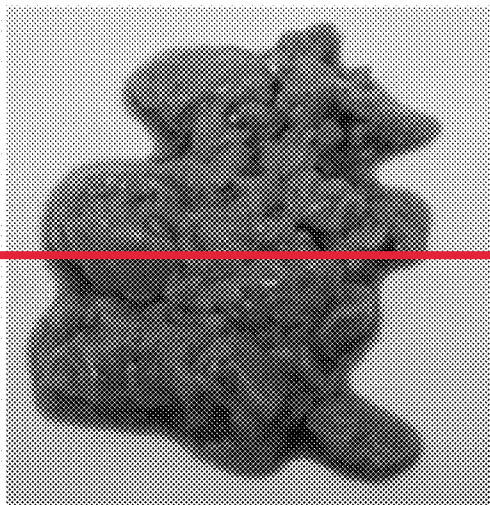


Figure 11

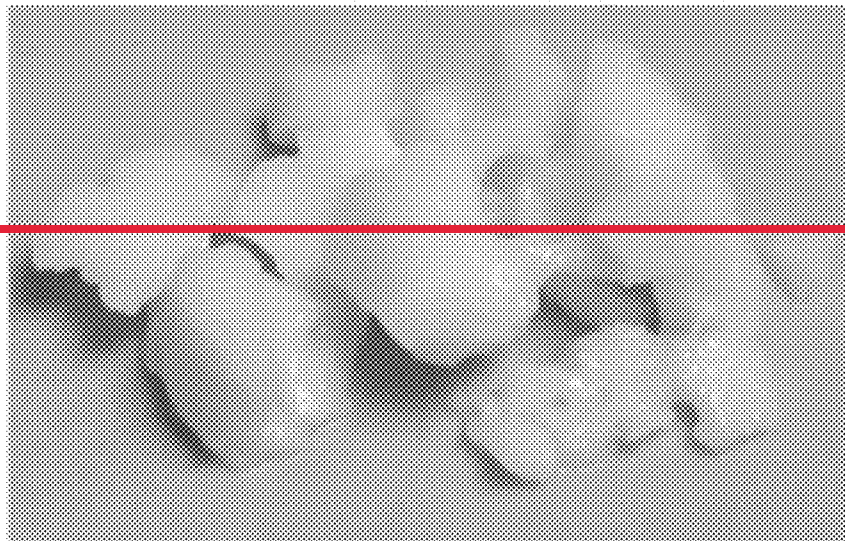


Figure 12

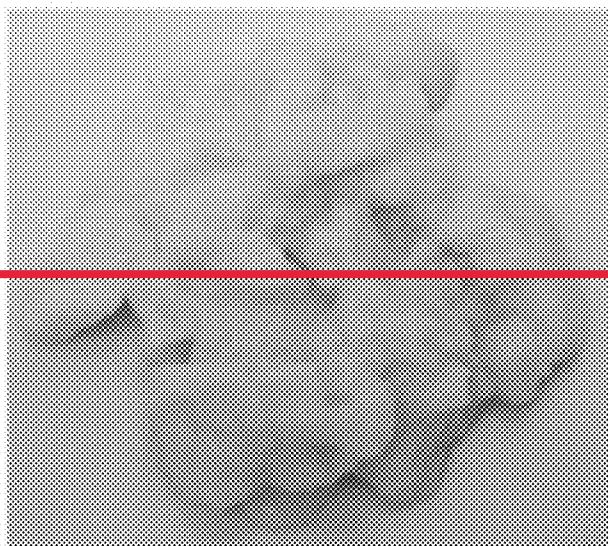


Figure 13

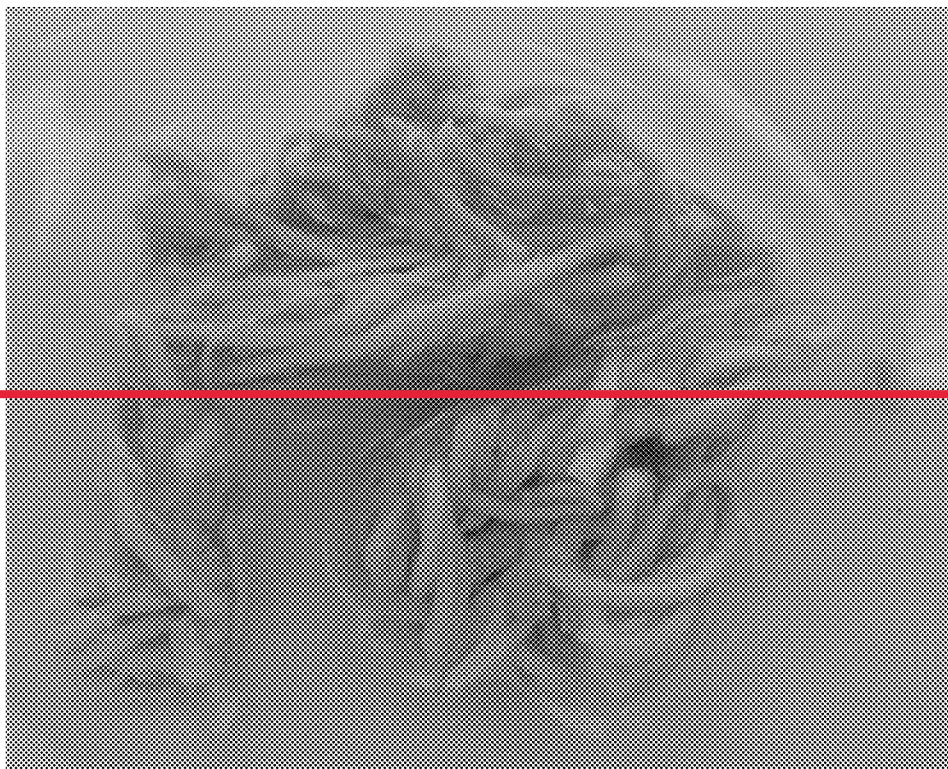


Figure 14

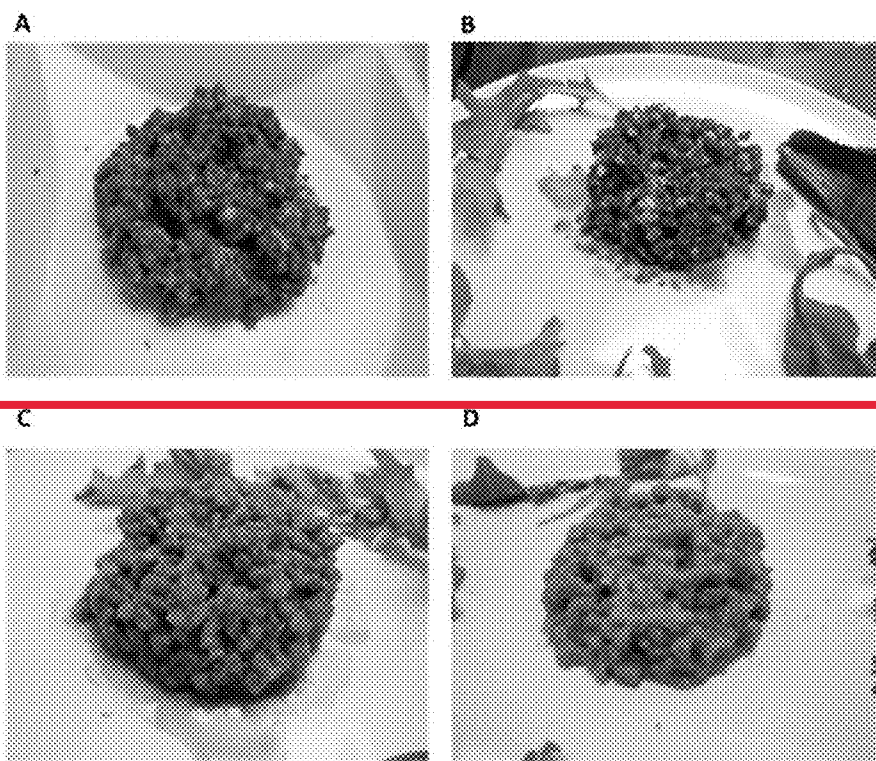


Figure 15

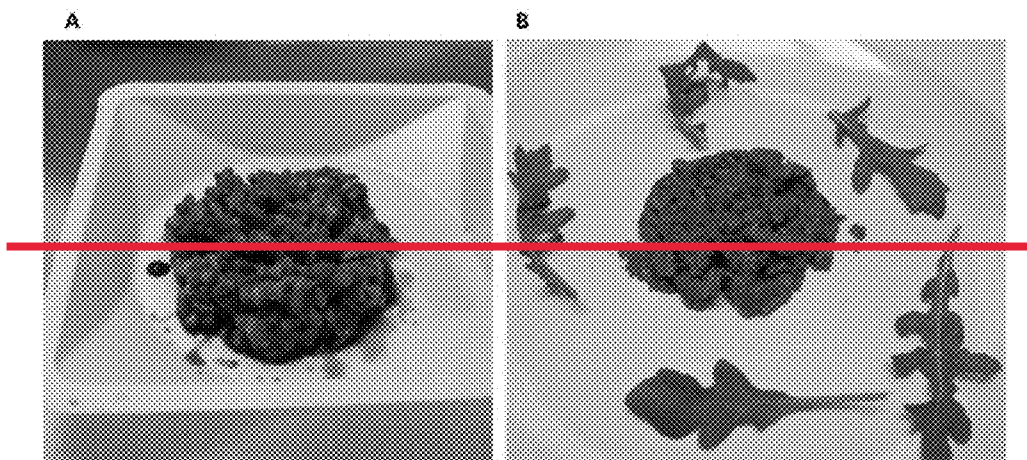


Figure 16

FIGURE 1

SEQ ID NO:1 *Vigna radiata*

MTTTLERGFTEEQEALVVKSWNVMMKKNSGELGLKFFLKIFEIAPSAQKLFSFLRDSTVP
LEQNPCLKPHAVSVFVMTCDASVQLRKAGKVTVRESNLKKLGATHFRTGVANEHFEVTK
FALLETIKEAVPEMWSPAMKNAWGEAYDQLVDAIKYEMKPPSS

SEQ ID NO:2 *Methylococcus thermophilus*

MIDQKEKELIKESWKRIEPNKNEIGLLFYANLFKEEPTVSVLFFQNPISSQSRKLMQVLG
ILVQGIDNLEGLIPTLQDLGRRHKQYGVVDSHYPLVGDCLLKSIIQEYLGQGFTEEAKAA
WTKVYGIAAQVMTAE

SEQ ID NO:3 *Aquifex aeolicus*

MLSEETIRVIKSTVPLLKEHGTEITARMYELLFSKYPKTKELFAGASEEQPKKLANAII
AYATYIDRLEELDNAISTARSHVRNVKPEHYPLVKECLLQAIEEVLNPGEEVLKAW
EAYDFLAKTLITLKKLYSQP

SEQ ID NO:4 *Glycine max*

MGAFTEKQEALVSSSFEEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLSNGVDPSNPKL
TGHAELKFLGLVRDSAGQLKANGTVVADAALGSIHAQKAITDPQFVVVKEALLKTIKEAV
GDKWDELSSAWEVAYDELAIAIKKAF

SEQ ID NO:5 *Hordeum vulgare*

MSAAEGAVVFSEEKEALVLKSWAIMKKDSANLGLRFFLKIFEIAPSARQMFPFLRDSV
PLETNPKLKTHAVSVFVMTCEAAAQLRKAGKITVRETTTLKRLGGTHLKYGVADGHFEVT
RFALLETIKEALPADMWGPENRANWGEAYDQLVAAIKQEMKPAE

Magnaporthe oryzae, (SEQ ID NO:6)

1 mdgavrlawt gldltgheih dgvpasrvq vmvsfplfk dhiimsskes psrksstigi
61 strngscqad tqkgqlppvg ekpkpvkenp mklkemsqr plptqhgdt yptekklgti
121 gedlkhirgy dvktilamvk sklkgelkd dktlmervm qlvarlptes kkraeltdsl
181 inelwesldh pplnylgpeh syrtpdgsyn hpfnplgaa gsryarsvip tvtpgalpd
241 pglifdsimg rtpnsyrkhp nnvssilwyw atiihdifw tdprdintnk sssyldlapl
301 ygnsgemqds irtfkdgmk pdcyadkrla gmpgvsavl imfnrfhnhv aenlalineg
361 grfnkpsdl egeareaaaw kynddlfqa rlvtsglyin itlvdyvrni vnlrvdttw
421 tldprqdaga hvgtadgaer gtgnavsae nlcyrwhsci sekdskfvea qfqnifgkpa
481 sevrpdemwk gfakmeqntp adpggrtfgg fkrqpdgkfd dddlvrcise avedvagafg
541 arnvpgamkv vetmgiiqgr kwnvagnlef rkhfhlpys tfedinsdpv vaealrrlyd

601 hpdnvelypg lvaeedkqpm vpgvgipty tisrvvlsda vclvrgdrfy ttdftprnlt
 661 nwgkyevdyd lsvnhgcvfy klfirafpnh fkqnsvyahy pmvvpksenr ilealgradl
 721 fdfeapkyip prvnitsygg aeyiletqek ykvtwheglg flmgeggklf mlsqddplha
 781 qqrkcmaaq l ykdgwteavk afyagmmeel lvksyflgn nkhrhvdiir dvgnmvvhvf
 841 asqvfglplk taknptgvft eqemygilaa ifttiffdld psksfplrtk trevcqklak
 901 lveanvklm kipwsrgmfv gkpakdepls iygktmikgl kahglstdyi awshvvptsg
 961 amvpngaqvf aqavdyylsp agmhyipeih mvalqpstpe tdalllgyam egirlagtfg
 1021 syreaavddv vkedngrqvp vkagdrvfvs fvdaardpkh fdpdevvnpr rpakkyihyg
 1081 vgphaclgrd asqiaitemf rclfrrrnvr rvpqpqgelk kvprpggyf ymredwggf
 1141 pfpvtmrvmw dde

Fusarium oxysporum (SEQ ID NO:7)

1 mkgsatlafa lvqfsaasql vwpskwdeve dllymqggfn krgfadalrt cefgsnvpgt
 61 qntaewlrta fhdaithdak agtgglldasi ywessrpenp gkafnntfgf fsgfhnprat
 121 asdltagtv lavgacngpr ipfragrida ykagpagvpe pstnlkdtfa aftkagftke
 181 emtamvacgh aiggvhsvdf peivgikadp nndtnvpfqq dvssfhngiv teylagtskn
 241 plvasknatf hsdkrifdnd katmkkllst agfnsncadi ltrmidvtpk svqltpvlea
 301 ydvrypyitel slnnknkihf tgsrvrvritn nirdnndlai nliyvgdrgk kvtpvtqqvt
 361 fgggtsfgag evfanfefdt tmdakngitk ffigevkpst katvthdnqk tggkqvddtv
 421 lyqlqqscav leklnaplv vtamvrdera kdaltlrva kkpvkgsivp rfqtaitnfk
 481 atgkksstyt gfqaktmfee qstyfdilvg gspasgvqfl tsqampsqcs

Fusarium graminearum (SEQ ID NO:8)

1 masatrqfar aatratrngf aiaprqvirk qgrryyssep aqkssawiw ltgaavagga
 61 gyyfygnsas satakvnps kedyqkyne iaarleekdd yddgsygpvl vrlawhasgt
 121 ydketgtggs ngatmrfafe sdhganagla aardflqpvk ekfpwitysd lwilagvcai
 181 qemlgpaipy rpgrsdrdvs gctpdgrlpd askrqdhlrg ifgrmgfndq eivalsgaha
 241 lgrchtdrsg ysgpwtfspt vltndyfrll veekwqwkww ngpaqyedks tkslmmllpsd
 301 ialiedkkfk pwvekyakdn daffkdfsns vlrlfelgvp faqgtenqrw tfkpthqe

SEQ ID NO: 9 *Chlamydomonas eugametos*

MSLFAKLGGREAVEAAVDKFYNKIVADPTVSTYFSNTDMKVQRSKQFAFLAYALGGASE
 WKGKDMRTAHKDLVPHLSDVHFQAVARHLSDTLTELGVPPEDITDAMAVVASTRTEVLN
 MPQQ

SEQ ID NO:10 *Tetrahymena pyriformis*

MNKPQTIYEKLGGENAMKAAVPLFYKKVLADERVKHFFKNTDMDHQTKQQTDFLTMLLG
 GPNHYKGKNMTEAHKGMNLQNLHFDAL IENLAATLKLGVTDVINEAAKVIEHTRKDM
 LGK

SEQ ID NO:11 *Paramecium caudatum*

MSLFEQLGGQAAVQAVTAQFYANIQADATVATFFNGIDMPNQTNKTA AFLCAALGGPNA
WTGRNLKEVHANMGVSNAQFTTVIGHLSALTGAGVAAALVEQTVAVAETVRGDVVTV

SEQ ID NO:12 *Aspergillus niger*

MPLTPEQIKI IKATVPVLQEYGTKITTA FYMMSTVHPELNAVFNTANQVKGHQARALA
GALFAYASHIDDLGALGPAVELICNKHASLYIQADEYKIVGKYLLEAMKEVLGDACTDD
ILDWGAAYWALADIMINREAALYKQSQG

SEQ ID NO:13 *Zea mays*

MALAEADDGAVVFGEEQEALVLKSWAVMKKDAANLGLRFFLKVFEIAPS AEQMFSFLRD
SDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRDTTLKRLGATHLRYGVADGHF
EVTGFALLETIKEALPADMWSLEMKKAWAEAYSQLVAAIKREMKPDA

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

MALVEGNNGVSGGAVSFSEEQEALVLKSWAIMKKDSANIGLRFFLKIFEVAPSASQMFS
FLRNSDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRDTTLKRLGATHFKYGVG
DAHFEVTRFALLETIKEAVPVDMMWSPAMKSAWSEAYNQLVAAIKQEMKPAE

