

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

~~CROSS-REFERENCE TO RELATED~~

~~APPLICATIONS~~

~~This application is a continuation of U.S. application Ser. No. 14/797,006 filed on Jul. 10, 2015 which is a continuation of and claims priority to PCT/US2014/011347 which claims priority to U.S. application Ser. No. 13/941,211, filed Jul. 12, 2013, U.S. Application Ser. No. 61/908,634, filed Nov. 25, 2013, and to U.S. Application Ser. No. 61/751,816, filed Jan. 11, 2013, and is related to the following patent applications: Application Serial No. PCT/US12/46560; Application Serial No. PCT/US12/46552; Application Ser. No. 61/876,676, filed Sep. 11, 2013; and Application Ser. No. 61/751,818, filed Jan. 11, 2013, all of which are incorporated herein by reference.~~

~~TECHNICAL FIELD~~

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

BACKGROUND OF THE INVENTION

(0001] Animal farming had 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. If the world's human population could shift from a meat containing diet to a vegetarian diet 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.

10002] 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 staggering.

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

[0004] Plant based meat substitutes have largely failed to cause a shift to a vegetarian diet. Usually meat substitutes involve extrusion of soy/grain mixture. Texture too homogenous. Sold pre-cooked - taste/aroma built in (not a product of cooking). These meat substitutes fail to replicate the experience of eating meat.

[0005] Thus, it is clear that there is a great need in the art for an improved method and system for reducing animal farming while avoiding the shortcomings and drawbacks of the previous meat substitutes.

SUMMARY OF THE INVENTION

INCORPORATION BY REFERENCE

[0006] 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.

~~Food is any substance that is either eaten or drunk by any animal, including humans, for nutrition or pleasure. It is usually of plant or animal origin,~~

and can contain essential nutrients, such as carbohydrates, fats, proteins, vitamins, or minerals. The substance is ingested by an organism and assimilated by the organism's cells in an effort to produce energy, maintain life, or stimulate growth.

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

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

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

SUMMARY

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

As provided herein, and without being bound by theory, certain characteristic meaty flavors and/or aromas (e.g., beefy, baeony, 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 include heterocyclic aromatic rings composed of one or more (2, 3, or 4 more) pyrrole, pyrrole-like, and/or pyrroline subunits. The highly conjugated heterocyclic ring complexed to an iron ion is referred to herein as an iron complex. In some embodiments, the heme moiety can be a heme cofactor such as a heme moiety bound to a protein; a heme moiety bound to a non-proteinaceous polymer; a heme moiety bound to a solid support; or a heme moiety encapsulated in a liposome. In some embodiments, the flavors and/or aromas are not generated in the absence of the iron complex (e.g., in the absence of a ferrous chlorin) or are not generated in the absence of a heme cofactor (e.g., in the absence of a heme-containing protein). Accordingly, as described herein, the iron complexes such as isolated chlorin-iron complexes or heme cofactors (e.g., heme-containing proteins) can be used to generate meaty flavors and/or aromas in a variety of food products, such as during the cooking process.

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

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

Food products for use in the present disclosure include those that have an iron complex (e.g., a heme cofactor such as a heme-containing protein), and one or more flavor precursor molecules included therein. The iron complex such as a heme cofactor (e.g., a heme-containing protein) and the one or more flavor precursor molecules can be homogeneously 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, meat loaf, bacon, strips, fingers, nuggets, cutlets, or cubes.

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

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

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

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

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

In one aspect, this document features a food product that includes an iron complex such as a heme moiety, or a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a 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, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, canola oil, olive oil, sunflower oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture to form one or more flavor compounds selected from the group consisting of phenylacetaldehyde, 1-octen-3-one, 2-n-heptylfuran, 2-thiophenecarboxaldehyde, 3-thiophenecarboxaldehyde, butyrolactone, 2-undecenal, pyrazine, methyl-, furfural, 2-decanone, pyrrole, 1-octen-3-ol, 2-acetylthiazole, (E)-2-octenal, decanal, benzaldehyde, (E)-2-nonenal, pyrazine,

1. hexanol, 1-heptanol, dimethyl trisulfide, 2-nonanone;
2. pentanone, 2-heptanone, 2,3-butanedione, heptanal, nonanal, 2-octanone, 1-octanol, 3-ethylcyclopentanone, 3-octen-2-one, (E,E)-2,4-heptadienal, (Z)-2-heptenal, 2-heptanone, 6-methyl-, (Z)-4-heptenal, (E,Z)-2,6-nonadienal, 3-methyl-2-butenal, 2-pentyl-furan, thiazole, (E,E)-2,4-decadienal, hexanoic acid, 1-ethyl-5-methyleclopentene, (E,E)-2,4-nonadienal, (Z)-2-decenal, dihydro-5-pentyl-2(3E)-furanone, trans-3-nonen-2-one, (E,E)-3,5-octadien-2-one, (Z)-2-octen-1-ol, 5-ethyldihydro-2(3E)-furanone, 2-butenal,
1. penten-3-ol, (E)-2-hexenal, formic acid, heptyl ester,
2. pentyl-thiophene, (Z)-2-nonenal, 2-hexyl-thiophene, (E)-2-decenal, 2-ethyl-5-methyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine, 2-ethyl-1-hexanol, thiophene, 2-methyl-furan, pyridine, butanal, 2-ethyl-furan, 3-methyl-butanal, trichloromethane, 2-methyl-butanal, methacrolein, 2-methyl-propanal, propanal, acetaldehyde, 2-propyl-furan, dihydro-5-propyl-2(3E)-furanone, 1,3-hexadiene, 4-decyne, pentanal,
1. propanol, heptanoic acid, trimethyl-ethanethiol, 1-butanol, 1-penten-3-one, dimethyl-sulfide, 2-ethyl-furan, 2-pentyl-thiophene, 2-propenal, 2-tridecen-1-ol, 4-octene,
2. methyl-thiazole, methyl-pyrazine, 2-butanone, 2-pentyl-furan, 2-methyl-propanal, butyrolactone, 3-methyl-butanal, methyl-thiirane, 2-hexyl-furan, butanal, 2-methyl-butanal, 2-methyl-furan, furan, octanal, 2-heptenal, 1-octene, formic acid heptyl ester, 3-pentyl-furan, and 4-penten-2-one. The heme moiety can be a heme-containing protein, a heme moiety bound to a non-peptidic polymer, or a heme moiety bound to a solid support. The method can include combining cysteine, ribose, lactic acid, lysine, and/or thiamine with the heme-containing protein.

In another aspect, this document features a method for producing a flavor compound. The method includes combining an iron complex, such as a heme-containing protein, and one or more flavor precursor molecules to form a mixture, the one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid,

succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, methionine, cysteine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture to form one or more flavor compounds set forth in Tables 3, 8, or 9. For example, the flavor precursors can include cysteine, a sugar, and one or more other precursors.

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

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

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this

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invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

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

BRIEF DESCRIPTION OF THE DRAWINGS

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

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

[0007] 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.

[0008] 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:

[0009] Figure 1 depicts a sliced open pea root nodule.

[0010] Figure 2 depicts leghemoglobin isolated from 1 oz of pea roots. The red color commonly attributed to meat is evident in the color photo.

DETAILED DESCRIPTION OF THE INVENTION

[0010] Methods and compositions for the production of consumables are described herein. The consumables can be for human or 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, 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.

[0011] The consumables of the present invention 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 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 the product appears the same as a traditional cooked meat.

[0012] 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.

[0013] 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.

[0014] 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.

[0015] The consumables require fewer resources to produce than animal farming. Accordingly, the present invention provides for meat replicates which require less water or energy to produce than meat. For example a consumable can require 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.

[0016] 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.

[0017] 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 replicates 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.

[0018] 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 a animal or human can distinguish the consumable from meat.

[0019] 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 “goopy” 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.

[0020] In some embodiments the consumable is compared to meat using fMRI. For example, the experience of eating meat is measured using fMRI. A map of regions of the brain associated with eating meat is created. The brain map can be refined by giving the person other types of foods and determining meat fMRI patterns. Additionally the person can be given pleasurable and distasteful foods to further refine the map. fMRI literature can be used to further refine the map. Once the experience of eating meat is defined statistically, meat replicas can be tested to determine how similar the meat replica is to meat. This process can be used to test various versions of the consumables to best approximate the experience of eating meat. In some embodiments the consumable is a meat replica that, when consumed, causes an fMRI pattern similar to the pattern found when the person consumes meat.

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

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

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

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TABLE 4

Flavor Precursor Molecules
Sugars, sugar alcohols, sugar acids, and sugar derivatives: glucose, fructose, ribose, sucrose, arabinose, glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, inositol, maltose, molasses, maltodextrin, glycogen, galactose, lactose, ribitol, gluconic acid and glucuronic acid, amylose, amylopectin, or xylose
Oils: coconut oil, mango oil, sunflower oil, cottonseed oil, safflower oil, rice bran oil, cocoa butter, palm-fruit oil, palm oil, soybean oil, canola oil, corn oil, sesame oil, walnut oil, flaxseed, jojoba oil, castor, grapeseed oil, peanut oil, olive oil, algal oil, oil from bacteria or fungi
Free fatty acids: caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, arachidic acid, arachidonic acid, behenic acid, or erucic acid
Amino acids and derivatives thereof: cysteine, cystine, a cysteine sulfoxide, alliein, 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, glutamic acid, a glutamic acid derivative, alanine, methionine, IMP, GMP, lactic acid, and mixtures thereof (e.g., glucose and cysteine; cysteine and ribose; cysteine, glucose or ribose, and thiamine; cysteine, glucose or ribose, IMP, and GMP; cysteine, glucose or ribose, and lactic acid). For example, the one or more flavor precursors can be alanine, arginine, asparagine, aspartate, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, glucose, ribose, maltodextrin, thiamine, IMP, GMP, lactic acid, and creatine.

As used herein, the term “heme containing protein” can be used interchangeably with “heme containing polypeptide” or “heme protein” or “heme polypeptide” and includes any polypeptide that can covalently or noncovalently bind a heme moiety. In some embodiments, the heme-containing polypeptide is a globin and can include a globin fold, which comprises a series of seven to nine alpha-helices. Globin-type proteins can be of any class (e.g., class I, class II, or class III), and in some embodiments, can transport or store oxygen. For example, a heme-containing protein can be a non-symbiotic type of hemoglobin or a leghemoglobin. A heme-containing polypeptide can be a monomer, i.e., a single polypeptide chain, or can be a dimer, a trimer, tetramer, and/or higher order oligomers. The life-time of the oxygenated Fe²⁺ state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption. The life-time of the unoxygenated Fe³⁺ state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption.

Non-limiting examples of heme containing polypeptides can include an androglobin, a cytoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a myoglobin, an erythrocyruorin, a beta-hemoglobin, an alpha-hemoglobin, a protoglobin, a cyanoglobin, a cytoglobin, a histoglobin, a neuroglobins, a chlorocruorin, a truncated hemoglobin (e.g., FlbN or FlbO), a truncated 2/2 globin, a hemoglobin 3 (e.g., Glb3), a cytochrome, or a

peroxidase.

Heme-containing proteins that can be used in the compositions and food products described herein can be from mammals (e.g., 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 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, *Vigna unguiculata* varieties (cow peas), *Vigna radiata* (mung beans), *Lupinus albus* (lupin), or *Medicago sativa* (alfalfa); *Brassica napus* (canola); *Triticum* spp. (wheat, including wheat berries, and spelt); *Gossypium hirsutum* (cotton); *Oryza sativa* (rice); *Zizania* spp. (wild rice); *Helianthus annuus* (sunflower); *Beta vulgaris* (sugarbeet); *Pennisetum glaucum* (pearl millet); *Chenopodium* sp. (quinoa); *Sesamum* sp. (sesame); *Linum usitatissimum* (flax); or *Hordeum vulgare* (barley). Heme-containing proteins can be isolated from fungi such as *Saccharomyces cerevisiae*, *Pichia pastoris*, *Magnaporthe oryzae*, *Fusarium graminearum*, *Aspergillus oryzae*, *Trichoderma reesei*, *Myceliophthora thermophila*, *Kluyveromyces fragilis*, or *Fusarium oxysporum*. Heme-containing proteins can be isolated from bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Synechocystis* sp., *Aquifex aeolicus*, *Methylobacterium* sp., 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 heme.protein.info/heme.php.

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

Any of the heme-containing proteins described herein that can be used for producing food products can have at least 70% (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%) sequence identity to the amino acid sequence of the corresponding wild-type heme-containing protein or fragments thereof that contain a heme-binding motif. For example, a heme-containing protein can have at least 70% sequence identity to an amino acid sequence set forth in FIG. 1, including a non-symbiotic hemoglobin such as that from *Vigna radiata* (SEQ ID NO:1), *Hordeum vulgare* (SEQ ID NO:5), *Zea mays* (SEQ ID NO:13), *Oryza sativa* subsp. *japonica* (rice) (SEQ ID NO:14), or *Arabidopsis thaliana* (SEQ ID NO:15), a FliII's gate globin I such as that from *Methylobacterium* sp. (SEQ ID NO:2), a flavohemoprotein such as that from *Aquifex aeolicus* (SEQ ID NO:3), a leghemoglobin such as that from *Glycine max* (SEQ ID NO:4), *Pisum sativum* (SEQ ID NO:16), or *Vigna unguiculata* (SEQ ID NO:17), a heme-dependent peroxidase such as from *Magnaporthe oryzae*, (SEQ ID NO:6) or *Fusarium oxysporum* (SEQ ID NO:7), a cytochrome c peroxidase from *Fusarium graminearum* (SEQ ID NO:8), a truncated hemoglobin from *Chlamydomonas 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 (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. B12seq performs a comparison between two amino acid sequences using the BLASTP 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); 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 e:\seq1.txt-j e:\seq2.txt-p blastp-o e:\output.txt. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology, then the designated output file will not present aligned sequences. Similar procedures can be following for nucleic acid sequences except that blastn is used.

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

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

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

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

In some embodiments, heme-containing proteins are isolated and purified from other components of the source material (e.g., other animal, plant, fungal, algal, or bacterial proteins). As used herein, the term "isolated and purified" indicates that the preparation of heme-containing protein is at least 60% pure, e.g., greater than 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% pure. Without being bound by theory, isolating and purifying proteins can allow the food products to be made with greater consistency and greater control over the properties of the food product as unwanted material is eliminated. Proteins can be separated on the basis of their molecular weight, for example, by size exclusion chromatography, ultrafiltration through membranes, or density centrifugation. In some embodiments, the proteins can be separated based on their surface charge, for example, by isoelectric

precipitation, anion-exchange chromatography, or cation-exchange chromatography. Proteins also can be separated on the basis of their solubility, for example, by ammonium sulfate precipitation, isoelectric precipitation, surfactants, detergents or solvent extraction. Proteins also can be separated by their affinity to another molecule, using, for example, hydrophobic interaction chromatography, reactive dyes, or hydroxyapatite. Affinity chromatography also can include using antibodies having specific binding affinity for the heme-containing protein, nickel NTA for His-tagged recombinant proteins, lectins to bind to sugar moieties on a glycoprotein, or other molecules which specifically binds the protein.

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

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

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

Modulating Flavor and/or Aroma Profiles

In some embodiments, the food product contains between 0.01% and 5% by weight of a heme protein. In some embodiments, the food product contains between 0.01% and 5% by weight of leghemoglobin. Some meat also contains myoglobin, a heme protein, which accounts for most of the red color and iron content of some meat. It is understood that these percentages can vary in meat and the food products can be produced to approximate the natural variation in meat.

In some embodiments, the food product comprises about 0.05%, about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, or more than about 2% of an iron-carrying protein (e.g., a heme-containing protein) by dry weight or total weight. In some cases, the iron-carrying protein has been isolated and purified from a source.

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

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

14

TABLE 2-continued

Flavor Types		
5	buttery metallic yeasty vegetable broth	umami raisin goaty

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

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

TABLE 3

Compounds Produced

1. butenal, 2-ethyl-acetonitrile

(E)-2-Hexenal

4-ethyl-phenol,

2-octanone-styrene

liran, 3-pentyl-formic acid, heptyl ester (E)-2-Heptenal 6-methyl-5-hepten-2-one n-caproic acid-vinyl ester

1-ethyl-2-hexenal

hepten-3-ol

1-ethyl-1-methyl-cyclopentane

~~2~~ ethyl-2-methyl-1,3-hexadiene
~~3~~ pentyl-thiophene (Z)-2-nonenal
~~4~~ n-octylfuran
~~5~~ hexyl-thiophene
~~6~~ cyclopentene-1,3-dione
~~7~~ nonanol (E)-2-decenal
~~8~~ ethyl-benzaldehyde 1,7-octadien-3-ol octanoic acid
~~9~~ ethyl-5-methyl-pyrazine
~~10~~ ethyl-2,5-dimethyl-pyrazine
~~11~~ cycloheptatriene 2-ethyl-1-hexanol
~~12~~ methyl octanoic acid-m-aminophenylacetylene-benzene
thiophene
2-methyl-furan
pyridine
furan
butanal
~~13~~ ethyl-fur-an-carbon-disulfide-Furan, 2-hexyl-2
~~14~~ methyl-butanal 2-methyl-butanal-methacrolein-octane-ethanol
2-methyl-propanal
acetone
propanal
methyl-thiirane
acetaldehyde
2-propenal
2-propyl-furan

1. — hexadiene
 2. — decyne-pentanal
 3. — propanol-heptanoic acid-ethanethiol
 4. — methyl-1-heptene (E)-4-octene-2-methyl-2-heptene-pentanoic acid-nonanoic acid
 5. — dimethyl-benzene

toluene-1-butanol
 1. — trimethyl-1-pentane-isopropyl-alcohol
 2. — pentamethyl-1-heptane-phenol
 3. — penten-3-one-dimethyl-sulfide-thiirane
 4. — (E)-2-octen-1-ol-2,4-dimethyl-1-heptene
 5. — bis(1,1-dimethylethyl)-benzene-heptane
 6. — 4,7-dimethyl-undecane
 7. — acetophenone
 8. — tridecane
 9. — thiophosphoramidate, s-methyl ester
 10. — methyl-thiazole
 11. — (1-methylethoxy)-propanenitrile;
 12. — bis(1,1-dimethylethyl)-phenol-3-ethyl-2,2-dimethyl-1-pentane
 13. — ethyl-pentane
 14. — trim-ethyl-pentane
 15. — trimethyl-octane
 16. — dimethyl-nonane-2-hexyl-fur-an
 17. — methyl-5-thiazoleethanol
 18. — penten-2-one-4-methylthiazole-2-methyl-3-pentanone
 19. — pentanedione (E)-2-tridecen-1-ol-2-thiophenemethan-amine (Z)-2-nonanal;
 methyl-thioacetate-methyl-ethanoate-isothiazole
 20. — dimethyl-hexane-4-methyl-heptane
 21. — dimethyl-heptane

TABLE 3-continued

Compounds Produced

dihydro-5-pentyl-2(3h)-furanone	dihydro-5-propyl-2(3H)-furanone	2,3,4-trimethyl-heptane
trans-3-nonene-2-one	dihydro-3-(2H)-2,2,6-trimethyl-decane	2-methyl-heptane
(E,E)-3,5-octadien-2-(Z)-2-octen-1-ol	3,3'-dithiobis[2-1-heptene	2-methyl-3-furanthiol
2-ethyl-dihydro-2(3h)-butenal	1,3-octadiene	4-amino-1,2,5-oxadiazole-3-1,2-benzisothiazol-3(2H)-one
1-penten-3-ol	1-nonene	2-acetyl-propen-2-ol;
1-(ethylthio)-2-buta-1,3-diene		1-decen-3-one

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing pro-15 tein) described herein is heated in the presence of ground chicken, to increase specific volatile flavor and odorant components typically elevated in beef. For example, propa-nal, butanal, 2-ethyl-furan, heptanal, octanal, trans-2-(2-pentenyl)furan, (Z)-2-heptenal, (E)-2-octenal, pyrrole, 2,4-20 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 pro-25 tein) described herein is heated in the presence of cysteine and glucose or other combinations of flavor precursors to provide a different profile of volatile odorants than when any subset of the three components are used individually. Volatile flavor components that are increased under these con-30 ditions include but are not limited to furan, acetone, thiazole, furfural, benzaldehyde, 2-pyridinecarboxaldehyde, 5-methyl-2-thiophenecarboxaldehyde, 3-methyl-2-thiophenecarboxaldehyde, 3-thiophenemethanol and decanol. See, e.g., Tables 8 and 9. Under these conditions, cysteine-35 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-40

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein is heated in the presence of cysteine and ribose to provide a different profile of volatile odorants. Heating in the presence of ribose created some additional 45 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 presence of 50 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 55 chlorin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the presence of a nucleotide such as inosine monophosphate and/or guanosine monophosphate to control the formation of flavor compounds such as (E)-4-octene, 2-ethyl-furan, 2-pentanone, 60 2,3-butanedione, 2-methyl-thiazole, methyl-pyrazine, tridecane, (E)-2-octenal, 2-thiophenecarboxaldehyde, and/or 3-thiophenecarboxaldehyde. These compounds are known to be present in meat and have a beefy, meaty, buttery, and or savory flavor notes. —65

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-penyl-thiophene, 2-nonanal-furfural, 1-octanol, 2-nonanal, thiazole, 2-acetylthiazole, phenylacetaldehyde, and/or 2-acetylthiazole. These compounds are known to be present in meat and some have a beefy, meaty, and or savory flavor.

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

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

In some embodiments, a particular type of heme-containing protein is chosen to control the formation of flavor compounds. See, for example, the results of Table 9, which shows that the addition of different types of heme proteins (LegH, Barley, B. myoglobin, or A. aeolicus) in flavor reaction mixtures containing one or more flavor precursor compounds results in many of the same key meat flavors, including but not limited to pentanone, 3-methylbutanal, 2-methylbutanal, 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-pentylthiophene. These differences in flavor compounds can change the overall taste profile.

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

In some embodiments, any undesirable flavors can be minimized by deodorizing with activated charcoal or by removing enzymes such as lipoxygenases (LOX), which can be present in trace amounts when using preparations of plant proteins, and which can convert unsaturated triacylglycerides (such as linoleic acid or linolenic acid) into smaller and more volatile molecules. LOX are naturally present in legumes such as peas, soybeans, and peanuts, as well as rice, potatoes, and olives. When legume flours are fractionated into separate protein fractions, LOX can act as undesirable “time-bombs” that can cause undesirable flavors on aging or storage. Compositions containing plant proteins (e.g., from ground plant seeds) can be subjected to purification to remove LOX using, for example, an affinity resin that binds to LOX and removes it from the protein sample. The affinity resin can be linoleic acid, linolenic acid, stearic acid, oleic acid, propyl gallate, or 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 (3-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, meat loaf, bacon, strips, fingers, nuggets, cutlets, or cubes.