SEQ ID NO:15 *Arabidopsis thaliana*

MESEKIVFTEEQEALVVKSWVMKKNSAELGLKLFIKIFEIAPT TTKMFSFLRDSPI P
AEQNPCLKPHAMSVFVMCCESAVQLRKTGKVTVRDTTLKRLGASHSKYGVVDEHFEVAK
YALLETIKEAVPEMWSPEMKVAWGQAYDHLVAAIKAEMNLSN

SEQ ID NO:16 *Pisum sativum*

MGFTDKQEALVNSSWESFKQNLSGNSILFYTTILEKAPAAKGLFSFLKDTAGVEDSPKL
QAHAEQVFGLVRDSAAQLRTKGEVVLGNATLGAIHVQRGVTDPHFVVVKEALLQTIKKA
SGNNWSEELNTAWEVAYDGLATAIKKAMT

SEQ ID NO:17 *Vigna unguiculata*

MVAFSDKQEALVNGAYEAFKANIPKYSVVFYTTILEKAPAAKNLFSFLANGVDATNPKL
TGHAEKLFGLVRDSAAQLRASGGVVADAALGAVHSQKAVNDAQFVVVKEALVKTLEAV
GDKWSDELGTAVELAYDELAAAIKKAY

SEQ ID NO:18 *Bos taurus*

MGLSDGEWQLVLNVAWGKVEADVAGHGQEVLIIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGNTVLTALGGILKKKGHHEAEVKHLAESHANKHKIPVKYLEFISDAIIHVLHA
KHPSDFGADAQAAMSKALELFRNDMAAQYKVLGFHG

SEQ ID NO:19 *Sus scrofa*

MGLSDGEWQLVLNVWGKVEADVAGHGQEVLIIRLFKGHPETLEKFDKFKHLKSEDEMKA
EDLKKHGNTVLTALGGILKKKGHHEAELTPAQSHATKHKIPVKYLEFISEAIIQVLQS
KH PGDFGADAQGAMSKALELFRNDMAAKYKELGFQG

SEQ ID NO: 20 *Equus caballus*

MGLSDGEWQQVLNVWGKVEADIAGHGQEVLIIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPKYLEFISDAIIHVLHS
KH PGDFGADAQGAMTKALELFRNDIAAKYKELGFQG

SEQ ID NO: 21 *Nicotiana benthamiana*

MSSFTEEQEALVVKSWDSMKNAGEWGLKLFLKIFEIAPSAKKLFSFLKDSNVPL
EQNAKLKPHSKSVFVMTCEAAVQLRKAGKVVRDSTLKKLGATHFKYGVAD
HFEVTKFALLETIKEAVPEMWSVDMKNAWGEAFDQLVNAIKTEMK

SEQ ID NO: 22 *Bacillus subtilis*

MGQSFNAPYEAIGEELLSQLVDTFYERVASHPLLKPIFPSDLTETARKQKQFLTQY
LGGPLYTEEHGHPMLRARHLPFPITNERADAWLSCMKDAMDHVGLERGEIREFL
FGRLELTARHVMVNQTEAEDRSS

SEQ ID NO: 23 *Corynebacterium glutamicum*

MTTSENFYDSVGGEETFSLVHRFYEQVPNDDILGPMYPPDDFEGAEQRLKMFLS
QYWGGPKDYQEQRGHPRLMRHVNYPYIGVTAAERWLQLMSNALDGVDLTAEQ
REAIWEHMOVRAADMLINSNPDPHA

SEQ ID NO: 24 *Synechocystis* PCC6803

MSTLYEKLGGTTAVDLAVDKFYERVLQDDRIKHFFADVDMAKQRAHQKAFLTY
AFGGTDKYDGRYMREAHKELVENHGLNGEHFDAVAEDLLATLKEMGVPEDLIA
EVAAGAPAHKRDVLNQ

SEQ ID NO: 25 *Synechococcus* sp. PCC 7335

MDVALLEKSFEQISPRAIIEFSASFYQNLFHHPKPLFAETSQTIQEKKLIFSLAAI
IENLRNPDLQPALKSLGARHAEVGTIKSHYPLVGQALITFAEYLAADWTEQLA
TAWVEAYDVIASMTIEGADNPAAYLEPELTFYEWLDLYGEESPKVRNAIATLTH
FHYGEDPQDVQRDSRG

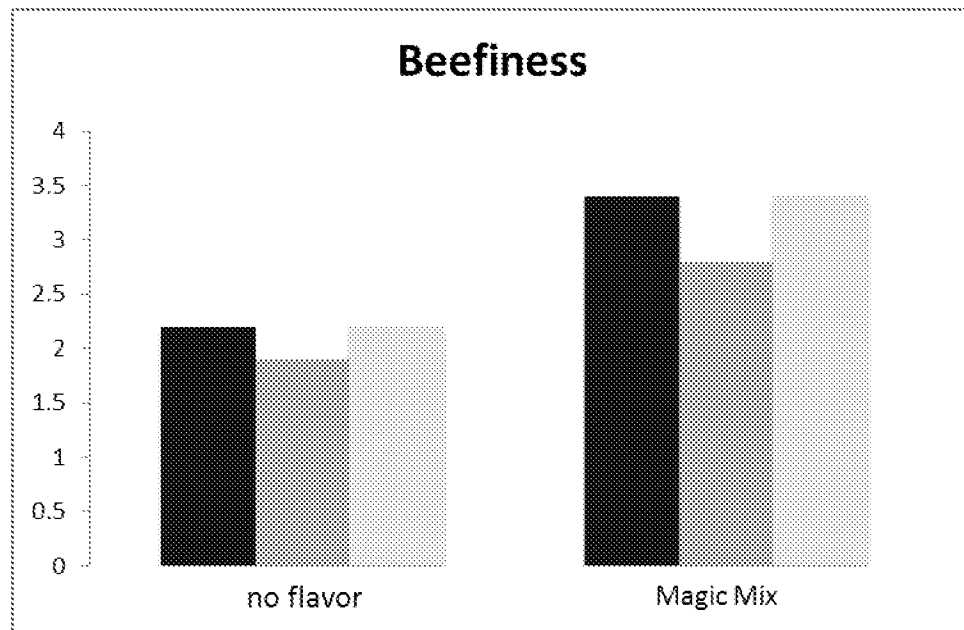
SEQ ID NO: 26 *Nostoc commune*

MSTLYDNIGGQPAIEQVVDELHKRIATDSLLAPVFAGTDMVKQRNHLVAFLAQIF
EGPKQYGGRPMDKTHAGLNLQPHFDAIAKHLGERMAVRGVSAENTKAALDR
VTNMKGAILNK

SEQ ID NO: 27 *Bacillus megaterium*

MREKIHSPYELLGGEHTISKLVDAFYTRVGQHPHELAPIFPDNLTTETARKQKQFLTQ
YLGGPSLYTEEHGHPMLRARHLPFEITPSRAKAWLTCMHEAMDEINLEGPERDE
LYHRLILTAQHMINSPQETDEKGFH

FIGURE 2



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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 5 following co-pending patent applications: Application Serial No. PCT/US12/46560; Application Serial No. PCT/US 12/46552; Application Serial No. 61,876,676, filed September 11, 2013; and Application Serial No. 61/751,818, filed January 11, 2013, all of which are incorporated herein by reference.

10 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.

15

BACKGROUND OF THE INVENTION

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

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

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

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 25 different portions, depending upon the purpose of the food. Often, certain portions of the plant,

such as the seeds or fruits, are more highly prized by humans than others and these are selected for human consumption, while other less desirable portions, such as the stalks of grasses, are typically used for feeding animals.

~~{0005} Plant~~ Current plant-based meat substitutes have largely failed to cause a shift to a vegetarian diet. ~~The current state of the art for meat~~ Meat substitute compositions ~~involves the extrusion of soy/ grain mixture, resulting in products~~ are typically extruded soy/ grain mixtures ⁵ which largely fail to replicate the experience of cooking and eating meat. Common

limitations of ~~these~~ plant-based meat substitute products are a texture and ~~mouthfeel~~ mouth- feel that are more homogenous than that of equivalent meat products. Furthermore, as ~~the~~ these products must largely be sold ~~precooked~~ pre-cooked, with artificial flavors and aromas ~~built-in~~ pre- incorporated, they fail to replicate the aromas, flavors, and other key features, such as texture and mouth- ¹⁰ feel, associated with cooking or cooked meat. As a result, these products appeal ~~mainly~~ largely to a limited consumer base that is already committed to vegetarianism/veganism, but have failed to appeal to the larger consumer segment accustomed to eating meat. It would be useful to have improved plant-based meat substitutes which better replicate the aromas and flavors of meat, particularly during and/or after cooking.

15

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 ²⁰ 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 ²⁵ 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 bacteriochlorophyll; a corphin; a chlorophyllin; a bacteriochlorin; or an isobacteriochlorin moiety complexed to an iron ion). Such highly conjugated heterocyclic moieties 5 include heterocyclic aromatic rings composed of one or more (2, 3, or 4 more) pyrrole, pyrrole-like, and/or pyrroline subunits. The highly conjugated heterocyclic ring complexed to an iron ion is referred to herein as an iron complex. In some embodiments, the heme moiety can be a heme cofactor such as a heme moiety bound to a protein; a heme moiety bound to a non-proteinaceous polymer; a heme moiety bound to a solid 10 support; or a heme moiety encapsulated in a liposome. In some embodiments, the flavors and/or aromas are not generated in the absence of the iron complex (e.g., in the absence of a ferrous chlorin) or are not generated in the absence of a heme-cofactor (e.g., in the absence of a heme-containing protein). Accordingly, as described herein, the iron complexes such as isolated chlorin-iron complexes or heme-cofactors (e.g., heme- 15 containing proteins) can be used to generate meaty flavors and/or aromas in a variety of food products, such as during the cooking process.

Combining one or more iron complexes such as a heme-cofactor (e.g., a heme-containing protein, including, for example a plant-derived heme protein such as a plant leghemoglobin (legH)), with one or more flavor precursor molecules or compositions 20 (see, e.g., Table 1 or Table 13) can generate or provide a range of savory and meaty

aromas and tastes (see, e.g., Tables 2, 7, and/or 11) in a cooked consumable food product. Flavor precursor molecules or compositions can be added to the uncooked food product in purified form and/or can be derived from ingredients in the uncooked consumable food product that contain and/or are enriched with one or more of the particular flavor 25 precursors or compositions, including, for example, yeast extract, vegetable oil, com oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, canola oil, sunflower oil, coconut oil, mango oil, or an algal oil. The resultant flavor and/or aroma profile

can be modulated by the type and concentration of the flavor precursors, the pH of the reaction, the length of cooking, the type and amount of iron complex (e.g., a heme cofactor such as a heme-containing protein), the

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

One or more flavor precursor molecules or compositions can be added along with a iron complex (e.g., ferrous chlorophyllin or a heme cofactor such as a heme-containing protein), to an uncooked food product, before and/or during the cooking process, to give the cooked consumable food product a particular meaty taste and smell, for example, the taste and smell of beef, bacon, pork, lamb, or chicken. Consumable food products can be animal or non-animal based (e.g., plant) food products, or combinations of an animal and non-animal based food product. For example, a plant based veggie burger or an animal-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,

25 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 30 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 5 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 10 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 15 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 20 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- 25 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 30 bacteriochlorophyll, 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 bacteriochlorophyll, a corphin, a chlorophyllin, a bacteriochlorin, or an isobacteriochlorin complexed to an iron) and one or more flavor precursor molecules to form a mixture, the one or more flavor precursor molecules selected from the group consisting of glucose, fructose, arabinose, ribose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose,

sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, com oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, canola oil, olive oil, sunflower oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture to form one or more flavor compounds selected from the group consisting of phenylacetaldehyde, 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, 5 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- 10 methyl-propanal,

butyrolactone,, 3-methyl-butanal, methyl-thiirane, 2-hexyl-furan,

butanal,, 2-methyl-butanal, 2-methyl-furan, furan, octanal, 2-heptenal, 1-octene, formic acid heptyl ester, 3-pentyl-furan, and 4-penten-2-one. The heme moiety can be a heme-containing protein, a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support. The method can include combining cysteine, ribose, lactic acid, 15 lysine, and/or thiamine with the heme-containing protein.

In another aspect, this document features a method for producing a flavor compound. The method includes combining an iron complex, such as a heme-containing protein, and one or more flavor precursor molecules to form a mixture, the one or more flavor precursor molecules selected from the group consisting of glucose, fructose, 20 ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, com oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, 25 olive oil, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, methionine, cysteine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture to form one or more

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

This document also features a method for imparting a meat like flavor (e.g., beeflike, chicken like, pork-like, lamb-like, turkey-like, duck-like, deer-like, or bison-like) to a food product. The method includes contacting the food product with a flavoring

composition, the flavoring composition comprising i) an iron complex, such as a heme moiety (e.g., a heme-containing protein); and ii) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, 10 maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP,

lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, com oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine,

15 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- 20 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.

25 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,

30 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, 5 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 10 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, 15 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 20 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 25 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 heterocyclic rings complexed to an iron ion (referred to as an iron 5 complex). “Heme” refers to a prosthetic group bound to iron (Fe^{2+} or Fe^3) in the center of a porphyrin ring. Thus, an iron complex can be a heme moiety, or a porphyrin, porphyrinogen, corrin, corrinoid, chlorin, bacteriochlorophyll, corphin, chlorophyllin, bacteriochlorin, or isobacteriochlorin moiety complexed to iron ion. The heme moiety that can be used to modulate the taste and/or aroma profile of food products can be a 10 heme cofactor such as a heme-containing protein; a heme moiety bound to a non-peptidic

polymer or other macromolecule such as a liposome, a polyethylene glycol, a carbohydrate, a polysaccharide, a cyclodextrin, a polyethylenimine, a polyacrylate, or derivatives thereof; a siderophore (i.e., an iron chelating compound); or a heme moiety bound to a solid support (e.g., beads) composed of a chromatography resin, cellulose, 15 graphite, charcoal, or diatomaceous earth.

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

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

25 TABLE 1

Flavor Precursor Molecules

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

lactose, ribitol, gluconic acid and glucuronic acid, amylose, amylopectin, or xylose

Oils: coconut oil, mango oil, sunflower oil, cottonseed oil, safflower oil, rice bran oil, cocoa butter, palm fruit oil, palm oil, soybean oil, canola oil, com oil, sesame oil, walnut oil, flaxseed, jojoba oil, castor, grapeseed oil, peanut oil, olive oil, algal oil, oil from bacteria or fungi

Free fatty acids: caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic, oleic acid, linoleic acid, alpha linolenic acid, gamma linolenic acid, arachidic acid, arachidonic acid, behenic acid, or emcic acid

Amino acids and derivatives thereof: cysteine, cystine, a cysteine sulfoxide, allicin, selenocysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, 5-hydroxytryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, or tyrosine

Nucleosides and Nucleotides: inosine, inosine monophosphate (IMP), guanosine, guanoside monophosphate (GMP), adenosine, adenosine monophosphate (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 hydrolysates, 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, 5 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, 10 isoleucine, leucine, lysine, methionine, phenylalanine, pro line, threonine, tryptophan, tyrosine, valine, glucose, ribose, maltodextrin, thiamine, IMP, GMP, lactic acid, and creatine.

As used herein, the term “heme containing protein” can be used interchangeably with “heme containing polypeptide” or “heme protein” or “heme polypeptide” and 15 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 20 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,

25 stored, handled or prepared for consumption. The life-time of the unoxygenated 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 Non-limiting examples of heme-containing polypeptides can include an 30 androglobin, a cytoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a

myoglobin, an erythrocyte protein, 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.

5 Heme-containing proteins that can be used in the compositions and food products described herein can be from mammals (e.g., farm animals such as cows, goats, sheep, pigs, ox, or rabbits), birds, plants, algae, fungi (e.g., yeast or filamentous fungi), ciliates, or bacteria. For example, a heme-containing protein can be from a mammal such as a farm animal (e.g., a cow, goat, sheep, pig, ox, or rabbit) or a bird such as a turkey or 10 chicken. Heme-containing proteins can be from a plant such as *Nicotiana tabacum* or *Nicotiana glauca* (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,

15 *Vigna unguiculata* varieties (cow peas), *Vigna radiata* (Mung beans), *Lupinus albus* (lupin), or *Medicago sativa* (alfalfa); *Brassica napus* (canola); *Triticum sp.* (wheat, including wheat berries, and spelt); *Gossypium hirsutum* (cotton); *Oryza sativa* (rice); *Zizania sp.* (wild rice); *Helianthus annuus* (sunflower); *Beta vulgaris* (sugarbeet); *Pennisetum glaucum* (pearl millet); *Chenopodium sp.* (quinoa); *Sesamum sp.* (sesame);

20 *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*, *Myceliophthora thermophila*, *Kluyvera lactis*, or *Fusarium oxysporum*. Heme-containing proteins can be isolated from bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, 25 *Bacillus megaterium*, *Synechocystis sp.*, *Aquifex aeolicus*, *Methylobacterium thermophilum*, 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.

5 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% 10 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 *Methylophilum thermophilum* (SEQ ID NO:2), a flavohemoprotein such as that 15 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 reinhardtii* (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 glauca* (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 30 (B12seq) program from the stand-alone version of BLASTZ containing BLASTP version

2.0.14. This stand-alone version of BLASTZ can be obtained from Fish & Richardson's web site (e.g., www.fr.com/blast/) or the U.S. government's National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov). Instructions explaining

how to use the B12seq program can be found in the readme file accompanying BLASTZ.
5 B12seq performs a comparison between two amino acid sequences using the BLAST?
algorithm. To compare two amino acid sequences, the options of B12seq are set as follows: -i is
set to a file containing the first amino acid sequence to be compared (e.g., C:\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); 10 and all other options are left at
their default setting. For example, the following

command can be used to generate an output file containing a comparison between two
amino acid sequences: C:\B12seq-i c:\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 15 share homology, then
the designated output file will not present aligned sequences.

Similar procedures can be following for nucleic acid sequences except that blastn is used.

Once aligned, the number of matches is determined by counting the number of
positions where an identical amino acid residue is presented in both sequences. The percent
identity is determined by dividing the number of matches by the length of the 20 full-length
polypeptide amino acid sequence followed by multiplying the resulting value by 100. It is noted
that the percent identity value is rounded to the nearest tenth. For example, 78.11, 78.12, 78.13,
and 78.14 is rounded down to 78.1, while 78.15, 78.16, 78.17, 78.18, and 78.19 is rounded up to
78.2. It also is noted that the length value will always be an integer.

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

Heme-containing proteins can be extracted from the source material (e.g.,
extracted from animal tissue, or plant, fungal, algal, or bacterial biomass, or from the culture
supernatant for secreted proteins) or from a combination of source materials (e.g., multiple plant
species). Leghemoglobin is readily available as an unused by-product of 5 commodity legume

crops (e.g., soybean, alfalfa, or pea). The amount of leghemoglobin in the roots of these crops in the United States exceeds the myoglobin content of all the red meat consumed in the United States.

In some embodiments, extracts of heme-containing proteins include one or more non-heme-containing proteins from the source material (e.g., other animal, plant, fungal, 10 algal, or bacterial proteins) or from a combination of source materials (e.g., different animal, plant, fungi, algae, or bacteria).

In some embodiments, heme-containing proteins are isolated and purified from other components of the source material (e.g., other animal, plant, fungal, algal, or bacterial proteins). As used herein, the term “isolated and purified” indicates that the 15 preparation of heme-containing protein is at least 60% pure, e.g., greater than 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% pure. Without being bound by theory, isolating and purifying proteins can allow the food products to be made with greater consistency and greater control over the properties of the food product as unwanted material is eliminated. Proteins can be separated on the basis of their molecular weight, for example, by size 20 exclusion chromatography, ultrafiltration through membranes, or density centrifugation. In some embodiments, the proteins can be separated based on their surface charge, for example, by isoelectric precipitation, anion exchange chromatography, or cation exchange chromatography. Proteins also can be separated on the basis of their solubility, for example, by ammonium sulfate precipitation, isoelectric precipitation, surfactants, 25 detergents or solvent extraction. Proteins also can be separated by their affinity to another molecule, using, for example, hydrophobic interaction chromatography, reactive dyes, or hydroxyapatite. Affinity chromatography also can include using antibodies having specific binding affinity for the heme-containing protein, nickel NTA for His- tagged recombinant proteins, lectins to bind to sugar moieties on a glycoprotein, or other 30 molecules which specifically binds the protein.

Heme-containing proteins also can be recombinantly produced using polypeptide expression techniques (e.g., heterologous expression techniques using bacterial cells, insect cells, fungal cells such as yeast, plant cells such as tobacco, soybean, or *Arabidopsis*, or mammalian cells). In some cases, standard polypeptide synthesis 5 techniques (e.g., liquid-phase polypeptide synthesis techniques or solid-phase

polypeptide synthesis techniques) can be used to produce heme-containing proteins synthetically. In some cases, *in vitro* transcription-translation techniques can be used to produce heme-containing proteins.

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

In some embodiments, the isolated and purified protein is substantially in its native fold and water soluble. In some embodiments, the isolated and purified protein is more than 50, 60, 70, 80, or 90% in its native fold. In some embodiments, the isolated 15 and purified protein is more than 50, 60, 70, 80, or 90% water soluble.

Modulating Flavor and/or Aroma Profiles

As described herein, different combinations of flavor precursors can be used with one or more iron complexes (e.g., a ferrous chlorin, a chlorin-iron complex, or a heme- 20 cofactor such as a heme-containing protein or heme bound to a non-peptidic polymer such as polyethylene glycol or to a solid support) to produce different flavor and aroma profiles when the flavor precursors and iron complexes are heated together (e.g., during cooking). The resultant flavor and/or aroma profile can be modulated by the type and concentration of the flavor precursors, the pH of the reaction, the length of cooking, the 25 type and amount of iron complex (e.g., a heme-cofactor such as heme-containing protein, heme bound to non-peptidic polymer or macromolecule, or heme bound to a solid support), the temperature of the reaction, and the amount of water activity in the product, among other factors. In embodiments in which a heme moiety is bound to a solid support such as cellulose or a chromatography resin, graphite, charcoal, or diatomaceous earth,

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

Table 2 provides non-limiting examples of flavor types that can be generated by 5 combining one or more flavor precursors and one or more heme co-factors (e.g., heme- containing

proteins). See also Tables 7 and/or 11.

TABLE 2

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

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.

10

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 -thiophenecarboxaldehy de	4-ethyl-phenol,	heptanoic acid
1-octene	3-octanone	ethanethiol
butyrolactone	styrene	2-methyl-1 -heptene
2-undecenal	furan, 3-pentyl-	(E)-4-octene
propyl-cyclopropane	formic acid, heptyl ester	2-methyl-2-heptene
methyl-pyrazine	(E)-2-Heptenal	pentanoic acid
1 -hydroxy-propanone	6-methyl-5-hepten-2-one	nonanoic acid
acetic acid	n-caproic acid vinyl ester	1,3-dimethyl-benzene
furfural	2-ethyl-2-hexenal	
2-decanone	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	thiophosphoramidate, 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-ethyl-dihydro-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- 5 pentenyl)furan, (Z)-2-heptenal, (E)-2-octenal, pyrrole, 2,4-dodecadienal, 1-octanal, (Z)-2- decenal, or 2-undecenal can be increased in the presence of the heme-containing protein, which can impart a more beefy flavor to the chicken.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein is heated in the presence of cysteine 10 and glucose or other combinations of flavor precursors to provide a different profile of volatile odorants than when any subset of the three components are used individually. Volatile flavor components that are increased under these conditions include but are not limited to furan, acetone, thiazole, furfural, benzaldehyde, 2-pyridinecarboxaldehyde, 5- methyl-2-thiophenecarboxaldehyde, 3 -methyl-2-thiophenecarboxaldehyde, 3 - 15 thiophenemethanol and decanol. See, e.g., Tables 8 and 9. Under these conditions, cysteine and glucose alone or in the presence of iron salts such as ferrous gluconate 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 5 and ribose to provide a different profile of volatile odorants. Heating in the presence of ribose created some additional compounds as compared to when a heme-containing protein and glucose were heated together. See Tables 8 and 9.

In some embodiments, an iron complex (e.g., a ferrous chlorophyllin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the 10 presence of thiamine and a sugar to affect the formation of 5-Thiazoleethanol, 4-methyl- furan, 3,3'-dithiobis[2-methyl-furan, and/or 4-Methylthiazole. These compounds are known to be present in meat and have beefy, meaty taste notes.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of a 15 nucleotide such as inosine monophosphate and/or guanosine monophosphate to control the formation of flavor compounds such as (E)-4-octene, 2-ethyl-furan, 2-pentanone, 2,3-butanedione, 2-methyl-thiazole, methyl-pyrazine, tridecane, (E)-2-octenal, 2-thiopenecarboxaldehyde, and/or 3-thiopenecarboxaldehyde. These compounds are known to be present in meat and have a beefy, meaty, buttery, and or savory flavor notes.

20 In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of lysine, a sugar such as ribose, and cysteine to control the formation of flavor compounds such as dimethyl trisulfide, nonanal, 2-pentyl thiophene, 2-nonenal furfural, 1-octanol, 2- nonenal, thiazole, 2-acetylthiazole, phenylacetaldehyde, and/or 2-acetylthiazole. These 25 compounds are known to be present in meat and some have a beefy, meaty, and or savory flavor.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of lactic acid, a sugar such as ribose, and cysteine to control the formation of the flavor 30 compounds nonanal, thiazole, 2-acetylthiazole, and/or 8-methyl 1-undecene. These compounds are known to be present in meat and have beefy, savory, browned, bready, and malty notes.

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of 5 amino acids, sugars such as glucose, ribose, and maltodextrin, lactic acid, thiamine, IMP, GMP, creatine, and salts such as potassium chloride and sodium chloride, to control the formation of flavor compounds such as 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.

10 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 15 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.

20 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 25 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 30 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 epigallocatechin gallate attached to a solid support such as a bead or resin. See, e.g., WO2013138793. In addition, depending on the protein component of the food product, certain combinations of antioxidants and/or LOX inhibitors can be used as effective agents to minimize off-flavor or off-odor generation especially in the presence of fats and oils. Such compounds can include, for example, one or more of P- carotene, a-tocopherol, caffeic acid, propyl gallate, or epigallocatechin gallate.

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

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

Food Products

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

In addition, food products described herein can be used to modulate the taste and/or aroma profile of other food products (e.g., meat replicas, meat substitutes, tofu, mock duck or other gluten based vegetable product, textured vegetable protein such as textured soy protein, pork, fish, lamb, or poultry products such as chicken or turkey 5 products) and can be applied to the other food product before or during cooking. Using the food products described herein can provide a particular meaty taste and smell, for example, the taste and smell of beef or bacon, to a non-meat product or to a poultry product.

Food products described herein can be packaged in various ways, including being 10 sealed within individual packets or shakers, such that the composition can be sprinkled or spread on top of a food product before or during cooking.

Food products described herein can include additional ingredients including food- grade oils such as canola, com, sunflower, soybean, olive or coconut oil, seasoning agents such as edible salts (e.g., sodium or potassium chloride) or herbs (e.g., rosemary, 15 thyme, basil, sage, or mint), flavoring agents, proteins (e.g., soy protein isolate, wheat gluten, pea vicilin, and/or pea legumin), protein concentrates (e.g., soy protein concentrate), emulsifiers (e.g., lecithin), gelling agents (e.g., k-carrageenan or gelatin), fibers (e.g., bamboo filer or inulin), or minerals (e.g., iodine, zinc, and/or calcium).

Food products described herein also can include a natural coloring agent such as 20 turmeric or beet juice, or an artificial coloring agent such as azo dyes, triphenylmethanes, xanthenes, quinines, indigoids, titanium dioxide, red #3, red #40, blue #1, or yellow #5.

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

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

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

Assessment of Food Products

Food products described herein can be assessed using trained human panelists.

The evaluations can involve eyeing, feeling, chewing, and tasting of the product to judge product appearance, color, integrity, texture, flavor, and mouth feel, etc. Panelists can be 5 served samples under red or under white light. Samples can be assigned random three- digit numbers and rotated in ballot position to prevent bias. Sensory judgments can be scaled for “acceptance” or “likeability” or use special terminology. For example, letter scales (A for excellent, B for good, C for poor) or number scales may be used (1 = dislike, 2 = fair, 3 = good; 4 = very good; 5=excellent). A scale can be used to rate the 10 overall acceptability or quality of the food product or specific quality attributes such beefiness, texture, and flavor. Panelists can be encouraged to rinse their mouths with water between samples, and given opportunity to comment on each sample.

In some embodiments, a food product described herein can be compared to another food product (e.g., meat or meat substitute) based upon olfactometer readings. In 15 various embodiments, the olfactometer can be used to assess odor concentration and odor thresholds, odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of appreciation, or relative intensity of odors.

In some embodiments, an olfactometer allows the training and automatic evaluation of expert panels. In some embodiments, a food product described herein 20 causes similar or identical olfactometer readings. In some embodiments, the differences between flavors generated using the methods of the invention and meat are sufficiently small to be below the detection threshold of human perception.

In some embodiments, volatile chemicals identified using GCMS can be evaluated. For example, a human can rate the experience of smelling the chemical 25 responsible for a certain peak. This information could be used to further refine the profile of flavor and aroma compounds produced using a heme-containing protein and one or more flavor precursors.

Characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions molecules including amino acids, fats and sugars 30 which are found in plants as well as meat. Therefore, in some embodiments, a food

product is tested for similarity to meat during or after cooking. In some embodiments human ratings, human evaluation, olfactometer readings, or GCMS measurements, or combinations thereof, are used to create an olfactory map of the food product. Similarly, an

olfactory map of the food product, for example, a meat replica, can be created. These 5 maps can be compared to assess how similar the cooked food product is to meat.

In some embodiments, the olfactory map of the food product during or after cooking is similar to or indistinguishable from that of cooked or cooking meat. In some embodiments the similarity is sufficient to be beyond the detection threshold of human perception. The food product can be created so its characteristics are similar to a food 10 product after cooking, but the uncooked food product may have properties that are different from the predicate food product prior to cooking.

These results will demonstrate that the compositions of the invention are judged as acceptably equivalent to real meat products. Additionally, these results can demonstrate that compositions of the invention are preferred by panelist over other 15 commercially available meat substitutes. So, in some embodiments the present invention provides for consumables that are significantly similar to traditional meats and are more meat like than previously known meat alternatives.

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

20

EXAMPLES

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

Replica burgers containing the ingredients in Table 4 and the flavor precursors 5 cysteine (10 mM), glutamic acid (10 mM), glucose (10 mM), and thiamine (1 mM) were prepared. Water was added to make up the balance. See, for example, U.S. Provisional Application No. 61/751,816, filed January 11, 2013. Control burgers were prepared as in Table 4 with precursors cysteine (10 mM), glutamic acid (10 mM), glucose (10 mM), and thiamine (1 mM) except LegH was omitted.

10 After cooking for 5 minutes at 150 C, the replica burgers were evaluated by a trained sensory panel. Panelists were served samples under red lights and each panelist individually evaluated the samples. Samples were assigned a random three-digit number and rotated in ballot position to prevent bias. Panelists were asked to evaluate cooked replica burger samples on multiple flavor, aroma, taste, texture and appearance attributes 15 including but not limited to: beefiness, bloody quality, savory quality, and overall acceptability using a 7-point scale from 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 20 burgers without LegH, the samples containing LegH were rated significantly beefier, bloodier, more savory, and overall preferred compared to those that did not include LegH. See Table 5.

TABLE 4

Replica Burger Ingredients	
Replica burger	"< precooked u w
Pea vicilin	3.86
Soy protein concentrate (SPC)	2.52
Bamboo fiber	0.34

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

TABLE 5

Sensory evaluation of replica burger with Heme

	<u>Beef</u>	<u>No</u>	<u>r.</u>
<u>Allribule</u>	<u>20 SO</u>	<u>1 leme</u>	<u>1 leme</u>
<u>lleel\iess</u>			
<u>mean</u>	<u>5.33</u>	<u>1.30</u>	<u>3.20</u>
<u>STDEV</u>	<u>1.58</u>	<u>0.67</u>	<u>0.79</u>
<u>Blood}</u>			
<u>mean</u>	<u>4.00</u>	<u>1.10</u>	<u>2.78</u>
<u>STDEV</u>	<u>1.32</u>	<u>0.32</u>	<u>1.64</u>
<u>Sa\or\</u>			
<u>mean</u>	<u>4.67</u>	<u>3.00</u>	<u>5.10</u>
<u>STDEV</u>	<u>1.22</u>	<u>1.63</u>	<u>0.57</u>

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

Replica burgers containing a flavor precursor mixture of glucose, cysteine, thiamine, and glutamic acid and 1% LegH pre-cooked w/w (see Table 4) were prepared as described in Example 1, and evaluated by a trained sensory panel after the burgers 10 were cooked for 5 minutes at 150 C. Control burgers included LegH and all other ingredients except for the flavor precursor mixture.

Panelists were asked to evaluate the samples overall improvement in taste and

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 5 burgers which included LegH and the flavor precursor mixture were described as having bouillon, gravy, meaty, bloody, savory, and beefy notes on taste, and were preferred to the same replica burger with LegH but no added flavor precursor mixture. See, Table 6

TABLE 6
Improvement of overall taste with precursors added to LegH burgers

	with precursors	without precursors
Average	3.5	1.8
STDV	0.6	0.5

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

Replica burgers (see Table 4) were cooked with different precursor mixes (see Table 7) and 1% LegH and evaluated by a trained sensory panel after the burgers were 15 cooked for 5 minutes at 150 C. Control burgers contained LegH and all of the other ingredients except for the flavor precursors. Panelists were asked to evaluate each sample and descriptively analyze of each sample. 5-point scale from 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 20 burger with a precursor mixture of 10 mM glucose, 10 mM ribose, 10 mM cysteine, 1 mM thiamine, 1 mM glutamic acid, 1 mM GMP, and LegH was described as having a bacon aroma and taste, and overall meatiness, savory quality, a very umami quality, a brothy quality, and slight beefy notes. See Table 7 for a summary of the flavor description for the various combinations of flavor precursors and heme-containing 25 protein.

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, breadcrumb
	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, grassy,
glucose (10 mM)	ribose (10 mM)	cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	IMP (2 mM)	bacon, very umami, savory, brothy, slight beef
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)		beef jerky, bloody, meaty, brothy
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	lactic acid (1 mM)	savory, beefy, bloody, meaty, savory, gravy

Figure 8

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

Figure 8

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

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

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

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

TABLE 8

Flavor compounds generated with cysteine, LegH, and either glucose or ribose in

the flavor reaction mixture.

LegH 1%		
Compounds evolved	cysteine (10 m\l). glucose (20 m\l)	cysteine (10 m\l). ribose (20 m\l)
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

Figure 8

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

<u>pyrrole</u>	<u>X</u>	<u>X</u>
<u>2-thiophenecarboxaldehyde</u>		<u>X</u>
<u>3 -thiophenecarboxaldehy de</u>		<u>X</u>

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

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

Flavor reaction mixtures at pH 6 containing LegH (1%), cysteine (10 mM), thiamine (1 mM), either glucose or ribose (20 mM), and with or without glutamic acid 10 (10 mM) were prepared and subsequently heated to 150 C for 3 minutes. These flavor

reaction samples then were evaluated by GCMS for the flavor compounds generated and evaluated by a trained panel for their sensory profiles. Volatile chemicals were isolated from the head space around the flavor reactions. GCMS showed 4-methyl-5- thiazoleethanol, 3,3'-dithiobis[2-methyl]-furan, and 4-methylthiazole compounds were 15 created by a mixture of LegH with thiamine, a sugar (either glucose or ribose), and cysteine. The same flavor reaction mixtures without thiamine did not generate these compounds; additionally these compounds were not generated when heme-proteins were not present in the flavor reaction mixtures.

The flavor reaction samples also were evaluated by a blinded trained sensory 20 panel, which described the samples with the addition of thiamine as more complex in taste and more beefy, meaty, and savory.