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

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

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

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

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

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

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

Assessment of Food Products

Food products described herein can be assessed using trained human panelists. The evaluations can involve eyeing, feeling, chewing, and tasting of the product to judge product appearance, color, integrity, texture, flavor, and mouth feel, etc. Panelists can be served samples under red or under white light. Samples can be assigned random three-digit numbers and rotated in ballot position to prevent bias. Sensory judgments can be scaled for “acceptance” or “like-ability” or use special terminology. For example, letter scales (A for excellent, B for good, C for poor) or number scales may be used (1=dislike, 2=fair, 3=good, 4=very good, 5=excellent). A scale can be used to rate the overall acceptability or quality of the food product or specific quality attributes such as 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) [0021] 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, ~~an~~ the olfactometer allows the training and automatic evaluation of expert panels. ~~In~~ So in some embodiments, ~~a food~~ the consumable is a product described herein that causes similar ~~or identical~~ olfactometer readings.

~~In some embodiments, the differences between flavors generated using the methods of the invention and meat are sufficiently small to be below the detection threshold of human perception. In some embodiments, volatile chemicals identified using GCMS can be evaluated. For example, a human can rate the experience of smelling the chemical responsible for a certain peak. This information could be used to further refine the profile of flavor and aroma compounds produced using a heme-containing protein and one or more flavor precursors.~~

[0022] Gas chromatography-mass spectrometry (GCMS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to separate and identify different substances within a test sample. GCMS can, in some embodiments, be used to evaluate the properties of a consumable. For example volatile chemicals can be isolated from the head space around meat. These chemicals can be identified using GCMS. A profile of the volatile chemicals in the headspace around meat is thereby created. In some instances each peak of the GCMS can be further evaluated. For instance, a human could rate the experience of smelling the chemical responsible for a certain peak. This information could be used to further refine the profile. GCMS could then be used to evaluate the properties of the consumable. The GCMS could be used to refine the consumable. In some embodiments the consumable has a GCMS profile similar or identical to that of meat. [0023] Characteristic flavor and fragrance components are mostly produced during the cooking process by chemical reactions molecules including amino acids, fats and sugars which are found in plants as well as meat. Therefore, in some embodiments, a food product the consumable is tested for similarity to meat during or after cooking. In some embodiments human ratings, human evaluation fMRI, olfactometer readings, or GCMS measurements, or combinations thereof, are used to create an olfactory map of the food product cooked meat. Similarly, an olfactory map of the food product consumable, for example, instance a meat replica, can be created. These maps can be compared to assess how similar the cooked consumable it so meat. In some embodiments the olfactory map of the consumable during or after cooking is similar to or indistinguishable from cooked or cooking meat.

[0024] The consumable is, in some embodiments, a meat replica principally or entirely composed of ingredients derived from non-animal sources, including a muscle tissue replica, an adipose tissue replica and and/or a connective tissue replica, where these elements are combined in a manner that approximates the physical organization of meat. The meat can be white meat or dark meat. The meat can be from an animal, for example from farmed animals such as cattle, sheep, pigs, chickens, or turkey. Meat is typically from skeletal muscle of an animal but it is understood that meat can also come from other muscles or organs of the animal. For instance, in some embodiments the consumable is a meat replica that simulates a kidney. Meat can also come from fish or other seafood. For example, in some embodiments, the consumable is a meat replica that simulates crab, lobster, oysters, fish, or scallops.

[0025] 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 and and/or a connective tissue replica, where these elements are combined in a manner that approximates the physical organization of natural ground meat. Alternatively, the consumable can be a meat product which is supplemented with non-animal sources. For example, in some embodiments ground beef can be supplemented with ingredients derived from non-animal sources, for instance a muscle tissue replica, an adipose tissue replica, and and/or a connective tissue replica. This supplementing can result in a product with properties similar to ground beef but which costs less and has a smaller environmental impact. In another example, in some embodiments meat from

game animals such as venison or elk is supplemented with ingredients derived from non-animal sources to improve upon the properties of the meat. For example an adipose tissue replica can be added to improve the qualities of the cooked game. [0026] The physical organization of the consumable may be manipulated by controlling the localization, organization, assembly, or orientation of different components of the consumable. In some embodiments the consumable is designed so the components are associated with one another as in meat. In some embodiments the consumable is designed so that after cooking the components of the consumable are associated with one another as in cooked meat. In some embodiments a soft, elastic gel made from plant or microbial proteins and/or carbohydrates containing droplets of fat suspended in the proteinaceous gel is used to approximate the organization adipose tissue in meat. In some embodiments some or all of the components of the consumable are suspended in a gel. In various embodiments the gel can be a hydrogel, an organogel, or a xerogel. The gel can be made thick using an agent based on polysaccharides or proteins. For example fecula, arrowroot, cornstarch, katakuri starch, potato starch, sago, tapioca, alginin, guar gum, locust bean gum, xanthan gum, collagen, egg whites, furcellaran, gelatin, agar, carrageenan, or proteins derived from legumes, grains, nuts, other seeds, leaves, algae, bacteria, or fungi can be used alone or in combination to form 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 (PPLO)) can be used alone or in combination to form an architecture or structure for the consumable. In some embodiments multiple gels with different components are combined to form the consumable. For example a gel containing a plant-based protein can be associated with a gel containing a plant-based fat. In some embodiments fibers or stings of proteins are oriented parallel to one another and then held in place by the application of a gel containing plant based fats.

10027) The percentage of different components may also be controlled. For example plant-based substitutes for muscle, fat tissue, connective tissue, and blood components can be combined in different ratios and physical organizations to best approximate the look and feel of meat. The various can also components can be arranged to insure consistency between bites of the consumable. The components can be arranged to insure that no waste is generated from the consumable. For example, while a traditional cut of meat may have portions that are not typically eaten, a meat replicate can improve upon meat by not including these inedible portions. Such an improvement allows for all of the product made or shipped to be consumed, which cuts down on waste and shipping costs. Alternatively, a meat replica may include inedible portions to mimic the experience of meat consumption. Such portions can include bone, cartilage, connective tissue, or other materials commonly referred to as gristle, or materials included simulating these components. In some embodiments the consumable may contain simulated inedible portions of meat products which are designed to serve secondary functions. For example a simulated bone can be designed to disperse heat during cooking, making the cooking of the consumable faster or more uniform than meat. In other embodiments a simulated bone may also serve to keep the consumable at a constant temperature during shipping.

[0028] 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 nitrogenous compounds, such as amino acids, 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 comprises between 60-90% water, 10-30% protein, 1-20% fat, 0.1-5% carbohydrates; and 1-10 percent other soluble non-protein substances. Some meat also contains myoglobin which accounts for most of the red color and iron content of some meat. It is understood that these percentages can vary in meat and the meat replicas can be produced to approximate the natural variation in meat. Additionally, in some instances, the present invention provides for

improved meat replicas, which comprise these components in typically unnatural percentages. 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.

[0029] 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.

[0030] 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.

[0031] 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.

[0032] The protein in the consumable can come from a variety or combination of sources. Non-animal sources can provide some or all of the protein in the consumable. Non-animal sources can include vegetables, fruits, nuts, grains, algae, bacteria, or fungi. The protein can be isolated or concentrated from one or more of these sources. In some embodiments the consumable is a meat replica comprising protein only obtained from non-animal sources.

[0033] 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.

[0034] In some embodiments the consumable contains a protein or proteins that have all of the essential amino acids. In some embodiments the proteins added to the consumable are supplemented with amino acids.

[0035] Fat is important for the experience of eating cooked meat. Accordingly, in some embodiments the consumable has a fat component. In some embodiments the fat content of the consumable is 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, or 60% fat. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a replica of animal fat consisting of a soft, elastic gel made from plant or microbial proteins (and/or carbohydrates), containing droplets of fat suspended in the proteinaceous gel.

[0036] Fat used in some embodiments of the present invention can be from a variety of sources. For example fats used in the consumable may be derived from coconuts, palm oil, palm kernel oil, cocoa, sunflowers, corn, rapeseed, soy beans, peanuts, tree nuts, algae, olives, cotton, or flax. In some embodiment the fat is derived from pulp or seed oil. Yeast or molds may also be sources of fat for certain embodiments of the invention. For instance, in one embodiment the consumable is a meat replica comprising triglycerides derived from *Mortierella isabellina*.

[0037] In some embodiments plant oils are modified to resemble animal fats. The plant oils can be modified to taste and smell most similar to meat when 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. [0038] In some embodiments, a variety of plant lipids are combined to approximate the properties of animal fat. In some embodiments the lipids that are not used in making the consumable are used for other applications, for example applications in cosmetics, lubricants, waxes, polymers, surfactants, water repellents, or biofuels. Accordingly the present invention provides for a method for saving and recycling plant lipids which are byproducts of the production of the consumable. [0039] 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 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. 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. [0040] Odorants released during cooking are generated by reactions between fats and protein & 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 mostly produced during the cooking process by chemical reactions of amino acids, fats and sugars found in plants as well as meat.

[0041] The color of meat is an important part of the experience of eating meat. The main determinant of the nutritional definition of the color of meat is the concentration of 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 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.

[0042] Hemoglobin is 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 a method 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.

[0043] 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.

[0044] Examples of iron containing proteins include hemerythrin and leghemoglobin. 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 oxygen binding proteins (e.g. hemocyanin, hemerythrin, chlorocruorin, vanabins, erythrocrucorin, pinnaglobin, and leghemoglobin) are added to the consumable. In some embodiments the consumable comprises a hemoprotein (e.g. hemoglobin, myoglobin, neuroglobin, cytoglobin, leghemoglobin).

[10045] Leghemoglobin, similar in structure and physical properties to myoglobin, is readily available as an unused byproduct of commodity legume crops (eg., soybean, pea). The leghemoglobin in the roots of these crops in the US exceeds the myoglobin content of all the red meat consumed in the US. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, including a muscle tissue replica, an adipose tissue replica, a connective tissue replica, and leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a heme protein. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a leghemoglobin. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a member of the globin protein family. In some embodiments the consumable is a meat replica principally or entirely composed of ingredients derived from non-animal sources, with a high iron content from a heme protein. In some embodiments the iron content is similar to meat. In some embodiments the consumable has the distinctive red color of meat, such color provided by leghemoglobin.

[10046] 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. [0047] 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.

[0048] In some embodiments the present invention provides methods for obtaining leghemoglobin from plants. Leghemoglobin can be obtained from a variety of plants. For example leghemoglobin can be isolated from the roots nodules of legumes such as soy beans. The root nodule is obtained and homogenized in an aqueous solution, soluble protein including leghemoglobin are removed 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.

[0049] Heme proteins, for example leghemoglobin, can be combined with other plant based meat replica components. In some embodiments the heme proteins are captured in a gel which contains other components, for example lipids and or proteins. In some aspects a multiple gels are combined with non-gel based heme proteins. In some embodiments the combination of the heme proteins and the other compounds of the consumable are done to insure that the heme proteins are able to diffuse through the consumable. In some embodiments the consumable is soaked in a heme containing solution, for instance a leghemoglobin

enable these compounds to undergo chemical reactions during cooking to produce odorants and flavor-producing compounds. In some embodiments the

solution, until the consumable is saturated. 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. 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. 10050) A meat replica principally or entirely composed of ingredients derived from non-animal sources, containing a heme protein, a muscle tissue replica, an adipose tissue replica and and/or a connective tissue replica, wherein the heme protein is within the muscle tissue replica. [0051] The release of odorants upon cooking is an important aspect of meat consumption. 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

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. [0052] 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.