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

25 The addition of inosine monophosphate and guanosine monophosphate in mixes with heme protein and other precursors controlled the formation of flavor compounds (E)-4-octene, 2-ethyl-furan, 2-pentanone, 2,3-butanedione, 2-methyl-thiazole,, methyl- pyrazine, tridecane, (E)-2-octenal, 2-thiophenecarboxaldehyde, and 3- thiophenecarboxaldehyde. These compounds are known to be present in meat and have a beefy, meaty, buttery, and or

savory flavor notes.

Reactions containing heme protein at 1% (LegH) with cysteine (10 mM), and 5 glucose (20 mM), 1 mM IMP and 1 mM GMP, at pH 6.0 were prepared and heated to 150C for 3 minutes. Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors reacting heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the 10 flavor reaction and identified using GCMS, creating a profile of the volatile chemicals in the headspace around the flavor reaction mixture. GCMS showed 4-octene, 2-ethyl furan, 2-pentanone, 2,3-butanedione, 2-methyl-thiazole, methyl-pyrazine, tridecane, 2-octenal, 2-thiophenecarboxaldehyde, 3-thiophenecarboxaldehyde compounds were created by a mixture of hemeprotein LegH with IMP, GMP, glucose, and cysteine. The same samples 15 without IMP and GMP did not generate these compounds, additionally these compounds were also not created when heme-proteins were not present, just precursor molecules. Sensory evaluation by blinded trained panelist found the samples with the addition of inosine and guanosine as described as having more complexity in taste and more beefy, meaty, brothy and savory. Figure 2 shows the abundance of the novel flavor compounds 20 created with heme protein at 1% was mixed in a reaction at pH 6, with cysteine (10 mM), and glucose (20 mM), IMP (1 mM) and GMP (1 mM), and detected by solid phase microextraction (SPME) and then detected by GCMS.

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

25 The addition of lactic acid in mixes with heme protein, ribose, and cysteine controlled the formation of the flavor compounds nonanal, thiazole, 2-acetylthiazole, and 8-methyl-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.
30 Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors reacting heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction and identified using GCMS, creating a profile of the generated compounds. Nonanal,
5 thiazole, 2-acetylthiazole, and 8-methyl-1-undecene compounds were created by a

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.

15 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.

20 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.

25 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, 30 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.

5

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- 10 pentyl-furan, 2,3-Butanedione, Thiophene, 2-methyl-thiazole, Pyrazine, Furan, Pyrrole, 2-methyl-furan and distinct differences in the flavor compounds, including but not limited to 2-pentyl-thiophene, Nonanal, 2-Nonanone, and 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 15 reaction mixed with cysteine (10 mM) and ribose (10 mM) at pH 6. The pre- reaction mixture was heated to 150 C for 3 minutes; this reaction created flavor compounds known to be present in meat; see Table 9. The characteristic flavor and fragrance components are mostly produced during the cooking process where the flavor precursor molecules react with the heme-protein. Samples were evaluated by GCMS to identify the 20 flavor compounds generated after heating and also evaluated for their sensory profiles.

Volatile chemicals were isolated from the head space around the flavor reactions. Table 9 shows the similarity and differences in volatile flavor compounds created by the different types of heme-proteins.

TABLE 9

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

<u>Name</u>	<u>LegH</u>	<u>Barley</u>	<u><i>B. myoglobin</i></u>	<u><i>A. aeolicus</i></u>
<u>Furan</u>	X	X	X	X
<u>Thiazole</u>	X	X	X	X
<u>benzaldehyde</u>	X	X	X	X
<u>2-acetylthiazole</u>	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 -methy 1-butanal	X	X		X
2-methyl-thiazole	X	X	X	X
pentanal	X	X	X	X
1,3,5-cycloheptatriene	X	X	X	X
methacrolein	X	X	X	X
heptanal	X	X	X	X
2-methyl-butanal	X	X		X
isothiazole	X	X	X	X
thiophene	X	X	X	X
propanal	X	X	X	X
2-heptenal	X		X	X
methyl-pyrazine	X	X	X	X
1-octene	X		X	X
butanal	X	X	X	X
2-acetyl-propen-2-ol	X	X	X	X
pyrrole	X	X	X	X
2-methyl-furan	X	X	X	X
nonanal		X	X	X
2-propenal		X	X	X
2-decenal		X	X	X
2-nonanone		X		X
2-octanone		X	X	X
2-tridecen-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 (08:1), stearic acid (08:0), or myristic acid 5 (04:0)) and phospholipids (PL) (beef heart polar lipids extract, Biolipon95 (from Perimond), or NatCholinePC40 (from Perimond)) were tested for their ability to produce beefy flavor in the absence and in the presents of other precursors. Oils, FFAs, and PLs were added to 50 mM potassium phosphate buffer (PPB) pH 6.0 or a Maillard reaction mix (MRM) containing 50 mM potassium phosphate pH 6.0, 5 mM Cysteine, 10 mM 10 Glucose, 0.1 mM Thiamine, and 0.1% (w/v) LegHemoglobin. Lipids in combination with MRM were designed to capture the cross reactions of lipid degradation and Maillard reaction productions while lipids in phosphate buffer functioned as a lipid control. The oils were added at 3% of the total 1 mL volume of solution while FFAs and PLs were added at 1% of the total 1 mL volumes. All samples were cooked at 150°C for 3 mins, 15 cooled to 50°C and then analyzed using GCMS (SPME fiber sampling of headspace). After all samples were analyzed by GCMS the caps were removed and samples were smelled by a trained flavor scientist and aromas recorded.

Table 10

Legend showing components of each sample

<u>Sample Name</u>	<u>Solution</u>	<u>Additives</u>
<u>MRM None</u>	<u>Maillard Reaction Mix</u>	<u>None</u>
<u>MRM Linoelic Acid</u>	<u>Maillard Reaction Mix</u>	<u>1% linoleic acid</u>
<u>MRM Oleic Acid</u>	<u>Maillard Reaction Mix</u>	<u>1% oleic acid</u>
<u>MRMC 14</u>	<u>Maillard Reaction Mix</u>	<u>1% C14:0 free fatty acid</u>
<u>MRMC 18</u>	<u>Maillard Reaction Mix</u>	<u>1% C18:0 free fatty acid</u>
<u>MRM Canola</u>	<u>Maillard Reaction Mix</u>	<u>3% Canola Oil</u>
<u>MRM Coconut</u>	<u>Maillard Reaction Mix</u>	<u>3% Coconut Oil</u>
<u>MRM BeefHeart</u>	<u>Maillard Reaction Mix</u>	<u>1% BeefHeart Polar Lipids Extract</u>
<u>MRM Biolipon95</u>	<u>Maillard Reaction Mix</u>	<u>1% Biolipon95 (emulsifier)</u>

MRM_NatCholinePC40	Maillard Reaction Mix	1% NatCholinePC40 (emulsifier)
KPhosb Linoelic Acid	PPB, pH 6	1% linoelic acid
KPhosb Oleic Acid	PPB, pH 6	1% oleic acid
KPhos6_C14	PPB, pH 6	1% C14:0 free fatty acid
KPhos6_C18	PPB, pH 6	1% C18:0 free fatty acid
KPhosb Canola	PPB pH 6	3% Canola Oil
KPhosbCoconut	PPB, pH 6	3% Coconut Oil
KPhosb BeefHeart	PPB, pH 6	1% BeefHeart Polar Lipids Extract
KPhosb Biolipon95	PPB, pH 6	1% Biolipon95 (emulsifier)
KPhosb NatCholinePC40	PPB, pH 6	1% NatCholinePC40 (emulsifier)

[Table 11 contains the aroma descriptions and Table 12 contains the GCMS data from the most interesting samples analyzed. Many of the lipids introduced a “fatty” aroma to MRM that was otherwise absent. The combinations of Linoleic Acid or 5 NatCholinePC40 in MRM produced the greatest abundance of fatty compounds](#)

[suggesting that these lipids may improve the flavor perception of beef tallow. Linoleic Acid and NatCholinePC40 also showed high abundance of earthy-mushroom aromas.](#)

[The addition of lipids to MRM significantly increased the abundance of “nutty & roasted” aromas. Less desirable “green” aroma compounds were most prominent in 10 samples with unsaturated free fatty acids \(linoleic acid or oleic acid\) or phospholipids. In general, the addition of lipids significantly increased the number of target beef compounds made.](#)

[TABLE 11](#)

[15 Aroma descriptions of each sample after it was cooked.](#)

Sample Names	Aroma Descriptions
MRMjDnly	brothy, malty, beef stew
KPhos6 BeefHeart	fatty, creamy, beef tallow, slight sweet, slight roasted nutty
MRM BeefHeart	fatty, beef tallow, old meat, mushroom
KPhos6 Biolipon95	fatty, fresh
MRM Biolipon95	fatty, brothy, hay, malty green

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

Table 12

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

<u>Compounds in Beef</u>	<u>MRM only</u>	<u>MRM BeefHeart</u>	<u>MRM_NatCholinePC40</u>	<u>MRM Linoelic acid</u>
<u>(s)-isopropyl lactate</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>1-ethyl-5-methylcyclopentene</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
<u>1-heptanol</u>	<u>N</u>	<u>Y</u>	<u>N</u>	<u>N</u>
<u>1-hepten-3-ol</u>	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
<u>1-heptene</u>	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
<u>2-methyl-1-heptene</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>1-hexanol</u>	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
<u>2-ethyl-1-hexanol</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>1-nonanol</u>	<u>N</u>	<u>N</u>	<u>Y</u>	<u>N</u>
<u>1-nonene</u>	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>N</u>
<u>1-octanol</u>	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>N</u>
<u>1-octen-3-ol</u>	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>

1-octen-3-one		<u>Y</u>	<u>Y</u>	<u>Y</u>
1-octene	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
1-pentanol	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
1-penten-3-ol	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>N</u>
1-propanol	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
8-methyl-1 -undecene	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
1,3-hexadiene	<u>N</u>	<u>N</u>	<u>N</u>	<u>Y</u>
3-ethyl-2-methyl-1,3-hexadiene	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
1,3-octadiene	<u>III</u>	<u>N</u>	<u>N</u>	<u>Y</u>
1,3,5-cycloheptatriene	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
2,3-dihydro-5,6-dimethyl-1,4- dioxin	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
1,7-octadien-3-ol	<u>N</u>	<u>Y</u>	<u>N</u>	<u>N</u>
1h-pyrrole-2-carboxaldehyde	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
2-methyl-1 H-pyrrole	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
2-acetyl-2-thiazoline	<u>III</u>	<u>N</u>	<u>N</u>	<u>N</u>
2-acetylthiazole	<u>Y</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
2-butanone	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
2-butenal	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
2-ethyl-2-butenal	<u>N</u>	<u>N</u>	<u>N</u>	<u>Y</u>
3 -methyl-2-butenal	<u>N</u>	<u>N</u>	<u>Y</u>	<u>Y</u>
3 -methyl-2-cyclohexen-1 -one	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
2-decanone	<u>Y</u>	<u>Y</u>	<u>Y</u>	<u>N</u>
(E)-2-decenal	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
(Z)-2-decenal	<u>Y</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
2-furanmethanol	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
2-heptanone	<u>Y</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
6-methyl-2-heptanone	<u>N</u>	<u>N</u>	<u>Y</u>	<u>N</u>
(E)-2-heptenal	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
(Z)-2-heptenal	<u>N</u>	<u>N</u>	<u>N</u>	 <u>Y</u>
(E)-2-hexenal	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
2-ethyl-2-hexenal	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
2-methyl-2-heptene	<u>111</u> <u>111</u> <u>1</u>	<u>N</u>	<u>N</u>	<u>N</u>
2-n-heptylfuran	<u>III</u>	<u>N</u>	<u>N</u>	<u>N</u>
2-n-octylfuran	<u>Y</u>	<u>Y</u>	<u>Y</u>	<u>N</u>

2-nonanone	N	Y		N
(E)-2-nonenal	Y	Y	Y	Y
(Z)-2-nonenal	N	N	N	Y
2-octanone	Y	Y	Y	Y
(Z)-2-octen-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-ethyl-2(3H)-furanone	N	Y	Y	Y
5-methyl-2(3H)-furanone	N	N	N	N
dihydro-5-pentyl-2(3H)-furanone	N	N	Y	Y
dihydro-5-propyl-2(3H)-furanone	N	N	N	N
2(5H)-furanone	N	N	N	N
tetrahydro-6-methyl-2H-pyran-2-one	N	N	N	N
3-ethylcyclopentanone	N	Y	Y	Y
3-hexanone	N	N	N	N
3-methyl-2-thiophenecarboxaldehyde	N	N	N	N
3-octanone	Y	Y	N	Y
3-octen-2-one	N		Y	
3-thiophenecarboxaldehyde	N	Y	Y	Y

<u>(E,E)-3,5-octadien-2-one</u>	<u>N</u>	<u>N</u>	<u>Y</u>	<u>Y</u>
<u>dihydro-2-methyl-3 (2H)-furanone</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>4-cyanocyclohexene</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>4-cyclopentene-1,3-dione</u>	<u>N</u>	<u>N</u>		<u>N</u>
<u>4-decyne</u>	<u>N</u>		<u>N</u>	<u>N</u>
<u>(Z)-4-heptenal</u>	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
<u>4-methyloctanoic acid</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>(E)-4-octene</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one</u>	<u>Y</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>6-methyl-5-hepten-2-one</u>	<u>Y</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>acetaldehyde</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>Y</u>
<u>acetic acid</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>acetic acid ethenyl ester</u>	<u>Y</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>acetoin</u>	<u>Y</u>	<u>N</u>	<u>N</u>	<u>N</u>
	<u>1111</u>			
<u>acetone</u>	<u>11</u>	<u>N</u>	<u>N</u>	<u>Y</u>
<u>acetonitrile</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>Y</u>
<u>benzaldehyde</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
<u>4-ethyl-benzaldehyde</u>	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>N</u>
<u>benzene</u>	<u>1111</u>	<u>N</u>	<u>N</u>	<u>N</u>
	<u>1111</u>			
<u>benzoic acid, hydrazide</u>		<u>N</u>	<u>N</u>	<u>N</u>
<u>butanal</u>		<u>N</u>	<u>N</u>	<u>Y</u>
<u>2-methyl-butanal</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>3-methyl-butanal</u>		<u>N</u>	<u>N</u>	<u>N</u>
<u>butanoic acid</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>butyrolactone</u>	<u>Y</u>	<u>Y</u>	<u>N</u>	<u>Y</u>
<u>caprolactam</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>carbon disulfide</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>Y</u>
<u>1-ethyl-1-methyl-cyclopentane</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>	<u>B1111</u>
<u>propyl-cyclopropane</u>	<u>N</u>	<u>N</u>		<u>Y</u>
<u>decanal</u>	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>N</u>
<u>dihydro-3-(2H)-thiophenone</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>Dimethyl sulfide</u>	<u>Y</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>dimethyl sulfone</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>dimethyl trisulfide</u>	<u>Y</u>	<u>Y</u>	<u>N</u>	<u>N</u>
<u>ethanethiol</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>

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

<u>4-ethyl-phenol</u>	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>N</u>
<u>phenylacetaldehyde</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
<u>(p-hydroxyphenyl)- phosphonic acid</u>	<u>Y</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>propanal</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>Y</u>
<u>2-methyl-propanal</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>propanoic acid</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>2-methyl-propanoic acid</u>	<u>Y</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>propanoic acid, ethenyl ester</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>pyrazine</u>	<u>N</u>	<u>Y</u>	<u>N</u>	<u>Y</u>
<u>2-ethyl-5 -methyl-pyrazine</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>2-ethyl-6-methyl-pyrazine</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>2,3-dimethyl-pyrazine</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>2,5-dimethyl-pyrazine</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>3 -ethyl-2,5 -dimethy 1- pyrazine</u>	<u>Y</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>ethyl-pyrazine</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>methyl-pyrazine</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>trimethyl-pyrazine</u>	<u>Y</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>pyridine</u>	<u>Y</u>	<u>N</u>	<u>Y</u>	<u>N</u>
<u>pyrrole</u>	<u>IIII</u>	<u>Y</u>	<u>HH</u>	<u>Y</u>
<u>styrene</u>	<u>Y</u>	<u>N</u>	<u>Y</u>	<u>N</u>
<u>thiazole</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
<u>methyl-thiirane</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>thiophene</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>Y</u>
<u>2-hexyl-thiophene</u>	<u>Y</u>	<u>N</u>	<u>Y</u>	<u>N</u>
<u>2-pentyl-thiophene</u>	<u>N</u>	<u>Y</u>	<u>N</u>	<u>N</u>
<u>trans-2-(2-pentenyl)furan</u>	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>N</u>
<u>trans-3 -nonen-2-one</u>	<u>N</u>	<u>Y</u>	<u>Y</u>	<u>Y</u>
<u>undecanoic acid</u>	<u>N</u>	<u>N</u>	<u>N</u>	<u>N</u>
<u>Total # of Compounds Detected:</u>	<u>54</u>	<u>63</u>	<u>66</u>	<u>76</u>

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_01eic Acid, KPhos6_Linoleic acid and MRM Linoleic acid samples. For (E,E)-2,4-decadial, the strongest signal intensity was in the MRM_NatCholinePC40 sample, followed by the MRM Linoleic acid, KPhos6_Linoleic acid, MRM BeefHeart, MRM_BioLipon95, KPhos6_BeefHeart, 5 MRM Oleic Acid, and KPhos6_01eic Acid samples. For (E,E)-2,4-heptadienal, the strongest signal intensity was in the MRM_NatCholinePC40 sample followed by the MRM Canola sample. (E,E)-2,4-heptadienal also was detected in the MRM_BioLipon95, MRM BeefHeart, and MRM Linoleic acid samples. For (E,E)-2,4-nonadienal, the strongest signal intensity was in the MRM Canola and MRM Linoleic 10 acid samples. (E,E)-2,4-nonadienal also was detected in the Kphos6_Canola,

MRM_NatCholinePC40, MRM_BioLipon95, MRM BeefHeart, and KPhos6_Linoleic acid samples. For 2,4-decadial, the strongest signal intensity was in the MRM Linoleic acid sample. 2,4-decadial also was detected in KPhos6_Linoleic acid, MRM Canola, and KPhos6_01eic Acid samples.

15 In samples having earthy or mushroom aromas, 3-octen-2-one, 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_01eic Acid, MRM Oleic Acid, KPhos6_Linoleic acid, and MRM Linoleic acid samples. For 1-octen-3-ol, the strongest 20 signal intensity was in the MRM Linoleic acid sample, followed by

MRM_NatCholinePC40, KPhos6_Linoleic acid, MRM BeefHeart, KPhos6_BeefHeart, MRM Canola, MRM_BioLipon95, KPhos6_01eic Acid, and MRM Oleic Acid samples. 3-octanone was detected in the MRM Oleic Acid, KPhos6_Linoleic acid, and MRM Linoleic acid samples. For 1-octen-3-one, the strongest signal intensity was in the 25 MRM Linoleic acid and MRM BeefHeart samples, followed by KPhos6_Linoleic acid, MRM_NatCholinePC40, KPhos6_BeefHeart, MRM_BioLipon95, MRM Oleic Acid, and KPhos6_01eic Acid samples. For 3-octen-2-one, the strongest signal intensity was in the KPhos6_Linoleic acid sample, followed by MRM Linoleic acid, MRM_NatCholinePC40, KPhos6_BeefHeart, KPhos6_01eic Acid, MRM Oleic Acid,

30 MRM BeefHeart, MRM_BioLipon95, MRM Canola, Kphos_BioLipon95, and

Kphos_NatCholinePC40. Pyrazine was detected in the MRM Coconut, MRM C18, MRM C14, and MRM_BioLipon95 samples.

In samples having a nutty and roasted aroma, thiazole and 2-acetylthiazole were the most abundant compounds detected, along with pyrazine, methyl pyrazine, trimethyl 5 pyrazine, and 3-ethyl-2,5-dimethylpyrazine. 2-acetylthiazole was detected in all samples with MRM and most abundant in samples with MRM Beefheat, MRM_biolipon95,

MRM Canola, and MRM coconut. Thiazole was created in samples with MRMCoconut, MRM BeefHeat, MRM_Biolipon95, MRM C14, MRM C18, MRM Canola, MRM Oleic acid and MRM Linoleic acid and MRM_NatCholinePC40. Pyrazine was 10 present in the largest amount in samples with MRM-Coconut, followed by samples MRM BeefHeat, MRM_Biolipon95, MRM C14, MRM C18, MRM Canola having roughly equal amount, MRM Oleic acid and MRM Linoleic acid sample had even less. Methyl-pyrazine was present in MRM_Biolipon95 and MRM Coconut. 3-ethyl-2,5- dimethyl- pyrazine and trimethyl- pyrazine, were present only without phospholipids in 15 the MRM.

In samples having green, vegetable, or grass aromas, 1-heptanol, 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, 20 MRM C14, Kphos_C18, MRM C18, MRM Canola, MRM Coconut, KPhos6_01eic Acid, MRM Oleic Acid, KPhos6_Linoleic acid, and MRM Linoleic acid samples. For 2-pentyl-furan, the strongest signal intensity was in the KPhos6_BeefHeart sample, followed by the KPhos6_Linoleic acid, MRM_BioLipon95, MRM Linoleic acid, MRM BeefHeart, MRM Oleic Acid, MRM_NatCholinePC40, MRM Canola, 25 KPhos6_01eic Acid, and Kphos_NatCholinePC40 samples. For (E)-2-heptenal, the strongest signal intensity was in the MRM BeefHeart, MRM Canola, MRM Oleic Acid, and KPhos6_Linoleic acid samples, followed by the KPhos6_01eic Acid, MRM_BioLipon95, KPhos6_BeefHeart, MRM Linoleic acid, MRM_NatCholinePC40, Kphos_BioLipon95, and Kphos_NatCholinePC40 samples. For (Z)-2-heptenal, the 30 strongest signal intensity was in the MRM Linoleic acid sample. MRM Linoleic acid also was detected in the KPhos6_Linoleic acid sample. For heptanal, the strongest signal

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 5 signal intensity was in the MRM Linoleic acid sample, followed by the MRM_NatCholinePC40, KPhos6_Linoleic acid, and MRM Oleic Acid samples.

Example 11 - Creation of beefy flavors using complex precursor mixtures

A formulation was prepared (the “magic mix,” see Table 13 containing the 10 estimated concentrations of amino acids, sugars, and other small molecules in beef based on their values reported in literature. The magic mix was tested for its ability to produce beefy flavors in the presence of LegHemoglobin (LegH). The magic mix and 1% w/v LegH were added to the meat replica, pH 6.0 (see Table 4) and baked in a convection oven for 7 minutes at 160°C. A control sample was prepared by adding 1% w/v LegH to 15 the meat replica, pH 6.0 and baking in a convection oven for 7 minutes at 160°C.

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

To determine which chemical products were produced upon heating, a solution of Magic Mix was prepared with 1% w/v LegH at pH 6.0. The samples were cooked with 25 shaking at 150°C for three minutes, then Solid Phase Micro Extraction (SPME) was performed for twelve minutes at 50°C to extract the volatile compounds above the headspace of the reaction. A search algorithm was used to analyze the retention time and mass fingerprint information of the volatile compounds and assign chemical names to peaks. Table 14 shows the compounds identified in both the Magic Mix + LegH (MM, 30 average of two samples) and in the LegH alone in buffer (LegH, average of five samples) samples. The compounds in Table 14 are listed in order of the retention time (R.T., in

seconds), and are designated as having a zero peak area (0), or a small (S), medium (M), or large (L) average peak area. Hundreds of compounds were identified between the samples, many of which are characteristic of beefy aroma, including but not limited to 5 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

10 **Chemical entities added to the Magic Mix**

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

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

TABLE 14

Compounds identified with GC-MS analysis in samples with MM and LegH, or

LegH alone (average of five samples)

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

344.2	2,4-dimethyl-1 -heptene	M	0
346.3	methacrolein	M	0
357.4	methyl-thiirane	L	0
360.2	3-methyl-furan	S	0
363.7	butanone	L	S
368.9	2,3 -dihydro-5 -methyl-furan	M	S
376.4	2-methyl-butanal	L	M
381.1	3 -methy 1-butanal	L	M
390.6	isopropyl alcohol	0	S
399.6	ethanol	L	M
406.2	2-propenoic acid, methyl ester	M	0
408.2	benzene	S	0
414.4	methyl vinyl ketone	M	0
416.4	2,2,4,6,6-pentamethyl-heptane	M	0
422.6	2-ethyl-furan	S	0
438.4	2-ethylacrolein	M	0
449.9	2-pentanone	S	0
453.2	pentanal/2,3-butanedione	L	0
453.8	2,3-butanedione	L	M
472.8	4,7-dimethyl-undecane	M	S
485.9	2-methyl-pentanal	M	0
492.6	2-methyl-1 -penten-1 -one	S	0
496.6	(E)-3-penten-2-one	M	0
508.6	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

Figure 8

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

Figure 8

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

Figure 8

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

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

5 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 10 suspension to turn brown. To this was added 1g of FeCh.dFLO in 10 mL FLO. 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 5 fractionation.

Chlorophyll and Ferrous chlorin crude fractions were stored at -20°C. Crude extracts were fractionated by reverse-phase high-pressure liquid chromatography (RP-HPLC). HPLC conditions are outlined in Table 15. Both chlorophyll and ferrous chlorophyll were eluted from the column with a peak retention time of 7.6 minutes. 10 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).

15

TABLE 15

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

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

Preparation of Flavor Reaction Containing Ferrous Chlorin or Leghemoglobin

20

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 5 the cooking process when the flavor precursor molecules reacted with the heme-protein or the ferrous chlorophyll. Samples were evaluated by GCMS to identify the flavor compounds generated after heating. Volatile chemicals were isolated from the headspace around the flavor reactions. The profile of the volatile chemicals in the headspace around the flavor reaction mixtures that were similar between heme-protein and ferrous chlorin 10 are shown in Table 16. Notably, 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.

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

<u>3 -methyl-3 -buten-2-one</u>	<u>2-methyl-propanal</u>
<u>3 -thiophenecarboxaldehyde</u>	<u>pyrazine</u>
<u>(E)-4-octene,</u>	<u>2,3- dimethy 1-pyr azine</u>
<u>methyl-pyrazine</u>	<u>2,5-dimethyl-pyrazine</u>
<u>thiazole</u>	

Example 13 - Flavor creation by immobilized Hemin

Preparation of hemin linked CM Sepharose.

200 mg of bovine hemin (Sigma Aldrich) was loaded into a scintillation vial. A 5 small magnetic stir bar, 800 pL acetonitrile, 64 pL 4-methylmorpholine, and 71 mg of N-hydroxysuccinimide were added in that order. The vial was placed in an ice bath and chilled then 118 mg of N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride was added with stirring, followed by 845 pL of Jeffamine ED900. This was stirred while allowing the black mixture to warm to ambient temperature. Chloroform (10 mL) was 10 added to the mixture followed by water (4 mL). A flashlight was used to distinguish between organic and aqueous layers since both were black and the organic layer was pipetted off and concentrated to a dark black oil. The oil was dissolved in a 4:1 mixture of acetonitrile and ethanol to make an approximately 10% strength solution that was inky black in color.

15 2 mL of water swelled and equilibrated CM Sepharose was equilibrated in a BioRad minicolumn with 3 volumes of acetonitrile. The resin was resuspended in 1 mL acetonitrile and pipetted into a scintillation vial. This was followed with 44 microliters 4-methylmorpholine, 23 mg N-hydroxysuccinimide, and 39 mg of solid N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride. The mixture was vortexed 20 vigorously and then shaken for three hours. To this white solid was added 570

microliters of inky black 20% strength hemin coupled diamine. The black solid was vortexed and shaken for an hour. The slurry strongly resembled Turkish coffee. The mixture was poured into a BioRad minicolumn and filtered, washed with acetonitrile until what came out no longer resembled espresso, then switched to deionized water, and 25 finally 20 mM pH 9 sodium carbonate buffer. The black solid was washed until the effluent ran clear and then resuspended in 2 mL of buffer for storage until use.

Flavor reaction

The flavor reaction was created with heme protein (equine myoglobin -Sigma) at 0.35% in a phosphate buffer (20 mM) at pH 6.0 with 100 mM NaCl, this was mixed with 5 Magic Mix (Table 13). Another flavor reaction was created with Immobilized Hemin at 0.35% in a phosphate buffer (20 mM) at pH 6.0 with 100 mM NaCl, this was mixed with Magic Mix (Table 13). The flavor reaction mixtures were heated to 150°C for 3 minutes; this reaction created flavor compounds known to be present in meat.

The characteristic flavor and fragrance components were mostly produced during 10 the cooking process when the flavor precursor molecules reacted with the Heme-protein or the immobilized Hemin. Samples were evaluated by GCMS to identify the flavor compounds generated after heating. Volatile chemicals were isolated from the headspace around the flavor reactions. As can be seen in Table 17, immobilized hemin catalyzed production of compounds similar to those whose production was catalyzed by myoglobin 15 free in solution. Notably, the profiles of flavor compounds, measured by GCMS,

produced by cooking mixtures containing the immobilized hemin and the heme-protein, respectively, were very similar.

20

TABLE 17

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

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

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	large	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

12

Figure 8

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

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

Three samples were compared: precursor mix alone, 1% heme protein alone, and 5 precursor mix with 1% heme. The precursor mix was made of glucose (20 mM), ribose (20 mM), cysteine (10 mM), thiamine (1 mM), and glutamic acid (1 mM). Reactions were all at pH 6.0, prepared and heated to 150°C for 3 minutes. These three samples were run in duplicate. These samples were evaluated by GCMS for the flavor compounds generated. Characteristic flavor and fragrance components were mostly produced during 10 the cooking process where precursors could react with the heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction. The flavor compounds created in each sample is indicated in Table 18. As shown most of the flavor molecules were created on when the precursors are combined 15 with the heme protein.

Table 18
Flavor molecules created by the combination of LegH and precursor mix.

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

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

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction 5
with the detailed description thereof, the foregoing description is intended to illustrate and not limit
the scope of the invention, which is defined by the scope of the appended claims. Other aspects,
advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

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

SUMMARY OF THE INVENTION

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

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

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

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

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

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

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

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

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

[0016] In another embodiment, said 1-7 isolated and purified proteins are each isolated from different plant species. [0017] In some embodiments the meat substitute comprises less than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 individual proteins isolated from one or more plant species.

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

[0019] In another embodiment, said one or more isolated and purified proteins are selected from the group consisting of leghemoglobin, non-symbiotic hemoglobin, hemoglobin, myoglobin, chlorocruorin, erythrocrurorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2-globin, HbN, cyano-globin, HbO, G1b3, 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, prolamins, phaseolin (protein), proteinoplast, secalin, extensins, triticeae-gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

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

[0021] In another embodiment, said one or more isolated and purified proteins are isolated from a single plant source. [0022] In

another embodiment, said one or more isolated and purified proteins are isolated from multiple plant sources. [0023] In another

embodiment, wherein said one or more isolated, purified proteins are isolated from a genetically modified organism.

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

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

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

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

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

[0029] In some embodiments, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocrurorin, neuro-globin, cytoglobin, protoglobin, truncated 2/2-globin, HbN, cyanoglobin, HbO, G1b3, and Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

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

[0031] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 2. SEQ ID NO 2: MIDQKEKELI-KESWKRIEPN-KNEIGLLFYA-NLFKEEPTVS-VLFQN-PISSQ-SRKLMQVLGI

LVQGIDNLEG-LIPTLQDLGR-RHKQYGWDS-HYPLVGDCLL-KSIQEYLGQG-FTEEA-KAAWT-KVYGIAAQVM-TAE. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 80% homology to SEQ ID NO 2. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 90% homology to SEQ ID NO 2. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 98% homology to SEQ ID NO 2. [0032] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 3. SEQ ID NO 3: MRKQPTVFEEK-LGGQAAMHAA-VPLFYKKVLA-DDRVKHYFKN-TNMEHQAKQQ-EDFLTMLLGG-PNHYKGKNMA-EAH-KGMNLQN-SHEDAIIENL-AATLKELGVS-DQI-IGEAARKV-IEHTRKDCLG-K. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 80% homology to SEQ ID NO 3. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 90% homology to SEQ ID NO 3. In some embodiments said iron-containing protein comprises an amino acid sequence with at least 98% homology to SEQ ID NO 3. [0033] In some embodiments, the isolated and purified proteins are assembled into one or more gels. [0034] In some embodiments, the meat substitute composition further comprises one or more fats. [0035] In particular embodiments, said one or more fats are derived from a plant source. [0036] In another aspect, the invention provides a meat substitute product that comprises an indicator that indicates cooking progression from a raw state to a cooked state, wherein said meat substitute product is derived from nonanimal sources. [0037] In some embodiments, said indicator is a visual indicator that accurately mimics the color transition of a meat product during said cooking progression. [0038] In one embodiment, said color transition is from red to brown. [0039] In one embodiment, said color transition is from pink to white or tan. [0040] In one embodiment, said visual indicator transitions from a translucent to opaque color during said cooking progression. [0041] In some embodiments, the indicator is an olfactory indicator that indicates cooking progression. [0042] In one embodiment, said olfactory indicator is one or more volatile odorants released during cooking. [0043] In some embodiments, said indicator comprises one or more isolated, purified iron-containing proteins. [0044] In particular embodiments, said one or more isolated, purified iron-containing proteins is in a reduced state before cooking. [0045] In one embodiment, said one or more isolated and purified iron-containing proteins is selected from the group consisting of hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocrucorin, neuro-globin, cytoglobin, protoglobin, truncated 2/2-globin, HbN, cyanoglobin, HbO, G1b3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins. [0046] In a particular embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTKQDALVSSSFEAFKANIPQYSV-VFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAE-KLFALVRDSAGQLKASGTWADAALGS-VH-AQKAVTDPQF-VWKEALLKTIKAAV-GDKWSDE-LSRAWEVAYDELA AAIKKA. [0047] In some embodiments, said one or more isolated and purified iron-containing proteins are not isolated from an animal. In some embodiments compositions of the invention do not contain any proteins from an animal. [0048] In particular embodiments, said one or more isolated and purified iron-containing proteins are isolated from one or more plant sources. [0049] In yet more particular embodiments, said one or more isolated, purified proteins are isolated from the root nodules, roots, seeds, leaves, or stems of said one or more plant sources. [0050] In other particular embodiments, said one or more plant sources are soy or pea plants. [0051] In one embodiment, said one or more plant sources comprises one or more plants of the legume family. [0052] In some embodiments, said one or more isolated and purified iron carrying proteins in a reduced or oxidized state has a similar UV-VIS profile to a myoglobin protein derived from an animal source when in an equivalent reduced or oxidized state. [0053] In a particular embodiment, the difference between the peak absorbance wavelength of said one or more isolated and purified iron-containing proteins and the peak absorbance wavelength of myoglobin derived from an animal source is less than 5%. [0054] In some embodiments, said one or more isolated, purified proteins are isolated from a genetically modified organism. [0055] In one embodiment, said genetically modified organism is a genetically modified bacteria or yeast organism. [0056] In some embodiments, the meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum. [0057] In particular embodiments, the meat substitute product additionally contains less than 1% wheat gluten. [0058] In a more particular embodiment, said meat substitute product contains no wheat gluten. [0059] In other particular embodiments, said meat substitute product contains no soy protein isolate. [0060] In other particular embodiments, said meat substitute product contains no soy protein concentrate. [0061] In other particular embodiments, said meat substitute product contains no soy protein. [0062] In a more particular embodiment, said meat substitute product contains less than 5% carbohydrates. [0063] In other particular embodiments, said meat substitute product contains no tofu. [0064] In some embodiments, said meat substitute product contains no tofu, and no wheat gluten. [0065] In some embodiments, said meat substitute product contains no soy protein, and no wheat gluten. [0066] In some embodiments, said meat substitute product contains no animal products and less than 5% carbohydrates. [0067] In some embodiments, said meat substitute product contains less than 1% cellulose. [0068] In some embodiments, said meat substitute product contains less than 5% insoluble carbohydrates.