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~~created. These maps can be compared to assess how similar the cooked food product is to meat.~~

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

from the predicate food product prior to cooking.

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

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

EXAMPLES

Example 1: Addition of Elene-Protein Increases Beefy Qualities of Replica Burgers

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

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

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

TABLE 4

ReDlica Burser Insredients	
Replica burger	% precooked w/w
Pea vicilin	3.86
Soy protein concentrate (SPC)	2.52
Bamboo fiber	0.34
NaCl	0.54
Pea legumin	2

20

TABLE 4 continued

ReDlica Burner Insredients	
Replica burger	% precooked w/w
Soy Protein Isolate (SPI) (Solae, St. Louis, MO)	4.68
Wheat gluten	4.68
Cocunut oil	15
Soy lecithin	0.1
k-carrageenan	1
LegH	1

TABLE 5

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

Example 2: Replica Buigers 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% LegEl pre-cooked w/w (see Table 4) were prepared as described in Example 1, and evaluated by a trained sensory panel after the burgers were cooked for 5 minutes at 150 C. Control burgers included LegEl and all other ingredients except for the flavor precursor mixture.

Panelists were asked to evaluate the samples overall improvement in taste and descriptively analyze each sample using a 5-point scale from 1=dislike extremely, to 5=like extremely. Panelists were encouraged to rinse their mouths with water between samples, and to fill out a survey to record their evaluation of each sample. The replicate burgers which included LegEl 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 LegEl but no added flavor precursor mixture. See, Table 6

TABLE 6

Improvement of overall taste with precursors added to Lean burgers		
	with precursors	without precursors
Average	3.5	1.8
STDEV	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% LegEl and evaluated by a trained sensory panel after the burgers were cooked for 5 minutes at 150 C. Control burgers contained LegH and all of the other ingredients except for the flavor precursors. Panelists were asked to evaluate each sample and descriptively analyze of each sample. 5-point scale from 1=dislike extremely, to 5=like extremely. Panelists were encouraged to rinse their mouths with water between samples, and to fill out a survey to record their evaluation of each sample. A replica burger with a precursor mixture of 10 mM glucose, 10 mM ribose, 10 mM cysteine, 1 mM thiamine, 1 mM glutamic acid, 1 mM GMP, and LegH was described as having a bacon aroma and taste, and overall meatiness, savory quality, a very good quality, and slight beefy notes. See Table 7 for a summary of the

flavor description for the various combinations of flavor precursors and heme-containing protein:

to be present in meat; see Table 8. Similarly, a flavor reaction mixture made when LegH heme protein at 1% was mixed with cysteine (10 mM) and ribose (20 mM) at pH 6 and heated to 150 °C for 3 minutes created flavor compounds⁵ 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 after¹⁵ 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

TABLE 7

Flavors generated by addition of precursors to LegH (1%)			Precursor (concentration)		Flavor Description
	ribose	cysteine	(10 mM)	(10 mM)	some kind of cold-cut/sheed deli meat
	ribose	cysteine	(10 mM)	(10 mM)	IMV (2 mM) bread crust with beef drippings, umami
	ribose	cysteine	(10 mM)	(10 mM)	lactic acid (1 mM) bread, malty, browned, bread crust
	ribose	cysteine	(10 mM)	(10 mM)	lysine (5 mM) savory, beefy, little grassy, brothy,
	ribose	cysteine	(10 mM)	(10 mM)	alanine (5 mM) savory, weak beefy, brothy, little
	ribose	cysteine	(10 mM)	(10 mM)	I + G (2 mM) savory, weak beefy, brothy, sweet
	ribose	cysteine	(10 mM)	(10 mM)	methionine cooked potato
	ribose	cysteine	(10 mM)	(10 mM)	glutamic acid (5 mM) little meaty, pretzel, brothy, savory, chocolate
glucose	ribose	cysteine	(10 mM)	(10 mM)	thiamin (2 mM) slight beefy, browned, grassy,
glucose	ribose	cysteine	(10 mM)	(10 mM)	thiamin (2 mM) IMV (2 mM) bacon, very umami, savory, brothy,
glucose	ribose	cysteine	(10 mM)	(10 mM)	thiamin (2 mM) glutamic acid (5 mM) beef jerky, bloody, meaty, brothy
glucose	ribose	cysteine	(10 mM)	(10 mM)	thiamin (2 mM) glutamic acid (5 mM) lactic acid (1 mM) savory, beefy, bloody, meaty,
glucose	ribose	cysteine	(10 mM)	(10 mM)	thiamin (2 mM) glutamic acid (5 mM) lysine (5 mM) roast beef
glucose	ribose	cysteine	(10 mM)	(10 mM)	thiamin (2 mM) glutamic acid (5 mM) alanine (5 mM) boiled beef, sweet
glucose	ribose	cysteine	(10 mM)	(10 mM)	thiamin (2 mM) glutamic acid (5 mM) I + G (2 mM) beefy with a sulfury note
glucose	ribose	cysteine	(10 mM)	(10 mM)	thiamin (2 mM) glutamic acid (5 mM) I + G (2 mM) sweet, malty, umami, meaty
glucose	ribose	cysteine	(10 mM)	(10 mM)	thiamin (2 mM) glutamic acid (5 mM) I + G (2 mM) savory, roast beef, grassy
glucose	ribose	cysteine	(10 mM)	(10 mM)	thiamin (2 mM) glutamic acid (5 mM) umami, savory, meaty, sweaty, fermented

Example 4: Type of Sugar Modulates Flavor

Compounds Created in the Presence of

Hemeprotein

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

⁵⁵ 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 ⁶⁰ 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 ⁶⁵ 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% —

cysteine (10 mM), cysteine (10 mM), Compounds created—glucose (20 mM)
ribose (20 mM)

benzaldehyde	X	X
2-butanone	X	X
dimethyl trisulfide	X	X
2-pentyl-furan	X	X
2-methyl-propanal	X	X
thiazole	X	X
butyrolactone	X	X
2-acetylthi-azole	X	X
pentanal	X	X
5-methyl-butanol	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
butanol	X	X
2-methyl-butanol	X	X
2-ethyl-furan	X	X
2-octanone	X	X
propanal	X	X
trichloromethane	X	X
2-methyl-fur-an	X	X
furan	X	X
pyrazine	X	X
thiophene	X	X
1,3-dimethyl-benzene	X	X
octane	X	X
octanal	X	X
thiazole	X	X
2-pentanone	X	X
furfural	X	X
2-nonanone	X	X
(Z)-2-heptenal	X	X
(E)-2-heptenal	X	X
1-octene	X	X
formic acid, heptyl ester	X	X
2-pentyl-thiophene	X	X
1-octen-3-one	X	X
3-pentyl-furan	X	X
2-propenal	X	X
(E)-2-tridecen-1-ol	X	X
benzene	X	X
(E)-4-octene	X	X
1-penten-3-one	X	X
4-penten-2-one	X	X
2-methyl-thiazole	X	X
methyl-pyrazine	X	X
trans-2-(2,5-dimethyl-1,4-dioxane-6-carbonyl)-3-methyl-5-thiophenecarboxaldehyde	X	X
3-ethylcyclopentanone	X	X
pyrrole	X	X
2,5-dimethyl-1,4-dioxane-6-carbonyl-3-methyl-5-thiophenecarboxaldehyde	X	X
thiophenecarboxaldehyde	X	X

Example 5: Heme-Protein in the Presence of Thiamine Affects the Production of Certain Flavor Compounds

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

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

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

when heme-proteins were not present in the flavor reaction mixtures.

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

Example 6: Heme-Proteins with Nucleotides

Controls Particular Flavor Compound Production

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

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

Example 7: Flavor Generation with the Addition of a Particular Organic Acid

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

Reactions containing heme protein at 1%, cysteine (10 mM), and ribose (20 mM), and lactic acid (1 mM), pH 6.0, were prepared and heated to 150 C for 3 minutes. Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors reacting heme protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction and identified using GCMS, creating a profile of the generated compounds. Nonanal, thiazole, 2-acetylthiazole, and 8-methyl-1-undecene compounds were created by a mixture of LegH with lactic acid, ribose, and cysteine. The same samples without lactic acid did not generate these compounds, additionally these compounds were not created in the absence of heme proteins.

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

Example 8: Flavor Generated with the Addition of a Particular Amino Acid

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

Reactions containing heme protein at 1%, cysteine (10 mM), and ribose (20 mM), and lysine (1 mM), at pH 6.0, were prepared and heated to 150 C for 3 minutes. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors could react with the heme protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction. Dimethyl trisulfide, nonanal, 2-pentyl-thiophene, furfural, 2-nonanal, 1-octanol, 2-nonanal, thiazole, 2-acetylthiazole, phenyl acetaldehyde, 2-acetylthiazole compounds were created by a mixture of LegH with lactic acid, ribose, and cysteine. The same samples without lactic acid did not generate these compounds, additionally these compounds were not created when heme proteins were not present, just precursor molecules. Sensory evaluation by blinded trained panelist found the samples with the addition of lysine as described as roast beefy, savory, and browned. The addition of lysine increased the roasted browned notes.

Example 9—Flavor Compound Production by Different Heme-Proteins

The addition of different types of heme proteins (LegH, Barley, B. myoglobin, or A. aeolicus) in flavor reaction mixtures containing one or more flavor precursor compounds results in many of the same key meat flavors, including but not limited to 2-pentyl-furan, 2,3-Butanedione, Thiophene, 2-methyl-thiazole, Pyrazine, Furan, Pyrrole, 2-methyl-furan and distinct differences in the flavor compounds, including but not limited to 2-pentyl-thiophene, Nonanal, 2-Nonanone, and 1-Octen-3-one. These differences in flavor compounds can change the overall taste profile. The different types of heme protein were LegH,

Barley, B. myoglobin, or A. aeolicus used at 1% w/w in a reaction mixed with cysteine (10 mM) and ribose (10 mM) at pH 6. The pre-reaction mixture was heated to 150 C for 3 minutes; this reaction created flavor compounds known to be present in meat; see Table 9. The characteristic flavor and fragrance components are mostly produced during the cooking process where the flavor precursor molecules react with the heme protein. Samples were evaluated by GCMS to identify the flavor compounds generated after heating and also evaluated for their sensory profiles. Volatile chemicals were isolated from the head space around the flavor reactions. Table 9 shows the similarity and differences in volatile flavor compounds created by the different types of heme proteins.