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

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

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

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

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

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

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

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

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

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

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

[0080] In some embodiments, said one or more isolated proteins are ~~selected from the group consisting of~~ hemoglobin, myoglobin, chlorocruorin, erythrocrucorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyano-globin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose biphosphate 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, triticale gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

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

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

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

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

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

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

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

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

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

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

[0091] In some embodiments, said one or more isolated and purified iron-containing proteins is ~~selected from the group consisting of~~ hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chlorocruorin, erythrocrucorin, neuro-globin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

[0092] In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1: MVA~~TEKQDALVSSSFEAFKA~~NIPQYSWFYTSILEKAPAAKDLFS-FLANGVDPTNP~~KL~~TGHAE KLFALVRDSAGQLKAS-GTWADAALGSVHAQKAVTDPQFVWKEALLKTIKA-AVGDKWSDELSRAWEVAYDELA~~AAIKKA~~. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 80% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 90% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 95% homology to SEQ ID NO 1. In a particular embodiment, said one or more isolated and purified iron-containing proteins comprises an amino acid sequence with at least 98%

homology to SEQ ID NO 1.

[0093] In particular embodiments, the muscle tissue replica comprises a protein content, wherein (i) one isolated and purified protein that is not an iron-containing protein accounts for 40-95% of said protein content, (ii) one or more isolated and purified iron-containing proteins accounts for 1-20% of said protein content, and (iii) one or more cross-linking reagents accounts for 0.1-35% of said protein content. [0094] In one embodiment, said protein content accounts for 5-50% of said replica by weight or by weight/volume. [0095] In one embodiment, said one isolated and purified protein is moong bean 8S protein.

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

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

[0098] In some embodiments, the muscle tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum. [0099] In particular embodiments, the muscle tissue replica additionally contains less than 1% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 5% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 10% wheat gluten. In particular embodiments, the muscle tissue replica additionally contains less than 0.1% wheat gluten. [0100] In a more particular embodiment, said muscle tissue replica contains no wheat gluten.

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

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

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

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

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

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

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

[0108] In some embodiments, said muscle tissue replica contains no animal products and less than 5% carbohydrates. [0109] In some embodiments, said muscle tissue replica contains less than 1% cellulose.

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

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

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

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

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

[0115] In some embodiments, the muscle tissue replica contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum. [0116] In particular embodiments, the meat replica contains less than 1% wheat gluten. In particular embodiments, the meat replica contains less than 5% wheat gluten. In particular embodiments, the meat replica contains less than 10% wheat gluten. In particular embodiments, the meat replica contains less than 0.1% wheat gluten.

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

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

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

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

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

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

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

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

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

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

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

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

[0129] In some embodiments, the meat replica contains no soy protein, and less than 5% insoluble carbohydrates. [0130] In some embodiments, the meat replica contains no wheat gluten, and less than 1% cellulose.

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

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

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

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

[0135] In some embodiments, said one or more plant oils is ~~selected from the group consisting of~~ com oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi, triglycerides, monoglycerides, diglycerides, sphingosides, glycolipids, lecithin, lysolecithin, phos-phatidic acids, lysophosphatidic acids, oleic

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

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

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

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

[0139] In some embodiments, said one or more isolated, purified proteins are derived from a non-animal source. [0140] In some embodiments, said non-animal source is a plant source.

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

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

[0143] In some embodiments, said one or more isolated proteins are ~~selected from the group consisting of~~ hemoglobin, myoglobin, chlorocruorin, erythrocrucorin, neuroglobin, cytoglobin, protoglobins, truncated 2/2 globin, HbN, cyano-globin, HbO, Glb3, and cytochromes, HELL's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose biphosphate 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, prolamins, phaseolin (protein), proteinoplast, secalin, extensins, triticale gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[0163] In some embodiments, said fat tissue replica contains no animal products and less than 5% carbohydrates. [0164] In some embodiments, said fat tissue replica contains less than 1% cellulose.

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

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

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

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

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

[0170] In another aspect, the invention provides a connective tissue replica, comprising a protein content comprising one or more isolated, purified proteins, wherein said protein content has been assembled into structures approximating the texture and visual appearance of connective tissue or skin. [0171] In some embodiments, said protein content is derived from non-animal source.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[0197] In some embodiments, 50% or more of said protein content by weight are one or more isolated purified proteins. [0198] In some embodiments, each of said one or more isolated proteins is isolated and purified separately from different plant species.

[0199] In some embodiments, one or more of said isolated proteins is selected from the group consisting of: hemoglobin, myoglobin, chlorocruorin, erythrocrurorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyano-globin, HbO, Glb3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose biphosphate 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 (mbisco), ribulose-1,5-bisphosphate carboxylase oxygenase activase (mbisco activase), albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glutenin, hordein, prolamins, phaseolin (protein), proteinoplast, secalin, extensins, triticaceae gluten, zein, any seed storage protein, oleosins, caloleosins, steroleosins or other oil body proteins, vegetative storage protein A, vegetative storage protein B, moong seed storage 8S globulin.

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

[0201] In some embodiments, said one or more isolated and purified iron-containing proteins is ~~selected from the group consisting of~~ hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chloroermin, erythroermin, neuro-globin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, G1b3, 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: MVAFTKQDALVSSFEAFKA-NIPQYSWFYTSILEKAPAAKDLFS-FLANGVDPTNPKLTGHAE KLFALVRDSAGQLKAS-GTWADAALGSVHAQKAVTDPQFVWKEALLKTIKA AVGDKWSDE LSRWEVAYDELA AAIKKA

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

[0203] In particular embodiments, the meat substitute product additionally contains less than 1% wheat gluten. [0204] In a more particular embodiment, said meat substitute product contains no wheat gluten.

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

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

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

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

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

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

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

[0212] In some embodiments, said meat substitute product contains no animal products and less than 5% carbohydrates. [0213] In some embodiments, said meat substitute product contains less than 1% cellulose.

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

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

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

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

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

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

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

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

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

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

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

[0225] In some embodiments, said iron-containing protein is ~~selected from the group consisting of~~ hemoglobin, myoglobin, leghemoglobin, non-symbiotic hemoglobin, chloroermin, erythroermin, neuroglobin, cytoglobin, protoglobin, truncated 2/2 globin, HbN, cyanoglobin, HbO, G1b3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins.

[0226] In one embodiment, said iron-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1. SEQ ID NO 1: MVAFTKQDALVSSFEAFKANIPQYSV-VFYTSILEKAPAAKDLFSFLANGVDPTNPKLTGHAE KLFALVRDSAGQLKASGTWADAALGS-VHAQKAVTDPQF-VWKEALLKTIKAAV-GDKWSDE LSRWEVAYDELA AAIKKA

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

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

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

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

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

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

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

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

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

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

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

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

[0239] In some embodiments, said food product contains less than 5% insoluble carbohydrates.
[0240] In some embodiments, said food product contains no soy protein, and less than 1% cellulose.
[0241] In some embodiments, said food product contains no soy protein, and less than 5% insoluble carbohydrates. [0242] In some embodiments, said food product contains no wheat gluten, and less than 1% cellulose.
[0243] In some embodiments, said food product contains no wheat gluten, and less than 5% insoluble carbohydrates. [0244] In another aspect, the invention provides a method of making a meat substitute composition, comprising isolating and purifying one or more proteins; and assembling said one or more proteins into a physical organization that approximates the physical organization of meat.
[0245] In another aspect, the invention provides a method of making a muscle tissue replica, comprising isolating and purifying one or more proteins; and assembling said one or more proteins into a physical organization that approximates the physical organization of skeletal muscle.
[0246] In another aspect, the invention provides a method of making a fat tissue replica, comprising isolating and purifying one or more proteins; preparing a solution comprising one or more proteins; emulsifying one or more fats in said solution; and stabilizing said solution into a gelled emulsification with one or more cross-linking reagents.
[0247] In another aspect, the invention provides a method of making a connective tissue replica, comprising isolating and purifying one or more proteins; and precipitating said one or more proteins, wherein said precipitating results in said one or more proteins forming physical structures approximating the physical organization of connective tissue.
[0248] In some embodiments, said precipitating comprises solubilizing said one or more proteins in a first solution; and extruding said first solution into a second solution, wherein said one or more proteins is insoluble in said second solution, wherein said extruding induces precipitation of said one or more proteins.
[0249] In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by an animal.
[0250] In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by humans.
[0251] In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by an animal. In another aspect, the invention provides a food product comprising one or more isolated, purified iron-containing proteins, wherein said food product is configured for consumption by humans.
[0252] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0253] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.
[0254] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which: [0255] FIG. 1 depicts a portion of the root of a pea plant (***Pisum sativum***) with the root nodules sliced open to demonstrate the red color conferred by leghemoglobin contained therein. The sliced open root nodule appears red.
[0256] FIG. 2 depicts leghemoglobin isolated from 1 oz of pea roots. The red color commonly attributed to meat is evident in the color photo.
[0257] FIG. 3 shows that leghemoglobins from different species are homologs and have similar color properties. In FIG. 3, panel A shows an SDS-PAGE gels of lysed root nodules of three legume plant species (1) Fava bean (2) English Pea (3) Soybean. Arrows mark respective leghemoglobins. Panel B shows the similarity of UV-VIS spectral profile of leghemoglobins from two different plant species (Favabean and Soybean).
[0258] FIG. 4 shows a comparison of reduced (heme iron 2+) and oxidized (heme iron 3+) soybean leghemoglobin (FIG. 4 panel A) and equine heart muscle myoglobin (FIG. 4 panel B) showing similarity of UV-VIS absorption profiles of two proteins. We purified soybean leghemoglobin from soybean root nodules using here described protocol. Purified equine myoglobin was purchased from SigmaAldrich. Soybean leghemoglobin (FIG. 4 panel A) and equine myoglobin (FIG. 4 panel B) were reduced with 1 mM sodium hydrosulfite. Shown are UV-VIS absorption spectra of heme Fe3+ (blue line—the higher peak in FIGS. 4 and 5) and heme Fe2+ (red line) of soybean leghemoglobin (FIG. 4 panel A) and equine myoglobin (FIG. 4 panel B). Insets show a zoom-in of UV-VIS spectra in 450 nm to 700 nm region. (FIG. 4 panel C) Images of 10 ul liquid droplet of a 40 mg/ml solution of soybean leghemoglobin in the heme-Fe3+ state (left droplet) showing characteristic rusty red color and a 40 mg/ml solution of soybean leghemoglobin solution in the heme-Fe2+ state (right droplet) showing characteristic red color of and (right image) corresponding samples of equine myoglobin. [0259] FIG. 5 depicts examples of successful reduction of leghemoglobin heme iron with sodium hydrosulfite and titanium citrate. In FIG. 5 the UV-VIS spectrogram of purified soybean leghemoglobin in which the heme iron is in the oxidized (+3) state is represented by the blue curves in each panel (the blue curves have the higher peaks on the main graphs). The red curves in

each panel represent the UV-VIS spectra of the same leghemoglobin species after reduction to the (+2) state (red lines) by addition of (Panel A) 1 mM sodium hydrosulfite or (Panel B) 0.24% (wt/v) titanium citrate in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride buffer. The Insets show a zoom-in of UV-VIS spectra in 450-700 nm region. For this example, leghemoglobin was purified from soybean root nodules using 60/90% ammonium sulfate fractionation and exchanged into 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride buffer. Sodium hydrosulfite stock solution was prepared by dissolving 100 mM sodium hydrosulfite in 1 mM sodium hydroxide in water. Titanium citrate stock solution was prepared from 20% (wt/v) Ti-chloride in hydrochloric acid by mixing it with 0.2M sodium citrate (1:10 v/v). pH was adjusted using sodium carbonate to pH 7.0.

[0260] FIG. 6 depicts an example of the leghemoglobin purification flow from soybean root nodules. The figure shows SDS-PAGE fractionation of different soybean leghemoglobin purification steps (Lane 1) Soybean root-nodule lysate; (Lane 2) Soybean root-nodule lysate purified by 60/90% (wt/v) ammonium sulfate fractionation. Shown is the protein content of 90% ammonium sulfate fractionated protein pellet resuspended in 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mM EDTA buffer; Proteins from 90% ammonium sulfate pellet were further purified by anion-exchange chromatography (FFQ-GE Healthcare) in 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride. Leghemoglobin collected in the flowthrough is shown in Lane 3. Anion-exchange flowthrough was fractionated using size-exclusion chromatography (Sephacryl S-100 GE Healthcare) and resulting leghemoglobin fraction is shown in Lane 4. Leghemoglobin content at different purification steps was determined by determining the fraction of leghemoglobin band on SDS-PAGE gel in a respective sample using ImageDoe analysis software (BioRad). Purity (partial abundance) of leghemoglobin at respective steps in the purification steps was: lysate: 32.7% (lane 1), 60/90% (wt/v) ammonium sulfate fractionation 78% (lane 2), anion-exchange chromatography 83% (lane 3), and size-exclusion chromatography to 95% (lane 4).

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

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

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

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

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

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

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

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

[0269] FIG. 15 depicts a ground beef prototype patty was made by combining 62% (wt/wt) muscle analog (62% (wt/wt) "dark muscle analog" and 38% (wt/wt) "white muscle analog"), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (FIG. 15 panel A). A ground beef prototype patty was made by combining 62% muscle analog

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

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

DETAILED DESCRIPTION OF THE INVENTION

[0271] Methods and compositions for the production of consumables are described herein. The consumables can be for animal consumption. For example the consumable can be food fit for human consumption. The consumable can be approved by suitable regulatory authorities. The consumables can be sold in grocery stores or prepared in restaurants, schools, hospitals, military facilities, prisons, shelters, long-term care facilities, similar to already existing human foods. The consumables could also be food for domestic animals. For instance, dog food could be produced according to the present inventions. The consumables may also be food for wild animals. For instance, the consumables could be provided to non-domesticated predatory animals.

[0272] The consumables of the present invention can compete with, supplement or replace animal based foods. For instance the consumables can be meat replicas made entirely from plant sources. The consumables can be made to mimic the cut or appearance of meat as it is currently sold. For instance a consumable may be visually similar to or indistinguishable from ground beef or a particular cut of beef. Alternatively, the consumables can be made with a unique look or appearance. For instance the consumable could contain patterns or lettering that is based upon the structure of the consumable. In some instances the consumables look like traditional meat products after they are prepared. For example a consumable may be produced which is larger than a traditional cut of beef but which, after the consumable is sliced and cooked appears the same as a traditional cooked meat. 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 thicker) than the traditional cut. So in some embodiments a composition that can be cut repeatedly into traditionally meat shaped products is provided.

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

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

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

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

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

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

[0279] The consumable may have animal welfare benefits compared to an animal product it replaces in the diet. For instance it may

be produced without requiring confinement, forced feeding, premature weaning, disruption of maternal-offspring interactions, or slaughter of animals for their meat. [0280] The consumable may have a smaller "carbon footprint" than the meat products they replace. For example the consumable may result in net greenhouse gas emissions of 1%, 5%, 10%, 25%, 50% or 75% of the greenhouse gas emissions attributable to the animal product it replaces. [0281] The consumable may provide alternatives to animal products or combinations of animal products whose consumption is forbidden by religious beliefs. For example, the consumable may be a kosher pork chop.

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

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

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

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

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

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

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

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

[0290] These results will demonstrate that the compositions of the invention are judged as acceptably equivalent to real meat products. Additionally these results can demonstrate that compositions of the invention are preferred by panelist over other—

commercially-available meat substitutes. So, in some embodiments the present invention provides for consumables that are significantly similar to traditional meats.

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

[0292] In some embodiments composition of the invention have a similar cook-loss characteristic as meat. In one embodiment a consumable of the invention with a similar fat and protein content as ground beef has the same reduction in size when cooked as real ground beef. Similar similarities in size loss profiles can be achieved for various compositions of consumables described herein matched to various meats. [0293] In some embodiments the consumable is compared to real meat based upon olfactometer readings. In various embodiments the olfactometer can be used to assess odor concentration and odor thresholds, odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of appreciation, or relative intensity of odors. In some embodiments the olfactometer allows the training and automatic evaluation of expert panels. So in some embodiments the consumable is a product that causes similar or identical olfactometer readings. In some embodiments the similarity is sufficient to be beyond the detection threshold of human perception.

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

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

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

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

benefits for the consumer.

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

Proteins and Protein Sources

[0299] In some embodiments, any of the meat substitute products, muscle tissue replica, fat replica, or connective tissue replica, comprise one or more isolated, purified proteins. In some embodiments, the meat substitute products are comprised of one or more of a muscle replica, a fat replica, and/or connective tissue replica which comprise one or more isolated, purified proteins. In other embodiments, the muscle replica, fat replica, and/or connective tissue replica comprises one or more isolated, purified proteins. In some embodiments, about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component is comprised of one or more isolated, purified proteins. For the purposes of this document, "purified protein" will refer to a preparation in which the cumulative abundance by mass of protein components other than the specified protein, which can be a single monomeric or multimeric protein species, is reduced by a factor of 2 or more, 3 or more, 5 or more, 10 or more, 20 or more, 50 or more, 100 or more or 1000 or more relative to the source material from which the specified protein was isolated. [0300] In some embodiments, the one or more isolated, purified proteins are derived from non-animal sources. Nonlimiting examples of non-animal sources include plants, fungi, 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.

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

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

[0303] In some embodiments, the one or more isolated proteins includes an abundant protein that is found in high levels in the seeds of plants. In some embodiments, the abundant protein comprises about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or 90% or more of the total protein content of the seeds of the source plant. In some embodiments, the abundant protein comprises about 0.5-10%, about 5-40%, about 10-60%,

about 20%–60%, or about 30–70% or >70% of the total protein content of the seeds of the source plant. Non-limiting examples of proteins found in high levels in the seeds of plants are seed storage proteins, e.g., albumins, glycinins, conglycinins, globulins, vicilins, conalbumin, gliadin, glutelin, gluten, glu-tenin, hordein, prolamin, phaseolin (protein), proteinoplast, secalin, triticeae gluten, zein, any seed storage protein, ole-osins, caloleosins, steroleosins or other oil body proteins [0304] In some embodiments, the one or more isolated proteins includes proteins that interact with lipids and help stabilize lipids in a structure. Without wishing to be bound by a particular theory, such proteins may improve the integration of lipids and/or fat replicas with other components of the meat substitute product, resulting in improved mouthfeel and texture of the final product. A non-limiting example of a lipid-interacting plant protein is the oleosin family of proteins. Oleosins are lipid-interacting proteins that are found in oil bodies of plants. Other non-limiting examples of plant proteins that can stabilize emulsions include seed storage proteins from Great Northern Beans, albumins from peas, globulins from peas, 8S globulins from Moong bean, 8S globulins from Kidney bean.