TABLE 9

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

Name	Leg H	Barley aeolius	B. myoglobin A.	
Furan	X	X	X	X
Thiazole	X	X	X	X
benzaldehyde	X	X	X	X
2-acetylthiazole	X	X	X	X
2-methyl-propan	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	X	X	X	X
3-methyl-butanal	X	X		X
2-methyl-thiazole	X	X	X	X
pentanal	X	X	X	X
1,3,5-triazole	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-	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-			X	X
octanal			X	X
2-undecenal				X
butyrolactone				X
1-octen-3-one				X
3-methylheptyl				X
2-pentyl-				X
thiophene				

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 (04:0)) and phospholipids (PL) (beef heart polar lipids extract, Biolipon95 (from Perimond), or NatCholinePC40 (from Perimond)) were tested for their ability to produce

TABLE 10

Sample Name	Solution	Additives
MRM_None	Maillard Reaction	None
MRM_Linoleic Acid	Maillard Reaction	1% linoleic acid
MRM_Oleic Acid	Maillard Reaction	1% oleic acid
MRM_C14	Maillard Reaction	1% C14:0 free fatty acid
MRM_C18	Maillard Reaction	1% C18:0 free fatty acid
MRM_Canola	Maillard Reaction	3% Canola Oil
MRM_Coconut	Maillard Reaction	3% Coconut Oil
MRM_BeefHeart	Maillard Reaction	1% BeefHeart Polar Lipids Extract
MRM_Biolipon95	Maillard Reaction	1% Biolipon95 (emulsifier)
MRM_6_NatCholinePC40	Maillard Reaction	1% NatCholinePC40 (emulsifier)
KPhos6_Linoleic	PPB, pH 6	1% linoleic acid
KPhos6_Oleic Acid	PPB, pH 6	1% oleic acid
KPhos6_C14	PPB, pH 6	1% C14:0 free fatty
KPhos6_C18	PPB, pH 6	1% C18:0 free fatty
KPhos6_Canola	PPB, pH 6	3% Canola Oil
KPhos6_Coconut	PPB, pH 6	3% Coconut Oil
KPhos6_BeefHeart	PPB, pH 6	1% BeefHeart Polar Lipids Extract
KPhos6_Biolipon95	PPB, pH 6	1% Biolipon95 (emulsifier)
KPhos6_NatCholinePC40	PPB, pH 6	1% NatCholinePC40 (emulsifier)

1-hexanol	N	Y	Y	Y
2-ethyl-1-hexanol	N	N	N	N
1-nonanol	N	N	Y	N
1-nonene	N	Y	Y	N
1-octanol	N	Y	Y	N
1-octen-3-ol	N	Y	Y	Y
1-octen-3-one	Y	Y	Y	Y
1-octene	N	N	N	N
1-pentanol	N	Y	Y	Y
1-penten-3-ol	N	Y	Y	N
1-propanol	N	N	N	N
8-methyl-1-octene	N	Y	Y	Y
1,3-hexadiene	N	N	N	Y
3-ethyl-2-methyl-1,3-hexadiene	N	Y	Y	Y
1,3-octadiene	Y	N	N	Y
1,5-octadiene	N	N	N	N
1,5-dimethyl-1,7-octadien-3-ol	N	N	N	N
1,7-octadien-3-ol	N	Y	N	N
lh-pyrrole-2-carboxaldehyde	N	N	N	N
2-methyl-1H-2-acetyl-2-acetylthiazole	Y	N	N	N
2-butanone	N	Y	Y	Y
2-butenal	N	Y	Y	Y
2-ethyl-2-butenal	N	N	N	Y
3-methyl-2-butenal	N	N	Y	Y
3-methyl-2-cyclohexen-1-one	N	N	N	N
2-decanone	Y	Y	Y	N
(E)-2-decenal	N	N	N	N

TABLE 11
Aroma descriptions of each sample after it was cooked.

Sample Names	Aroma Descriptions
MRM - Only K-Phos	brothy, malty, beef stew fatty, creamy, beef tallow,
MRM - BeefHeart	sweet, slight roasted nutty fatty, beef tallow, old meat,
K-Phos6 - Biolipon9	mushroom
MRM - Biolipon95	fatty, fresh
K-Phos	fatty, brothy, hay, malty
MRM - NatCholine	light fatty, fresh
K-Phos6 - C14	fatty, beef tallow, brothy
MRM - C14	light/faint plastic/waxy
K-Phos6 - C18	brothy, beefy, minty, fresh
MRM - C18	light/faint plastic/waxy
K-Phos6 - Canola	beefy with cucumber &/or pepper aroma
MRM - Canola	fresh, cucumber
K-Phos6 - Coconut	fatty, brothy, oil, roasted nuts
MRM - Coconut	nothing
K-Phos6 - Oieie	brothy, beefy, slight fatty,
MRM - Oieie Acid	fresh, cucumber,
K-Phos6 - Linoelic	minty-like
MRM - Linoelic	herbal, plastic, slight cheesy,
MRM - Linoelic Acid	light plastic
	fatty, light waxy, brothy,
	herbal

TABLE 12
List of aromatic compounds found in Beef by GCMS and a chart shows; which detected in each lipid plus MRM

Compounds in Beef	MRM - Only	MRM - BeefHeart	MRM - NatCholine	MRM - C14	MRM - C18	MRM - Canola	MRM - Coconut	MRM - Oieie	MRM - Linoelic	MRM - Linoelic Acid
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List of aromatic compounds found in Beef by GCMS and a chart

showins; which detected in each lipid plus MRM				
Compounds in Beef	MRM only Beef	MRM only Beef Heart	MRM NatChol in PC40	MRM Limoleic acid
(Z)-2-decenal	Y	Y	Y	Y
2-furanmethanol	N	N	N	N
Z-heptanone	Y	Y	Y	Y
6-methyl-2-	N	N	Y	N
(E)-2-heptenal	N	Y	Y	Y
(Z)-2-heptenal	N	N	N	Y
(E)-2-hexenal	N	Y	Y	Y
2-ethyl-2-hexenal	N	N	N	N
Z-methyl-2-heptene	Y	N	N	N
Z-n-heptylfuran	Y	N	N	N
Z-n-octylfuran	Y	Y	Y	N
Z-nonanone	N	Y	Y	N
(E)-2-nonenal	Y	Y	Y	Y
(Z)-2-nonenal	N	N	N	Y
Z-octanone	Y	Y	Y	Y
(Z)-2-octen-1-ol	Y	Y	Y	Y
(E)-2-octenal	N	Y	Y	Y
Z-pentanone	N	Y	Y	N
1-propoxy-2-	N	N	N	N
1-(acetyl-oxy)-2-	Y	N	N	N
1-hydroxy-2-	Y	N	N	N
2-propenal	N	N	N	Y
2-undecenal	Y	Y	Y	Y
2,3-butanedione	N	N	N	Y
Z,3-pentanedione	N	N	N	N
(E,E)-2,4-	N	Y	Y	Y
2,4-decadienal	N	N	N	Y
(E,E)-2,4-	N	Y	Y	Y
(E,E)-2,4-	N	Y	Y	Y
2,6-	N	N	N	N
(E,Z)-2,6-	N	N	Y	N
3-ethylidihydro-	N	Y	Y	Y
2(3H)-	N	N	N	N
3-methyl-2(3H)-	N	N	Y	Y
4(3H)-5-pentyl-	N	N	N	N
2(3H)-	N	N	N	N
dihydro-5-	N	N	N	N
propyl-2-(3-H)-	N	N	N	N
2(5H)-furanone	N	N	N	N
tetrahydro-6-	N	N	N	N
methyl-2-H-	N	Y	Y	Y
3-	N	N	N	N
3-hexanone	N	N	N	N
3-methyl-2-	N	N	N	N
thiophenecarboxald	N	N	N	N
3-octanone	Y	Y	N	Y
3-octen-2-one	N	Y	Y	Y
(E,E)-3,5-octadien-	N	N	Y	Y
dihydro-2-	N	N	N	N
methyl-3-(2H)-	N	N	N	N
4-	N	N	N	N
4-cyclopenten-1,3-	N	N	Y	N
4-decyne	N	Y	N	N
(Z)-4-heptenal	N	Y	Y	Y
4-methyloctanoic	N	N	N	N
(E)-4-octene	N	N	N	N
2,3-dihydro-3,5-	Y	N	N	N
6-methyl-4(1H)-	Y	N	N	N
6-methyl-5-hepten-	Y	N	N	N
acetaldehyde	N	N	N	Y
acetic acid	N	N	N	N
acetic acid-ethenyl	Y	N	N	N
acetoin	Y	N	N	N
acetone	Y	N	N	Y
acetonitrile	N	N	N	Y
benzaldehyde	Y	Y	Y	Y
4-ethyl-	N	Y	Y	N
benzene	Y	N	N	N
benzoic acid	Y	N	N	N
butanal	Y	N	N	Y
Z-methyl-butanal	N	N	N	N

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

Compounds in Beef	MRM in Beef only	MRM in Beef Heart	MRM in NatChol ePC40	MRM in Linoleic acid
3-methyl-butanal	Y	N	N	N
butanoic acid	N	N	N	N
butyrolactone	Y	Y	N	Y
caprolactam	N	N	N	N
carbon disulfide	N	N	N	Y
1-ethyl-1-methyl-cyclopentane	Y	Y	Y	Y
propyl-decanal	N	N	Y	Y
decanal	N	Y	Y	N
dihydro-3-(2H)-thiophenone	N	N	N	N
Dimethyl sulfide	Y	N	N	N
dimethyl sulfone	N	N	N	N
dimethyl trisulfide	Y	Y	N	N
ethanethiol	N	N	N	N
ethanol	N	N	N	Y
1-(1H)-pyrrol-2-yl)-phenone	N	N	N	N
1-(2-furanyl)-ethosuximide	N	N	N	N
formic acid, heptyl	Y	Y	N	N
fur-an	Y	N	N	Y
2-ethyl-fur-an	Y	N	N	N
2-hexyl-furan	Y	N	N	Y
2-methyl-furan	N	N	N	Y
2-pentyl-furan	N	Y	Y	Y
2-propyl-fur-an	N	N	Y	Y
3-methyl-furan	Y	N	N	N
3-pentyl-furan	Y	Y	Y	Y
furfural	N	Y	Y	Y
heptanal	N	Y	Y	Y
heptanoic acid	N	N	N	Y
2-methyl-hex-2-yn	N	N	N	N
hexanoic acid	N	N	N	Y
hydrogen sulfide	N	N	N	N
m-maleic anhydride	N	N	N	N
methacrolein	N	N	N	N
methanethiol	N	N	N	N
methyl ethanoate	N	N	N	N
methyl isobutyl	Y	N	N	N
n-caproic acid	N	Y	Y	N
nonanal	N	Y	Y	Y
3-methyl-nonane	Y	N	N	N
nonanoic acid	Y	N	N	N
octanal	N	Y	Y	Y
octane	N	N	N	Y
octanoic acid	N	N	N	Y
oxalic acid,	Y	N	N	N
isobutyl-pentyl	N	N	N	N
p-cresol	N	N	N	N
pentanal	N	N	N	Y
pentanoic acid	Y	N	N	Y
4-ethyl-phenol	N	Y	Y	N
phenylacetaldehyde	Y	Y	Y	Y
(p-hydroxyphenyl)-phosphonic acid	Y	N	N	N
propanal	N	N	N	Y
2-methyl-propan-al	N	N	N	N
propanoic acid	N	N	N	N
2-methyl-propanoic	Y	N	N	N
propanoic acid,	N	N	N	N
ethenyl ester	N	Y	N	Y
pyrazine	N	Y	N	Y
2-ethyl-5-methyl-	N	N	N	N
2-ethyl-6-methyl-	N	N	N	N
2,5-dimethyl-	N	N	N	N
2,5-dimethyl-	N	N	N	N
3-ethyl-2,5-dimethyl-	Y	N	N	N
ethyl-pyrazine	N	N	N	N
methyl-pyrazine	N	N	N	N
trimethyl-pyrazine	Y	N	N	N