Muscle Replicas

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

[0306] Many meat products comprise a high proportion of striated skeletal muscle in which individual muscle fibers are organized mainly in an isotropic fashion. Accordingly, in some embodiments the muscle replica comprises fibers that are to some extent organized isotropically. In some embodiments the fibers comprise a protein component. In some embodiments, the fibers comprise about 1%, about 2%, about 5%, about 10%, about 15%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99% or more of a protein component. [0307] In some embodiments, the protein component comprises one or more isolated, purified proteins. For example the one or more isolated, purified protein can comprise the 8S globulin from Moong bean seeds, or the albumin or globulin fraction of pea seeds. These proteins provide examples of proteins with favorable properties for constructing meat replicas because of their ability to form gels with textures similar to animal muscle or fat tissue. Examples and embodiments of the one or more isolated, purified proteins are described herein. The list of potential candidates here is essentially open and may include Rubisco, any major seed storage proteins, proteins isolated from fungi, bacteria, archaea, viruses, or genetically engineered microorganisms, or synthesized in vitro. The proteins may be artificially designed to emulate physical properties of animal muscle tissue. The proteins may be artificially designed to emulate physical properties of animal muscle tissue. In some embodiments, one or more isolated, purified proteins accounts for about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more of the protein component by weight.

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

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

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

[0311] In some embodiments extrusion can be conducted using an MPF19 twin-screw extruder (APY Baker, Grand Rapids, Mich.) with a cooling die. The cooling die can cool the extrudate prior to return of the extrudate to atmospheric pressure, thus substantially inhibiting expansion or puffing of the final product. In the MPF19 apparatus, dry feed and liquid can be added separately and mixed in the barrel. Extrusion parameters can be, for example: screw speed of 200 rpm, product temperature at the die of 150 C., feedrate of

23 g/min, and water flow rate of 11 g/min. Product temperature can be measured during extrusion by a thermocouple at the end of the extrusion barrel. Observations can be made on color, opacity, structure, and texture for each collected sample. Collected samples can be optionally dried at room temperature overnight, then ground to a fine powder (<60 mesh) using a Braun food grinder. The pH of samples can be measured in duplicate using 10% (w/v) slurries of powdered sample in distilled water.

[0312] Fat Replica

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

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

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

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

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

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

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

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

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

[0322] In particular embodiments, the fat replica is an emulsion comprising a solution of one or more proteins and one or more fats suspended therein as droplets. In some embodiments, the emulsion is stabilized by one or more cross-linking enzymes into a gel. In more particular embodiments, the one or more proteins in solution are isolated, purified proteins. In yet more particular embodiments, the isolated, purified proteins comprise a purified pea albumin enriched fraction. In other more particular embodiments, the isolated, purified proteins comprise a purified pea globulin enriched fraction. In other more particular embodiments, the isolated, purified proteins comprise a purified Moong bean 8S globulin enriched fraction. In yet more particular embodiments, the isolated, purified proteins comprise a Rubisco enriched fraction. In other particular embodiments, the one or more fats are derived from plant-based oils. In more particular embodiments, the one or more fats are derived from one or more of: corn oil, olive oil, soy oil, peanut oil, walnut oil, almond oil, sesame oil, cottonseed oil, rapeseed oil, canola oil, safflower oil, sunflower oil, flax seed oil, algal oil, palm oil, palm kernel oil, coconut oil, babassu oil, shea butter, mango butter, cocoa butter, wheat germ oil, rice bran oil, oils produced by bacteria, algae, archaea or fungi or genetically engineered bacteria, algae, archaea or fungi; triglycerides, monoglycerides, diglycerides, sphingolipids, glycolipids, lecithin, lysophospholipids, phosphatidic acids, lysophosphatidic acids, oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, conjugated oleic acid, or esters of: oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or glycerol esters of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid, or triglyceride derivatives of oleic acid, palmitoleic acid, palmitic acid, myristic acid, lauric acid, myristoleic acid, caproic acid, capric acid, caprylic acid, pelargonic acid, undecanoic acid, linoleic acid, 20:1 eicosanoic acid, arachidonic acid, eicosapentanoic acid, docosohexanoic acid, 18:2 conjugated linoleic acid, or conjugated oleic acid. In yet even more particular embodiments, the one or more fats is a rice bran oil. In another particular embodiment, the one or more fats is a canola oil. In other particular embodiments, the cross-linking enzyme is transglutaminase, lysyl oxidase, or other amine oxidase. In yet even more particular embodiments, the cross-linking enzyme is transglutaminase. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of purified pea albumin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially purified moong bean 8S globulin emulsified with 40-80% rice bran oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially purified moong bean 8S globulin emulsified with 40-80% canola oil, stabilized with 0.5-5% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of purified pea albumin emulsified with 40-80% rice bran oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially purified moong bean 8S globulin emulsified with 40-80% rice bran oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel. In particular embodiments, the fat replica is a high fat emulsion comprising a protein solution of partially purified moong bean 8S globulin emulsified with 40-80% canola oil, stabilized with 0.0001-1% (wt/vol) transglutaminase into a gel.

[0323] Connective Tissue Replica

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

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

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

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

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

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

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

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

[0332] In some embodiments some or all of the components of the consumable are suspended in a gel. In various embodiments the gel can be a hydrogel, an organogel, or a xerogel. The gel can be made thick using an agent based on polysaccharides or proteins. For example fucella, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, algi-nin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, cellulose, methylcellulose, hydroxymethylcellulose, acacia gum, konjac, starch, pectin, amylopectin or proteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, or 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, lysyl oxidases, or other amine oxidases (e.g. *Pichia pastoris* lysyl

oxidase (PPO)) 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 strings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

[0333] The compositions of the invention can be puffed or expanded by heating, such as frying, baking, microwave heating, heating in a forced air system, heating in an air tunnel, and the like, according to methods well known in the art. [0334] In some embodiments multiple gels with different components are combined to form the consumable. For example a gel containing a plant-based protein can be associated with a gel containing a plant-based fat. In some embodiments fibers or strings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

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

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

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

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

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

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

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

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

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

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

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

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

[0347] In some embodiments the meat replica contains no animal products, and less than 5% insoluble carbohydrates. [0348] In some embodiments the meat replica contains no animal products, no soy protein, and less than 1% cellulose. [0349] In some embodiments the meat replica contains no animal products, no soy protein, and less than 5% insoluble carbohydrates.

[0350] In some embodiments the meat replica contains no animal products, no wheat gluten, and less than 1% cellulose. [0351] In some embodiments the meat replica contains no animal products, no wheat gluten, and less than 5% insoluble carbohydrates.

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

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

[0354] In some embodiments the consumable contains components to replicate the components of meat. The main component of meat is typically skeletal muscle. Skeletal muscle typically consists of roughly 75 percent water, 19 percent protein, 2.5 percent intramuscular fat, 1.2 percent carbohydrates and 2.3 percent other soluble non-protein substances. These include organic acids, sulfur compounds, nitrogenous compounds, such as amino acids and nucleotides, and inorganic substances such as minerals. Accordingly, some embodiments of the present invention provide for replicating approximations of this composition for the consumable. For example, in some embodiments the consumable is a plant-based meat replica can comprise roughly 75% water, 19% protein, 2.5% fat, 1.2% carbohydrates; and 2.3 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 60-90% water, 10-30% protein, 1-20% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. In some embodiments the consumable is a plant-based meat replica comprising between 60-90% water, 5-

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

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

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

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

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

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

[0360] Indicators of Cooking Meat

[0361] The release of odorants upon cooking is an important aspect of meat consumption. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking beef. In some embodiments, the consumable when cooked generates an aroma recognizable by humans as typical of cooking pork. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking bacon. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking chicken. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking lamb. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking fish. In some embodiments, the consumable is a meat replica entirely composed of non-animal products that when cooked generates an aroma recognizable by humans as typical of cooking turkey. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with an odorant that is released upon cooking. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing mixtures of proteins, peptides, amino acids, nucleotides, sugars and polysaccharides and fats in combinations and spatial arrangements that enable these compounds to undergo chemical reactions during cooking to produce odorants and flavor-producing compounds. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a volatile or labile odorant that is released upon cooking. In some embodiments the consumable is a method for preparing a meat replica where meat replicas principally or entirely composed of ingredients derived from non-animal sources are heated to release a volatile or labile odorant.

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

reactions involving one or more amino acids, fats, peptides, nucleotides, organic acids, sulfur compounds, sugars and other carbohydrates found in plants as well as meat.

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

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

[0365] Color Indicators

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

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

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

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

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

[0371] Leghemoglobin, similar in structure and physical properties to myoglobin, is readily available as an unused by-product of

commodity legume crops (eg., soybean, pea). The leghemoglobin in the roots of these crops in the US exceeds the myoglobin content of all the red meat consumed in the US. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, including a muscle tissue replica, an adipose tissue replica, a connective tissue replica, and leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a heme protein. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from nonanimal sources, containing a leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a member of the globin protein family. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from nonanimal sources, with a high iron content from a heme protein. In some embodiments the iron content is similar to meat. In some embodiments the consumable has the distinctive red color of meat, such color provided by leghemoglobin. [0372] Leghemoglobin is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked. [0373] A heme protein is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked. [0374] A heme protein from the group of: Hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin, non-symbiotic hemoglobin, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, is, in some embodiments, used as an indicator that the consumable is finished cooking. So, one embodiment of the invention is a method for cooking a consumable comprising detecting leghemoglobin which has migrated from the interior of the consumable to the surface when the product is cooked. Another embodiment of the invention is a method for cooking a consumable comprising detecting the change in color of from red to brown when the product is cooked.

Food Products Comprising Isolated, Purified Leghemoglobin

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

Sources of Leghemoglobin

[0376] In some embodiments the present invention provides methods for obtaining leghemoglobin from plants. Leghemoglobin can be obtained from a variety of plants. Various legumes species and their varieties, for example, Soybean, Fava bean, Lima bean, Cowpeas, English peas, Yellow peas, Lupine, Kidney bean, Garbanzo beans, Peanut, Alfalfa, Vetch hay, Clover, Lespedeza and Pinto bean, contain nitrogen-fixing root nodules in which leghemoglobin has a key role in controlling oxygen concentrations (for example root nodules from a pea plant, FIG. 1). FIG. 2 shows 100 μ mol of leghemoglobin solution isolated from 30 grams of pea root nodules. Leghemoglobins from different species are homologs and have similar color properties (FIG. 3). In FIG. 3, panel A shows an SDS-PAGE gels of lysed root nodules of three legume plant species (1) Fava bean (2) English Pea (3) Soybean. Arrows mark respective leghemoglobins. Note that leghemoglobin is the most abundant soluble protein in each lysate. Panel B shows the similarity of UV-VIS spectral profile of leghemoglobins from two different plant species (Fava bean and Soybean). We purified leghemoglobin from fava bean (green curve) and Soybean (red curve) root nodules using the protocol described elsewhere in the specification. UV-VIS spectra of both proteins shows that the heme iron is in the reduced (+2) state. Note that they are almost perfectly superimposed, consistent with their visually identical red color. The heme iron in the respective leghemoglobins was reduced to the +2 oxidation state by incubating Fava bean and Soybean leghemoglobin with 10 mM sodium hydrosulfite in 20 mM potassium-phosphate pH 7.4, 100 mM sodium chloride buffer. Sodium hydrosulfite was then removed from the leghemoglobin solution using gel exclusion chromatography. Inset shows a zoom-in of UV-VIS spectra in 450 nm to 700 nm region. Some plant species express several leghemoglobin isoforms (for example soybean has four leghemoglobin isoforms). Minor variations in precise amino acid sequence can modify overall charge of the protein at a particular pI and can modify precise structural conformation of iron containing heme group in leghemoglobin. Differences in structural conformation of heme group of different leghemoglobins can influence oxidation and reduction rates of the heme iron. These differences may contribute to color and flavor generation properties of different leghemoglobins. [0377] Leghemoglobin has a virtually identical absorbance spectrum and visual appearance to myoglobin from animal muscle. FIG. 4 shows a comparison of reduced (heme iron 2+) and oxidized (heme iron 3+) soybean leghemoglobin (FIG. 4 panel A) and equine heart muscle myoglobin (FIG. 4 panel B) showing similarity of UV-VIS absorption profiles of two proteins. We purified soybean leghemoglobin from soybean root nodules using here described protocol. Purified equine myoglobin was purchased from SigmaAldrich. Soybean leghemoglobin (FIG. 4 panel A) and equine myoglobin (FIG. 4 panel B) were reduced with 1 mM sodium hydrosulfite. Shown are UV-VIS absorption spectra of heme Fe3+ (blue line) and heme Fe2+ (red line) of soybean leghemoglobin (FIG. 4 panel A) and equine myoglobin (FIG. 4 panel B). Insets show a zoom-in of UV-VIS

spectra in 450 nm to 700 nm region. (FIG. 4 panel C) Images of 10 μ l liquid droplet of a 40 mg/ml solution of soybean leghemoglobin in the heme-Fe³⁺ state (left droplet) showing characteristic rusty red color and a 40 mg/ml solution of soybean leghemoglobin solution in the heme-Fe²⁺ state (right droplet) showing characteristic red color of and (right image) corresponding samples of equine myoglobin.

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

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

[0380] Leghemoglobin can be purified from legume root nodules, such as the root nodules of peas or soybeans (FIG. 1 shows Leghemoglobin isolated from pea root nodules). Root nodules from soy beans were thoroughly cleaned to remove soil and extraneous root tissues prior to root nodule lysis in 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mM EDTA and 1 mM ascorbic acid. Root nodules were lysed by grinding root nodules using a Vitamix blender. For some samples Polyvinylpyrrolidone polymer was added at 30% wt/v to aid in removal of plant phenolic small molecules that mediate oxidation of leghemoglobin heme iron. Root nodule lysate was fractionated using ammonium sulfate in two steps, first ammonium sulfate was added to 60% wt/v. Pellet was discarded and supernatant brought to 90% wt/v. ammonium sulfate. Leghemoglobin was collected as a precipitated pellet in 90% ammonium sulfate fraction. Ammonium sulfate precipitated leghemoglobin was resuspended in 20 mM potassium phosphate, 1 mM EDTA, 50 mM sodium chloride and ammonium sulfate was removed using dialysis or size-exclusion chromatography in the same buffer. In some instances this was the last purification step, while in other instances leghemoglobin was further purified using anion-exchange chromatography (FFQ GE Healthcare), which was sometimes followed by size-exclusion chromatography (Sephacryl S-100, GE Healthcare). Soybean leghemoglobin from 90% ammonium sulfate pellet was loaded on anion-exchange columns (FFQ or DEAE, GE Healthcare) in different buffers (20 mM potassium phosphate pH 7.4, containing 1 to 100 mM sodium chloride, 20 mM Tris pH 8 containing 0 to 100 mM sodium chloride, 20 mM sodium borax pH 9.8, 20 mM sodium chloride, 20 mM sodium carbonate pH 9, 20 mM sodium chloride) and purified either in flow-through or using sodium chloride (0-1M salt gradient). An example of the leghemoglobin purification flow from soybean root nodules is represented in FIG. 6. The figure shows SDS-PAGE fractionation of different soybean leghemoglobin purification steps (Lane 1) Soybean root nodule lysate; (Lane 2) Soybean root nodule lysate purified by 60/90% (wt/v) ammonium sulfate fractionation. Shown is the protein content of 90% ammonium sulfate fractionated protein pellet resuspended in 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride, 2 mM EDTA buffer; Proteins from 90% ammonium sulfate pellet were further purified by anion-exchange chromatography (FFQ GE Healthcare) in 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride. Leghemoglobin collected in the flowthrough is shown in Lane 3. Anion-exchange flowthrough was fractionated using size-exclusion chromatography (Sephacryl S-100 GE Healthcare) and resulting leghemoglobin fraction is shown in Lane 4. Leghemoglobin content at different purification steps was determined by determining the fraction of leghemoglobin band on SDS-PAGE gel in a respective sample using ImageDoc analysis software (BioRad). Purity (partial abundance) of leghemoglobin at respective steps in the purification steps was: lysate: 32.7% (lane 1), 60/90% (wt/v) ammonium sulfate fractionation 78% (lane 2), anion-exchange chromatography 83% (lane 3), and size-exclusion chromatography to 95% (lane 4).

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

weights (MW) of soybean leghemoglobin isoforms range from MW 15.4 to 15.8 kDa.

[0382] Leghemoglobin purified from soybean and fava root nodules, respectively was tasted by a panel of volunteers and in each case described as tasting like blood.

[0383] Leghemoglobin can be isolated from the roots nodules of legumes such as soy beans, fava beans, cow peas, lima beans, garbanzo beans, peas, lupine, lotus japonicum or other legumes. The root nodule (for example root nodules from a pea plant) is obtained and homogenized in an aqueous solution, soluble proteins including leghemoglobin are recovered after insoluble matter is removed by precipitation or filtration. Leghemoglobin can be purified by selective precipitation and/or chromatography and/or the use of molecules with specific affinity for leghemoglobin. (FIG. 1, showing 100 mis of solution of leghemoglobin isolated from 30 grams of pea root nodules).

[0384] Heme proteins, for example leghemoglobin, can be combined with other plant based meat replica components. In some embodiments the heme proteins are captured in a gel which contains other components, for example lipids and or proteins. In some aspects a multiple gels are combined with non-gel based heme proteins. In some embodiments the combination of the heme proteins and the other compounds of the consumable are done to insure that the heme proteins are able to diffuse through the consumable. In some embodiments the consumable is ed in a heme protein containing solution, for instance a leghemoglobin solution. In some embodiments the consumable is soaked in a heme protein containing solution, for instance a leghemoglobin solution for 1, 5, 10, 15, 20 or 30 hours. In some embodiments the consumable is soaked in a heme containing solution, for instance a leghemoglobin solution for 1, 5, 10, 15, 30, or 45 minutes.

[0385] FIG. 8 shows an example of 6 cubes of a commercial meat analog (Quorn chicken analog), about 1 cm on a side, 4 of which (Left and lower right) have been soaked in a solution of about 10 mg/ml soybean leghemoglobin in 20 mM Potassium phosphate pH 7.4 and 100 mM NaCl; the remaining two (Upper right) were soaked in the same buffer without leghemoglobin. Note the deep pink color of the leghemoglobin-infused cubes in contrast to the pale tan color of the uninfused cubes.

[0386] FIG. 9 shows the 4 leghemoglobin-infused cubes of Quorn chicken analog in the process of cooking in a pan at 350° C. The two lower cubes have been turned over to expose the grilled surface, which has turned brown. Note in the upper two cubes that the heated portion has turned grey-brown, while the cooler top surface retains its pink color. In some embodiments the consumable is injected with a heme containing solution, for instance a leghemoglobin solution, until the consumable is the color of uncooked meat.

[0387] Given the usefulness of heme proteins for coloring consumables it will be useful to detect whether a product contains a particular heme protein. Accordingly the present invention includes in some embodiments methods to determine whether a product contains a heme protein. Methods for detecting proteins are well known in the art. For example an ELISA or proximity ligation assay or luninex assay or western blot analysis can be performed to determine whether leghemoglobin is present in a food product such as meat or a meat replica. In one embodiment the detection methods are performed to determine whether meat has been altered with leghemoglobin.

EXAMPLES

[0388] An exemplary muscle replica composition comprising one or more isolated, purified plant proteins is described herein.

[0389] Protein Purification for Components of the Replica [0390] Moong bean seeds, Green Pea dry seed were purchased as milled flour and used for purification of respective seed storage proteins. Rubisco was purified from fresh alfalfa plant. Protein composition at individual fractionation steps was monitored by SDS-PAGE and protein concentrations were measured by standard UV-VIS and Pierce assay methods.

[0391] Moong bean 8S globulins: Moong bean flour was resuspended in in 50 mM potassium phosphate buffer pH 7 and 0.5M NaCl at 1:4 (wt/v) ratio, and mixture was incubated for 1 hr. Insoluble material was separated by centrifugation and proteins in the supernatant were fractionated by addition of ammonium sulfate in 2 steps: 50% (wt/v) followed by 90% (wt/v). Protein precipitated in 90% fraction contained the moong bean 8S globulins and was stored at -20 C until further use.

[0392] Pea albumins: Green pea dry seed flour was resuspended at 1:10 (wt/v) ratio in 50 mM sodium acetate buffer pH 5 and incubated for 1 hr. Insoluble material was separated by centrifugation and proteins in the supernatant were fractionated by ammonium sulfate precipitation in two steps: 50% (wt/v) followed by 90% (wt/v). Ammonium sulfate solutions were stirred for 1 hour and ammonium sulfate precipitated proteins removed by centrifugation. Proteins of interest precipitated in 90% (wt/v) ammonium sulfate. Pellet was stored at -20 C until further use.

[0393] Pea globulins: Green pea dry seed flour was resuspended at 1:10 (wt/v) ratio in 20 mM potassium phosphate buffer pH 8; 0.4M sodium chloride and stirred for 1 hr. After centrifugation, the supernatant was subjected to ammonium sulfate fractionation. First, supernatant was brought to 50% (wt/v) ammonium sulfate, and precipitated proteins removed. Second, 50% (wt/v) ammonium sulfate supernatant was brought to 80% (wt/v) ammonium sulfate saturation. The 80% (wt/v) ammonium sulfate pelleted protein contained globulins of interest. Pellet was stored at -20° C. until further use.

[0394] RuBisCO: RuBisCO was fractionated from alfalfa greens (or other green plants eg soybean plants, spinach etc) by first grinding leaves with 4 volumes of cold 50 mM KPhos-phate buffer pH 7.4 buffer (with (in lab) or without (in field) 0.5M NaCl+2 mM DTT+1 mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (erude lysate) was used in further purification steps. Proteins in the crude lysate were fractionated by addition of ammonium sulfate to 30% (wt/v) saturation. The solution was stirred for 1 hr and then centrifuged. The pellet from this step was discarded and additional ammonium sulfate was added to the supernatant to 50% (wt/v) ammonium sulfate saturation. The solution was centrifuged again after stirring for 1 hr. The pellet from this step contains RuBisCO, and was kept at -20 C until used.

[0395] Obtaining Plant Proteins.

[0396] Moong bean seed 8S protein was purified by ammonium sulfate fractionation as described. Pellet was resuspended in 20 mM potassium phosphate pH 7.4 and 0.5M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16000 g, 10 min and protein concentrated to desired concentration. Pea globulins purified by ammonium sulfate fractionation as described. Protein pellet was resuspended in 20 mM potassium phosphate pH 7.4 and 0.4M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16000 g, 10 min and protein concentrated to desired concentration. Pea albumin purified by ammonium sulfate fractionation as described. Protein pellet was resuspended in 20 mM potassium phosphate pH 7.4 and 0.1M sodium chloride and ammonium sulfate removed by dialysis against the same buffer. Any precipitate was removed by centrifugation at 16000 g, 10 min and protein concentrated to desired concentration.

[0397] Constructing a Muscle Tissue Analog [0398] Moong bean seed 8S protein was purified by ammonium sulfate fractionation as described above. For preparation of gels, 200 g of pellet was dissolved in 400 ml of dialysis buffer (20 mM potassium phosphate, 400 mM NaCl, pH 7.3) and the resulting solution dialyzed for 6 hours against 5l of dialysis buffer, replaced twice with fresh buffer. Protein solution was centrifuged at 12,000 g for 15 min to remove debris. Protein was concentrated by dialyzing for 3-6 hours against 5l of 30% w/w solution of PEG 8000 (polyethylene glycol, molecular weight 8000) in dialysis buffer. Final protein concentration was 150 mg/ml.

[0399] Leghemoglobin was purified from soybean root nodules. Legume root nodules were cleaned to remove soil and extraneous root tissues prior to root nodule lysis in 20 mM potassium phosphate pH 7.4, 100 mM sodium chloride, 1 mM EDTA and 1 mM ascorbic acid. Root nodules were lysed by grinding root nodules using juicer blender. Insoluble material was separated by centrifugation. Root nodule lysate was fractionated using ammonium sulfate in two steps, first ammonium sulfate was added to 60% wt/v and solution incubated for 1 hr, 4° C. Pellet was discarded and supernatant brought to 90% wt/v ammonium sulfate and incubated for 12 hr, 4° C. Leghemoglobin was collected as a precipitated pellet in 90% ammonium sulfate fraction and resuspended in 20 mM potassium phosphate, 1 mM EDTA, 100 mM sodium chloride. SDS-PAGE gel analysis determined that protein solution contains 70% leghemoglobin and 30% other root nodule proteins. Ammonium sulfate was removed using size-exclusion chromatography in the same buffer. Leghemoglobin was concentrated by dialyzing for 48 hr against 30% PEG 8000 (polyethylene glycol, molecular weight 8000) in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride. Total protein concentration was 57 mg/ml. UV-VIS spectra suggested that leghemoglobin was in heme-iron oxidized state. Thus, leghemoglobin was incubated with 5 mM sodium hydrosulfite for 5 min and sodium hydrosulfite was removed using size-exclusion chromatography in 20 mM potassium phosphate, 100 mM sodium chloride buffer. Leghemoglobin was further concentrated to 35.4 mg/ml. UV-VIS spectra analysis confirmed that leghemoglobin is in heme-iron reduced state.

[0400] Transglutaminase was obtained commercially from (Activa TI, Ajimoto). Stock solution (20% wt/v) was made in 20 mM potassium phosphate pH 7.3, 100 mM sodium chloride buffer.

[0401] To prepare "dark" muscle tissue analog (FIG. 10), 43 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 37 ml of leghemoglobin solution (46.5 mg/ml leghemoglobin and 20 mg/ml of other soybean root nodule protein) in 20 mM potassium phosphate, 100 mM NaCl, pH 7.3). 20 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly mixed, divided into two 50 ml Falcon tubes and incubated overnight at room temperature. Final protein concentrations were 65 mg/ml for moong bean protein, 18 mg/ml of leghemoglobin, 91 mg/ml total protein.

[0402] "White" muscle analog (FIG. 11) was prepared by mixing 43 ml moong bean protein solution (150 mg/ml) with 45 ml of 11.7 mg/ml solution of leghemoglobin and 0.8% (wt/v) of transglutaminase solution. Final protein concentrations were 63 mg/ml for moong bean protein, 5.2 mg/ml of leghemoglobin, 68 mg/ml total protein.

[0403] The "dark" muscle tissue analog formed an opaque gel of dark chocolate color, smooth uniform texture, with glistening surface, and a small amount (<1 ml) of dark red, venous blood colored liquid on top. The gel was freely standing, elastic but fragile, similar in appearance to thin Jell-O. The gel has a medium aroma with notes of beans and blood clearly discernible. The flavor is dominated by notes of beans and iron/blood, with weaker grassy and medicinal/chemical flavors. The taste is salty, with a long aftertaste of blood. [0404] The "white" muscle tissue analog was very similar, but with much lighter, cappuccino-like, color. It was also more fragile, 2-3 fold less strong against compression. [0405] Fat Tissue Analog

[0406] Fat tissue analog using moong bean 8S globulin fraction was prepared as follows: 15 ml of moong bean protein solution (150 mg/ml in dialysis buffer) were mixed with 15 ml of rice bran oil. 6 ml of transglutaminase solution (20% w/w) were added, solutions thoroughly emulsified using a homogenizer (VWR) at speed #2. Emulsion was aliquoted into 1.6 eppendorf tubes and incubated overnight at room temperature. After that, tubes were heated at 95° C. for 5 min in a heat block, and allowed to cool down to room temperature on a bench. Final concentrations were 75 mg/ml for moong bean protein, 50% w/w oil.

[0407] Fat tissue analog using pea globulin (100 mg/ml) was prepared by the same method. Additionally, fat tissue analog was prepared from pea globulin, and either rice bran or canola oil, in bulk by the same method, but without aliquoting emulsions into eppendorf tubes. Instead, emulsions in 50 ml Falcon tubes were rotated overnight on a nutator, and were subsequently incubated at 90° C. for 30 min.

[0408] Fat tissue analog based on moong beans (FIG. 12) and prepared in eppendorf tubes formed an opaque gel of off-white color, smooth uniform texture, with no visible discernible liquid that was not incorporated into the gel. The gel was freely standing, elastic and springy. The gel has a slight, pleasant aroma and a mild and pleasant flavor. The taste is mildly salty.

[0409] Fat tissue analog based on pea globulin (FIG. 13) and prepared in eppendorf tubes was very similar to moong bean-based fat

[0414] In a further test, the effect of cooking the ground beef replica patties was evaluated by grilling on a 350°F. pan. A ground beef patty analog was made by combining 62% (wt/wt) muscle tissue analog (62% (wt/wt) “dark muscle analog” and 38% (wt/wt) “muscle analog”), 29% (wt/wt) fat tissue analog (from pea globulin and canola oil), 5% (wt/wt) connective tissue analog (FIG. 16). The panel on the left shows the patty before cooking and the panel on the right shows the same patty after cooking for about 2 minutes. Observers described the aroma of the cooking ground beef replica as distinctly “beefy”.

- ~~a) a muscle replica;~~
- ~~b) a fat tissue replica; and~~
- ~~c) a connective tissue replica;~~

~~wherein said muscle replica, fat tissue replica, and/or connective tissue replica are assembled in a manner that approximates the physical organization of meat.~~

~~164. The meat substitute product of claim 163, comprising two or more of said muscle replica, fat tissue replica, and connective tissue replica.~~

~~165. The meat substitute product of claim 163, wherein (i) said muscle replica accounts for 40-90% of said product by weight, (ii) said fat tissue replica accounts for 1-60% of said product by weight, and (iii) said connective tissue replica accounts for 1-30% of said product by weight.~~

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

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

2. The food product of claim 1, wherein said highly conjugated heterocyclic ring complexed to an iron ion is selected from the group consisting of a heme moiety, and a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochlorophyll, 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. ~~166. The meat substitute~~ The food product of claim 1632, comprising: wherein said highly conjugated heterocyclic ring complexed to iron is ferrous chlorin.
5. A food product comprising:
- a) a heme-containing protein; and
 - b) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a hydrolyzed protein, a malt extract, a yeast extract, and a peptone.
6. The food product of any one of claims 1-5, wherein said food product comprises two to fifty flavor precursors.
7. The food product of any one of claims 1-5, wherein said food product comprises two to forty flavor precursors.
8. The food product of any one of claims 1-5, wherein said food product comprises two to thirty-five flavor precursors.
9. The food product of any one of claims 1-5, wherein said food product comprises two to ten flavor precursors.

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

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 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*.

- a) 60-90% water;
b) 5-30% protein content;
c) 1-20% of a fat or fat replica;
wherein said protein content comprises one or more isolated, purified plant proteins.
167. The meat substitute product of claim 166, wherein said protein content is derived from a non-animal source.
168. The meat substitute product of claim 167, wherein said non-animal source is a plant source, or a genetically modified yeast or bacteria.
169. (canceled)
170. The meat substitute product of claim 163, wherein 50% or more of said protein content by weight are one or more isolated purified proteins.
171. The meat substitute product of claim 170, wherein each of said one or more isolated proteins is isolated and purified separately from different plant species.
172. The meat substitute product of claim 170, wherein one or more of said isolated proteins is selected from the group consisting of: hemoglobin, myoglobin, chlorocruorin, erythrocrucorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2-globin, HbN, cyanoglobin, HbO, G1b3, and cytochromes, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, flavohemoglobins, ribosomal proteins, actin, hexokinase, lactate dehydrogenase, fructose biphosphate aldolase, phosphofructokinase, 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, triticale 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, erythrocrucorin, neuroglobin, cytoglobin, protoglobin, truncated 2/2-globin, HbN, cyanoglobin, HbO, G1b3, Hell's gate globin I, bacterial hemoglobins, ciliate myoglobins, and flavohemoglobins.

20. 175. The meat substitute composition of claim 174 wherein said iron-containing The food product of any one of claims 3-19, wherein the heme-containing protein comprises an amino acid sequence with at least 70% homology to SEQ ID NO 1, having at least 80% sequence identity to a polypeptide set forth in SEQ ID NOs: 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, com oil, palm fruit oil, palm seed oil, rice bran oil, safflower oil, olive oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and
- b) heating the mixture to form one or more flavor compounds selected from the group consisting of phenylacetaldehyde, 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-ethyl-dihydro-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 bacteriochlorophyll, 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, com oil, palm fruit oil, palm seed oil, rice bran oil, safflower oil, olive oil, canola oil, coconut oil, mango oil, a free fatty acid, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and
b) heating the mixture to form one or more flavor compounds set forth in Table 3, 8,9, 12, 14, 16, or 17.
35. A method for imparting a meat-like flavor to a food product comprising contacting said food product with a flavoring composition, said flavoring composition comprising i) a heme-containing protein; and ii) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6- phosphate, fructose

6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, cottonseed oil, soybean oil, com oil, palm fruit oil, palm seed oil, rice bran oil, safflower oil, olive oil, sunflower oil, canola oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a hydrolyzed protein, a malt extract, a yeast extract, and a peptone; wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product.

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

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

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

39. A method of making a food product, said method comprising combining an isolated heme-containing protein and one or more flavor precursor molecules to form a mixture, said one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, sunflower oil,

canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a hydrolyzed protein, a malt extract, a yeast extract, and a peptone; and heating the mixture.

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

a) incubating a heme-moiety bound to a solid support with one or more flavor precursor molecules, said one or more flavor precursor molecules selected from the group consisting of cysteine, ribose, glucose, fructose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, cottonseed oil, soybean oil, com oil, palm fruit oil, palm seed oil, rice bran oil, safflower oil, olive oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and b) heating the heme-moiety bound to said solid support and one or more flavor precursors to form one or more flavor compounds selected from the group consisting of phenylacetaldehyde, 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,
263 butyrolactone, 3-methyl-butanal, methyl-thiirane, 2-hexyl-furan, butanal,, 2-methyl-
264 butanal, trichloromethane, 2-methyl-furan, furan, octanal, 2-heptenal, 1-octene, heptyl
265 ester formic acid, 3-pentyl-furan, and 4-penten-2-one.

176. The meat substitute product of claim 163, wherein said meat substitute product contains no methylcellulose, no carrageenan, no caramel color, no konjac flour, no gum arabic, and no acacia gum.

177. The meat substitute product of claim 174, wherein said meat substitute product contains less than 1% wheat gluten.

178. The meat substitute product of claim 175, wherein said meat substitute product contains no wheat gluten.

179. The meat substitute product of claim 176, wherein said meat substitute product is characterized by one or more of the following: contains no soy protein isolate, no soy protein concentrate, or no tofu.

180. (canceled)

181. (canceled)

182. The meat substitute product of claim 179, wherein said meat substitute product contains less than 5% carbohydrates.

183. (canceled)

184. The meat substitute product of claim 163, wherein said meat substitute product is characterized by one or more of the following: contains no tofu, no soy protein, contains less than 1% cellulose, contains less than 5% insoluble carbohydrates, or no wheat gluten.

185. (canceled)

186. The meat substitute product of claim 163, wherein said meat substitute product contains no animal products and less than 5% carbohydrates.

187. (canceled)

188. (canceled)

189. (canceled)

190. (canceled)

191. (canceled)

192. The meat substitute product of claim 163, wherein said meat substitute product contains no wheat gluten, and less than 5% insoluble carbohydrates.

193-222. (canceled)