TABLE 12-continued

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

Compounds in Beef	MRM only	MRM-BeefHeart	MRM-NatCholinePC40	MRM-Linoleic acid
pyridine	Y	N	Y	N
pyrrole	Y	Y	Y	Y
styrene	Y	N	Y	N
thiazole	Y	Y	Y	Y
methyl-thiirane	N	N	N	N
thiophene	N	N	N	Y
2-hexyl-thiophene	Y	N	Y	N
2-pentyl-thiophene	N	Y	N	N
trans-2-octenal	N	Y	Y	N
trans-3-nonen-2-one	N	Y	Y	Y
undecanoic acid	N	N	N	N
Total # of Compounds Detected:	24	63	66	76

In samples having fatty or creamy aromas, 2,4-decadienal, (E,E)-2,4-nonadienal, (E,E)-2,4-heptadienal, and/or (E,E)-2,4-decadienal were detected in the KPhos6-BeefHeart, MRM-BeefHeart, MRM-BioLipon95, MRM-NatCholinePC40, KPhos6-Canola, MRM-Canola, KPhos6-01eic Acid, KPhos6-Linoleic acid and MRM-Linoleic acid samples. For (E,E)-2,4-decadienal, the strongest signal intensity was in the MRM-NatCholinePC40 sample, followed by the MRM-Linoleic acid, KPhos6-Linoleic acid, MRM-BeefHeart, MRM-BioLipon95, KPhos6-BeefHeart, MRM-01eic 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 acid samples. (E,E)-2,4-nonadienal also was detected in the KPhos6-Canola, MRM-NatCholinePC40, MRM-BioLipon95, MRM-BeefHeart, and KPhos6-Linoleic acid samples. For 2,4-decadienal, the strongest signal intensity was in the MRM-Linoleic acid sample. 2,4-decadienal also was detected in KPhos6-Linoleic acid, MRM-Canola, and KPhos6-01eic Acid samples.

In samples having earthy or mushroom aromas, 3-octen-2-one, 1-octen-3-one, 3-octanone, and/or 1-octen-3-ol were detected in the KPhos6-BeefHeart, MRM-BeefHeart, KPhos-BioLipon95, MRM-BioLipon95, KPhos-NatCholinePC40, MRM-NatCholinePC40, MRM-Canola, KPhos6-01eic Acid, MRM-01eic Acid, KPhos6-Linoleic acid, and MRM-Linoleic acid samples. For 1-octen-3-ol, the strongest signal intensity was in the MRM-Linoleic acid sample, followed by MRM-NatCholinePC40, KPhos6-Linoleic acid, MRM-BeefHeart, KPhos6-BeefHeart, MRM-Canola, MRM-BioLipon95, KPhos6-01eic Acid, and MRM-01eic Acid samples. 3-octanone was detected in the MRM-01eic Acid, KPhos6-Linoleic acid, and MRM-Linoleic acid samples. For 1-octen-3-one, the strongest signal intensity was in the MRM-Linoleic acid and MRM-BeefHeart samples, followed by KPhos6-Linoleic acid, MRM-NatCholinePC40, KPhos6-BeefHeart, MRM-BioLipon95, MRM-01eic 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-01eic Acid, MRM-BeefHeart, MRM-BioLipon95, MRM-Canola, KPhos-BioLipon95, and KPhos-NatCholinePC40. Pyrazine was detected in the MRM-Coconut, MRM-C18, MRM-C14, and MRM-BioLipon95 samples.

In samples having a nutty and roasted aroma, thiazole and 2-acetylthiazole were the most abundant compounds detected, along with pyrazine, methyl-pyrazine, trimethyl-pyrazine, and 3-ethyl-2,5-dimethylpyrazine. 2-acetylthiazole was detected in all samples with MRM and most abundant in samples with MRM-BeefHeart, MRM-BioLipon95, MRM-Canola, and MRM-Coconut. Thiazole was created in samples with MRM-Coconut, MRM-BeefHeart, MRM-BioLipon95, MRM-C14, MRM-C18, MRM-Canola, MRM-01eic acid and MRM-Linoleic acid and MRM-NatCholinePC40. Pyrazine was present in the largest amount in samples with MRM-Coconut, followed by samples MRM-BeefHeart, MRM-BioLipon95, MRM-C14, MRM-C18, MRM-Canola having roughly equal amount, MRM-01eic acid and MRM-Linoleic acid sample had even less. Methyl-pyrazine was present in MRM-BioLipon95 and MRM-Coconut. 3-ethyl-2,5-dimethyl-pyrazine and trimethyl-pyrazine, were present only without phospholipids in the MRM.

In samples having green, vegetable, or grass aromas, 1-heptanol, 1-hepten-3-ol, 1-hexanol, (E)-2-heptenal, (Z)-2-heptenal, (E)-2-hexenal, 2-pentyl-furan, and/or heptanal were detected in the KPhos6-BeefHeart, MRM-BeefHeart, KPhos-BioLipon95, MRM-BioLipon95, KPhos-NatCholinePC40, MRM-NatCholinePC40, KPhos-C14, MRM-C14, KPhos-C18, MRM-C18, MRM-Canola, MRM-Coconut, KPhos6-01eic Acid, MRM-01eic 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-01eic Acid, MRM-NatCholinePC40, MRM-Canola, KPhos6-01eic Acid, and KPhos-NatCholinePC40 samples. For (E)-2-heptenal, the strongest signal intensity was in the MRM-BeefHeart, MRM-Canola, MRM-01eic 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 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-01eic Acid sample, followed by the KPhos6-01eic Acid, MRM-C14, MRM-C18, MRM-Canola, MRM-BeefHeart, MRM-NatCholinePC40, MRM-Linoleic acid, and KPhos6-BeefHeart samples. For (E)-2-hexenal, the strongest signal intensity was in the MRM-Linoleic acid sample, followed by the MRM-NatCholinePC40, KPhos6-Linoleic acid, and MRM-01eic Acid samples.

Example 11—Creation of Beefy Flavors Using Complex Precursor Mixtures

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

The meat replica sample containing only LegH, was compared to the meat replica sample containing the magic mix and LegH by a sensory panel and GCMS analysis. Five tasters rated the flavored meat replicas for beefiness, bitterness, and levels of savory flavors, and off flavors. Each property was rated on a 7 point scale in which 7 was the

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highest amount of the specified property (e.g., a standard 80:20 ground beef would be rated 7 on the beefy scale). The Magic Mix flavor was rated one point higher in beefy character than the LegH only sample (FIG. 2).

To determine which chemical products were produced upon heating, a solution of Magic Mix was prepared with 1% w/v LegEl at pH 6.0. The samples were cooked with shaking at 150° C. for three minutes, then Solid Phase Micro Extraction (SPME) was performed for twelve minutes at 50° C. to extract the volatile compounds above the headspace of the reaction. A search algorithm was used to analyze the retention time and mass fingerprint information of the volatile compounds and assign chemical names to peaks. Table 14 shows the compounds identified in both the Magic Mix+LegEl (MM, average of two samples) and in the LegEl alone in buffer (LegEl, average of five samples) samples. The compounds in Table 14 are listed in order of the retention time (R.T., in seconds), and are designated as having a zero peak area (0), or a small (S), medium (M), or large (L) average peak area. Hundreds of compounds were identified between the samples, many of which are characteristic of beefy aroma, including but not limited to 1,3-bis(1,1-dim-ethylethyl)-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 LegEl.

TABLE 13

Chemical entities added to the Magic Mix

Chemical entity	mM
Alanine	5.6
Arginine	0.6
Asparagine	0.8
Aspartate	0.8
Cysteine	0.8
Glutamic acid	3.4
Glutamine	0.7
Glycine	1.3
Histidine	0.6
Isoleucine	0.8
Leucine	0.8
Lysine	0.7
Methionine	0.7
Phenylalanine	0.6
Proline	0.9
Threonine	0.8
Tryptophan	0.5
Tyrosine	0.6
Valine	0.9
glucose	5.6
Ribose	6.7
Maltodextrin	5.0
Thiamine	0.5
GMP	0.24
IMP	0.6
Lactic acid	1.0
creatine	1.0
NaCl	10
KCl	10
Kphos pH 6.0	10

TABLE 14

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

R.T.(s) Name	MM
	with LegH LegH alone

~~1.~~	~~carbon disulfide~~	~~L~~	~~S~~
~~1.~~	~~dimethyl sulfide~~	~~S~~	~~0~~

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TABLE 14-continued

Compounds identified with GC-MS analysis in samples with MM and

LegH, or LegH alone (averagesamples)

R.T.(s)	Name	MM with LegH LegH alone
265	oxalic acid, isobutyl pentyl ester	M 0
268.1	2,3,4-trimethyl-pentane	M 0
269.2	methanethiol	S 0
283.4	propanal	M 0
283.4	octane	M 0
287.1	furan	M 0
293.3	Z-methyl-prop-anal	L S
297.6	acetone	L S
319.3	2-propenal	M S
338.1	Z-methyl-fur-an	M S
342.1	butanal	L S
344.2	2,4-dimethyl-1-heptene	M 0
346.3	methacrolein	M 0
357.4	methyl-thiirane	L 0
360.2	3-methyl-fur-an	S 0
363.7	butanone	L S
368.9	2,3-dihydro-5-methyl-furan	M S
376.4	Z-methyl-butanal	L M
381.1	3-methyl-butanal	L M
390.6	isopropyl alcohol	0 S
399.6	ethanol	L M
406.2	Z-propenoic acid, methyl	M 0
408.2	benzene	S 0
414.4	methyl vinyl ketone	M 0
416.4	2,2,4,6,6-pentamethyl-	M 0
422.6	Z-ethyl-furan	S 0
438.4	Z-ethylacrolein	M 0
449.9	Z-pentanone	S 0
453.2	pentanal/Z,3-butanedione	L 0
453.8	2,3-butanedione	L M
472.8	4,7-dimethyl-undecane	M S
483.9	Z-methyl-p-entanal	M 0
492.6	Z-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
523.3	3-methyl-p-entanal	M 0
533.1	(E)-3-decene	M 0
536.3	toluene	M S
537.9	2-butenal	M S
543.8	4-penten-2-one	M 0
550.8	methyl thioacetate	M 0
683.7	p-xylene	S 0
727.4	dimethyl selenone	M 0
738.3	methyl isopropyl disulphide	M 0
753	Z-heptanone	M 0
758.7	heptanal	L 0
781.9	1,3-dioxoproxy-1,3-dithia-cyclobutane	S M
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	Z-pentyl-fur-an	L 0
831	Z-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
893.6	methyl-pyrazine	M 0
910.3	thiocyanic acid, methyl-ester	S 0
918.6	4-methylthiazole	M 0
921.4	Z-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
974.3	(E)-2-heptenal	M 0
987.4	3-methyl-1-undecene	M 0
993.8	Z-hexyl-fur-an	M 0

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

R.T.(s)	Name	MM with LegH
1.1	2-methyl-5-(methylthio)-furan,	S
1.1	2-butyl-1-decene	M
1.1	dimethyl trisulfide	L
1.1	2-nonanone	M
1.1	nonanal	L
1.1	1,3-bis(1,1-dimethylethyl)-benzene	M
1.1	(E)-2-octenal	M
1.1	1-heptanol	M
1.1	methional	L
1.1	acetic acid	M
1.1	furfural	M
1.1	2-decanone	M
1.1	decanal	M
1.1	1-decen-3-one	M
1.1	pyrrole	M
1.1	5-ethenyl-4-methyl-thiazole	M
1.1	benzaldehyde	L
1.1	2-n-octylfuran	M
1.1	(E)-2-nonenal	M
1.1	1-octanol	M
1.1	2-methyl-1(H)-pyrrole	S
1.1	2-undecanone	M
1.1	(E)-2-octen-1-ol	M
1.448	butyrolactone	S
1.1	(E)-2-decenal	M
1.1	phenylacetaldehyde	L
1.1	2-acetylthiazole	L
1.1	acetophenone	M
1.1	1-nonanol	M
1.487	methyl (methylthio)methyl disulfide	M
1.1	5-(2-chloroethyl)-4-methylthiazole	L
1.1	1-(ethylthio)-2-(methylthio)-buta-1,3-diene	L
1.512	3-thiophenecarboxaldehyde	M
1.1	2-nonen-4-one	M
1.1	2-thiophenecarboxaldehyde	S
1.1	dodecanal	M
1.1	4-ethyl-2-methyl-pyrrole	S
1.1	3-(methylthio)-propanenitrile	S
1.1	3-decen-2-one	M
1.1	bis(2-methyl-4,5-dihydro-3-furyl) disulfide	M
1.1	1,10-undecadiene	M
1.1	2-undecenal	S
1.1	2-phenylpropenal	M
1.1	(Z)-3-decen-1-ol, acetate	M
1.1	3-phenyl-furan	S
1.1	4-nitrophenyl-2-thiophenecarboxylic acid	S
1.1	ester	
1.1	5-formyl-4-methylthiazole	M
1.1	pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl-ester	M
1.1	benzyl alcohol	S
1.1	pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl-ester	S
1.1	dodecanal	M
1.1	(1-ethyl-1-propenyl)-benzene	S
1.1	1-undecanol	M
1.1	2-methyl-3-furanthiol	M
1.1	2-methyl-3-(methylthio)-furan	M
1.1	4-chloro-2,6-bis(1,1-dimethylethyl)-phenol	S
1.1	phenol	S
1.1	{(methylsulfonyl)methyl}-benzene	S
1.1	(e)-2-tridecen-1-ol	M
1.1	1-heptyl-1,1,2,3,4-tetrahydro-4-methyl-naphthalene	S
1.1	2,4-decadienal	S
1.1	3,3'-dithiobis[2-methyl]-furan	M
1.1	3,5-di-tert-butylbenzoic acid	S
1.1	4-ethoxy-benzoic acid, ethyl-ester	S
1.1	3-(phenylmethyl)-2,5-piperazinedione	S
1.1	9-octadecenal	M
1.1	3,5-bis(1,1-dimethylethyl)-phenol	M
1.1	4-methyl-5-thiazoleethanol	M

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

R.T.(s)	Name	MM with LeaH	LeaH alone
2007.8	1,1'-(1,2-cyclobutanediyl)bis-	S	0
2019.8	benzoic acid	S	S
2026.4	4-quinolinecarboxaldehyde	S	0
2027.8	m-aminophenylacetylene	M	0

Example 12—Ferrous Chlorin Catalyzes Production of Meat-Like Flavor Compounds

Fresh green spinach (10 lb) was added to 500 mL water and finely ground in a Vitamix blender to yield 2 L of green suspension. Acetone (8 L) was added with mixing and the material was allowed to extract for 1 hour. The material was filtered through Whatman filter paper and the acetone was removed on a rotary evaporator (Buchi). To the residual green suspension (500 mL) was added 2 mL of 10 M HCl, causing the suspension to turn brown. To this was added 1 g of FeCl₂·4H₂O in 10 mL H₂O. The solution was shaken then left at 4° C. for 16 hours. This suspension was extracted with diethyl ether (3x50 mL) 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 (1.1 g). The pellet was dissolved in chloroform for fractionation.

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

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

TABLE 15	
Sample:	Chlorophyll or Fe-chlorin (~2 mg/mL in CHCl ₃)
System:	Agilent 1100 with Chemstation
Column:	Zorbax Bonus-RP (4.6 x 250 mm, 5 μm)
Mobile phase:	acetonitrile, methanol, ethyl acetate (60:20:20) isocratic flow
Temperature:	30° C.
Flow Rate:	1.0 mL per minute
Injection volume:	0.05 mL

Preparation of Flavor Reaction Containing Ferrous Chlorin or Leghemoglobin

A solution of ferrous chlorophyll was mixed with the Magic Mix (Table 13) to a final concentration of 0.35% ferrous chlorin, 1% glycerol, 0.005% tween-20, 5% sunflower oil, 100 mM NaCl, 20 mM phosphate at pH 6. Leghemoglobin (0.35%) at pH 6 in phosphate buffer (20 mM), 100 mM NaCl, was mixed with the Magic Mix (Table 13), 1% glycerol, and 0.005% tween-20. The flavor reaction mixtures were heated to 150° C. for 3 minutes; this reaction created flavor compounds known to be present in meat, created by hemoglobin and also created by ferrous chlorin; see Table 16.

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

1-heptanol	acetone
1-hexanol	acetonitrile
1-octanol	benzaldehyde
1-octen-3-ol	butanal
1-octen-3-one	2-methyl-butanal
1-pentanol	dimethyl trisulfide
2-acetylthi-azole	ethyl acetate
2-butenal	furan
3-methyl-2-butenal,	2-ethyl-furan
(Z)-2-decenal	2-hexyl-furan
6-methyl-2-heptanone	2-pentyl-furan
(E)-2-heptenal	furfural
(E)-2-hexenal	heptanal
7-methyl-3-furanthio	ammonophenylacetyl
(E)-2-nonenal	methacrolein
(E)-2-octenal	methional
2-pentanone	octanal
1-hydroxy-2-propanone	octane
2-undecenal	oxalic acid, diallyl
3-methyl-3-buten-2-one	2,3-butanedione
(E)-2-octene,	2-methyl-propanal
thiazole	pyrazine
	2,3-dimethyl-
	2,5-dimethyl-
	pyrazine

Example 13—Flavor Creation by Immobilized

Hemin

Preparation of Hemin Linked CM Sepharose.

200 mg of bovine hemin (Sigma Aldrich) was loaded into a scintillation vial. A small magnetic stir bar, 800 μ L acetonitrile, 64 μ L 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 μ L of JEFFAMINE ED900. This was stirred while allowing the black mixture to warm to ambient temperature. Chloroform (10 mL) was added to the mixture followed by water (4 mL). A flashlight was used to distinguish between organic and aqueous layers since both were black and the organic layer was pipetted off and concentrated to a dark black oil. The oil was dissolved in a 4:1 mixture of acetonitrile and ethanol to make an approximately 10% strength solution that was inky black in color.

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

Flavor Reaction

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

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

TABLE 17

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

Flavor compound	myoglobin	hemin-linker-resin
2-methyl-5-(methylthio)-thiophene	Low	
dihydro-5-propyl-2(3H)-octanone	Low	
pyrrole	Low	Low
methanethiol	Low	Low
2-methyl-3-pyrazine	Low	Low
4-hydroxy-2-propanone	Low	Low
propanal	Low	Low
thiophene	Low	medium
pyridine	Low	Low
2-methyl-furan	Low	medium
oxalic acid, butyl propyl	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
tert-butanol	medium	
4,4'-dithiobis [2-methyl]-m-aminophenylacetylene	medium	medium
2,5-dihydro-3,4-dimethyl-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
4-methyl-2-butanal	medium	medium
thiazole	medium	large
2-methyl-3-furanthiol	large	large
2-propenal	large	large
3-methyl-2-butenal	large	medium
2-methyl-5-(methylthio)-ethyl acetate	large	large
	large	medium

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

Flavor compound	myoglobin	hemin-linker-resin
methacrolein	large	medium
methyl-thiirane	large	large
methionol	large	large
methyl alcohol	large	medium
2-butanone	large	Low
2,3-butanedione	large	medium
acetone	large	large
furan	large	medium
benzaldehyde	large	medium
methyl thiolacetate	large	medium
acetaldehyde	very large	very large
2-methyl-propanal	very large	very large
dimethyl trisulfide	very large	very large
3-methyl-butanol	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		medium
1-nonanol		large
1-octene		large
1-heptanol		large
1-dodecene		large
phorone		very large

Example 14. The Combination of Precursors with

Heme Protein Drives Flavor Reactions

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

TABLE 18

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

Compound	Precursor mix	Precursor LegH mix + Leg H
carbon disulfide	medium	high
isopropyl alcohol	medium	low

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

Compound	Precursor mix	LegH	Precursor mix + Leg H
2-methyl-furan	low		low
butanal	low		medium
thiophene	low		low
2,3-butanedione	low	low	high
furan	low		medium
2,4-dimethyl-1-heptene		high	high
acetone		high	high
dimethyl trisulfide		medium	medium
2-methyl-heptane		medium	medium
2-pentanone		medium	
pentanal		medium	medium
2-pentyl-furan		medium	medium
2-methyl-propanal		low	high
2-acetyl-1-propene		low	low
2-methyl-butanal		low	medium
1,3-dimethyl-benzene		low	low
octane		low	low
benzene		low	low
benzaldehyde			very high
2-butanone			very high
furfural			very high
thiazole			high
nonanal			high
thiazole			high
2-acetylthiazole			medium
3-methyl-butanal			medium
(Z)-2-heptenal			medium
heptanal			medium
methyl-thiurane			medium
3-ethyl-pentane			medium
phenylacetaldehyde			medium
2-hexyl-furan			medium
2-nonanone			medium
propanal			medium
pyrazine			medium
(Z)-2-heptenal			medium
2-methyl-1-heptene			medium
2-ethyl-furan			medium
octanal			medium
(E)-4-octene			low
(E)-2-octenal			low
2-methyl-thiazole			low
2-propenal			low
1-octen-3-one			low
1-octene			low
2-octanone			low
dimethyl-sulfide			low
3-pentyl-furan			low
2-n-octylfuran			low
2-pentyl-thiophene			low

Other Embodiments

50

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by ⁵⁵ the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 27

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

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145 150 155 160

Ser

<210> SEQ ID NO 2 <211> LENGTH:

133 <212> TYPE: PRT

<213> ORGANISM: *Methylobacterium thermophilum* <400>

SEQUENCE: 2

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

```

115 120 125

Val Met Thr Ala Glu 130

-continued

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Me Leu Ser Glu Glu Thr He Arg Val He Lys Ser Thr Val Pro Leu
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 20 25 30
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 35 40 45
 Glu Gin Pro Lys Lys Leu Ala Asn Ala He He Ala Tyr Ala Thr Tyr
 50 55 60
 He Asp Arg Leu Glu Glu Leu Asp Asn Ala He Ser Thr He Ala Arg
 65 70 75 80
 Ser His Val Arg Arg Asn Val Lys Pro Glu His Tyr Pro Leu Val Lys
 85 90 95
 Glu Cys Leu Leu Gin Ala He Glu Glu Val Leu Asn Pro Gly Glu Glu
 100 105 110
 Val Leu Lys Ala Trp Glu Glu Ala Tyr Asp Phe Leu Ala Lys Thr Leu
 115 120 125
 He Thr Leu Glu Lys Lys Leu Tyr Ser Gin Pro
 130 135

<210> SEQ ID

NO 4

<211> LENGTH:

<212> PR

<213> Glycine ma

ORGANISM: *

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

130 135 140

Phe

145

-continued

<400>SEQUENCE: 5

Me Ser Ala Ala Glu Gly Ala Val Val Phe Ser Glu Glu Lys Glu Ala
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 Leu Val Leu Lys Ser Trp Ala He Met Lys Lys Asp Ser Ala Asn Leu
 20 25 30
 Gly Leu Arg Phe Phe Leu Lys He Phe Glu He Ala Pro Ser Ala Arg
 35 40 45
 Gin Met Phe Pro Phe Leu Arg Asp Ser Asp Val Pro Leu Glu Thr Asn
 50 55 60
 Pro Lys Leu Lys Thr His Ala Val Ser Val Phe Val Met Thr Cys Glu
 65 70 75 80
 Ala Ala Ala Gin Leu Arg Lys Ala Gly Lys He Thr Val Arg Glu Thr
 85 90 95
 Thr Leu Lys Arg Leu Gly Gly Thr His Leu Lys Tyr Gly Val Ala Asn
 100 105 110
 Gly His Phe Glu Val Thr Arg Phe Ala Leu Leu Glu Thr He Lys Glu
 115 120 125
 Ala Leu Pro Ala Asp Met Trp Gly Pro Glu Met Arg Asn Ala Trp Gly
 130 135 140
 Glu Ala Tyr Asp Gin Leu Val Ala Ala He Lys Gin Glu Met Lys Pro
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Ala-Glu

<210> SEQ ID: 6

<211> LENGTH:

<212> TYPE:

<213> ORGANISM: Magnaporthe oryzae

> SM: e

SEQUENCE: 6

<400> CE:

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20 25 30

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 35 40 45
 Glu Ser Pro Ser Arg Lys Ser Ser Thr He Gly Gin Ser Thr Arg Asn
 50 55 60
 Gly Ser Cys Gin Ala Asp Thr Gin Lys Gly Gin Leu Pro Pro Val Gly
 65 70 75 80
 Glu Lys Pro Lys Pro Val Lys Glu Asn Pro Met Lys Lys Leu Lys Glu
 85 90 95
 Met Ser Gin Arg Pro Leu Pro Thr Gin His Gly Asn Gly Thr Tyr Pro
 100 105 110
 Thr Glu Lys Lys Leu Thr Gly He Gly Glu Asp Leu Lys His He Arg
 115 120 125
 Gly Tyr Asp Val Lys Thr Leu Leu Ala Met Val Lys Ser Lys Leu Lys
 130 135 140
 Gly Glu Lys Leu Lys Asp Asp Lys Thr Met Leu Met Glu Arg Val Me
 145 150 155 160
 Gin Leu Val Ala Arg Leu Pro Thr Glu Ser Lys Lys Arg Ala Glu Leu

165 170 175

Thr Asp Ser Leu Ile Asn Glu Leu Trp Glu Ser Leu Asp His Pro Pro 180 185
 190

Leu Asn Tyr 195 Leu Gly Pro Glu His Ser Tyr Arg Thr Pro Asp Gly Ser 200 205
 Tyr Asn His 210 Pro Phe Asn Pro 215 Gin Leu Gly Ala Ala Gly Ser Arg Tyr 220
 Ala Arg Ser 225 Val He Pro 230 Thr Val Thr Pro 235 Pro Gly Ala Leu Pro 240 As
 Pro Gly Leu He 245 Phe Asp Ser He 250 Met Gly Arg Thr Pro Asn Ser Tyr 255
 Arg Lys His 260 Pro Asn Asn Val Ser Ser He 265 Leu Trp Tyr Trp Ala Thr 270
 He He He 275 His Asp He Phe Trp Thr Asp Pro Arg Asp He Asn Thr 280 285
 As 290 Lys Ser Ser Ser Tyr 295 Leu Asp Leu Ala Pro 300 Leu Tyr Gly Asn Ser 305
 Gin Glu Met Gin Asp Ser 310 He Arg Thr Phe 315 Lys Asp Gly Arg Met Lys 320
 Pro Asp Cys Tyr 325 Ala Asp Lys Arg Leu Ala Gly Met Pro Pro Gly Val 330 335
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 As 355 Leu Ala Leu He Asn Glu Gly Gly Arg Phe Asn Lys Pro Ser 360 365 As
 Leu Leu Glu Gly Glu Ala 370 Arg 375 Glu Ala Ala Trp Lys Lys Tyr Asp 380 As
 As 385 Leu Phe Gin Val Ala Arg Leu Val Thr Ser Gly Leu Tyr He 390 395 As 400
 He Thr Leu Val Asp Tyr Val Arg Asn He 405 410 Val Asn Leu Asn Arg Val 415
 As 420 Thr Thr Trp Thr Leu Asp Pro Arg Gin Asp Ala Gly Ala His Val 425 430
 Gly Thr Ala Asp Gly Ala Glu Arg Gly Thr Gly Asn Ala Val Ser Ala 435 440 445
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 Ser Lys Phe Val Glu Ala 470 Gin Phe Gin Asn He 475 Phe Gly Lys Pro Ala 480
 Ser Glu Val Arg Pro Asp Glu Met Trp 485 490 Lys Gly Phe Ala Lys Met Glu 495
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 Arg Gly Pro Asp Gly Lys Phe Asp Asp Asp Asp Leu Val Arg Cys He 515 520 525
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 Pro Gin Ala Met Lys Val Val Glu Thr Met Gly He 545 550 555 He Gin Gly Arg 560
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580 585 590

Glu Ala Leu Arg Arg Leu Tyr Asp His Pro Asp Asn Val Glu Leu Tyr 595 600

605

—continued

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 645 650 655
 Arg Asn Leu Thr Asn Trp Gly Tyr Lys Glu Val Asp Tyr Asp Leu Ser
 660 665 670
 Val Asn His Gly Cys Val Phe Tyr Lys Leu Phe He Arg Ala Phe Pro
 675 680 685
 As His Phe Lys Gin Asn Ser Val Tyr Ala His Tyr Pro Met Val Val
 690 695 700
 Pro Ser Glu Asn Lys Arg He Leu Glu Ala Leu Gly Arg Ala Asp Leu
 705 710 715 720
 Phe Asp Phe Glu Ala Pro Lys Tyr He Pro Pro Arg Val Asn He Thr
 725 730 735
 Ser Tyr Gly Gly Ala Glu Tyr He Leu Glu Thr Gin Glu Lys Tyr Lys
 740 745 750
 Val Thr Trp His Glu Gly Leu Gly Phe Leu Met Gly Glu Gly Gly Leu
 755 760 765
 Lys Phe Met Leu Ser Gly Asp Asp Pro Leu His Ala Gin Gin Arg Lys
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 Cys Met Ala Ala Gin Leu Tyr Lys Asp Gly Trp Thr Glu Ala Val Lys
 785 790 795 800
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 865 870 875 880
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 885 890 895
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 900 905 910
 Pro Trp Ser Arg Gly Met Phe Val Gly Lys Pro Ala Lys Asp Glu Pro
 915 920 925
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 930 935 940
 Leu Ser Asp Tyr Asp He Ala Trp Ser His Val Val Pro Thr Ser Gly
 945 950 955 960
 Ala Met Val Pro Asn Gin Ala Gin Val Phe Ala Gin Ala Val Asp Tyr
 965 970 975
 Tyr Leu Ser Pro Ala Gly Met His Tyr He Pro Glu He His Met Val
 980 985 990
 Ala Leu Gin Pro Ser Thr Pro Glu Thr Asp Ala Leu Leu Leu Gly Tyr

995 1000 1005

Ala Met Glu Gly Ile Arg Leu Ala Gly Thr Phe Gly Ser Tyr Arg Glu 1010 1015
 1020

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Val	Lys	Ala	^{Gly} _{Δ_{ccn}}		Arg	Val	Phe	Val	Ser	Phe	Val	Asp	Ala	Ala	Arg
			1045						1050						1055
Asp	Pro	Lys	His	Phe	Pro	Asp	Pro	Glu	Val	Val	^{As} _n	Pro	Arg	Arg	Pro
			1060					1065							1070
Ala	Lys	Lys	Tyr	He	His	Tyr	Gly	Val	Gly	Pro	His	Ala	Cys	Leu	Gly
			1075				1080								1085
Arg	Asp	Ala	Ser	Gln	He	Ala	He	Thr	Glu	Met	Phe	Arg	Cys	Leu	Phe
			1090				1095					1100			
Arg	Arg	Arg	Asn	Val	Arg	Arg	Val	Pro	Gly	Pro	Gln	Gly	Glu	Leu	Lys
			1105				1110				1115				1120
Lys	Val	Pro	Arg	Pro	Gly	Gly	Phe	Tyr	Val	Tyr	^{Me} _t	Arg	Glu	Asp	Trp
			1125						1130						1135
Gly	Gly	Leu	Phe	Pro	Phe	Pro	Val	Thr	Met	Arg	Val	Met	Trp	Asp	Asp
			1140					1145							1150

Glu

<210> SEQ ID NO 7 <211> LENGTH:
 530 <212> TYPE: PRT
 <213> ORGANISM: Fusarium oxysporum

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 Leu Tyr Met Gin Gly Gly Phe Asn Lys Arg Gly Phe Ala Asp Ala Leu
 35 40 45
 Arg Thr Cys Glu Phe Gly Ser Asn Val Pro Gly Thr Gin Asn Thr Ala
 50 55 60
 Glu Trp Leu Arg Thr Ala Phe His Asp Ala He Thr His Asp Ala Lys
 65 70 75 80
 Ala Gly Thr Gly Gly Leu Asp Ala Ser He Tyr Trp Glu Ser Ser Arg
 85 90 95
 Pro Glu Asn Pro Gly Lys Ala Phe Asn Asn Thr Phe Gly Phe Phe Ser
 100 105 110
 Gly Phe His Asn Pro Arg Ala Thr Ala Ser Asp Leu Thr Ala Leu Gly
 115 120 125
 Thr Val Leu Ala Val Gly Ala Cys Asn Gly Pro Arg He Pro Phe Arg
 130 135 140
 Ala Gly Arg He Asp Ala Tyr Lys Ala Gly Pro Ala Gly Val Pro Glu
 145 150 155 160
 Pro Ser Thr Asn Leu Lys Asp Thr Phe Ala Ala Phe Thr Lys Ala Gly
 165 170 175
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 180 185 190
 Gly Gly Val His Ser Val Asp Phe Pro Glu He Val Gly He Lys Ala
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 210 215 220
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 225 230 235 240

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 275 280 285
 Pro Lys Ser Val Gin Leu Thr Pro Val Leu Glu Ala Tyr Asp Val Arg
 290 295 300
 Pro Tyr He Thr Glu Leu Ser Leu Asn Asn Lys Asn Lys He His Phe
 305 310 315 320
 Thr Gly Ser Val Arg Val Arg He Thr Asn Asn He Arg Asp Asn Asn
 325 330 335
 Asn Leu Ala He Asn Leu He Tyr Val Gly Arg Asp Gly Lys Lys Val
 340 345 350
 Thr Val Pro Thr Gin Gin Val Thr Phe Gin Gly Gly Thr Ser Phe Gly
 355 360 365
 Ala Gly Glu Val Phe Ala Asn Phe Glu Phe Asp Thr Thr Met Asp Ala
 370 375 380
 Lys Asn Gly He Thr Lys Phe Phe He Gin Glu Val Lys Pro Ser Thr
 385 390 395 400
 Lys Ala Thr Val Thr His Asp Asn Gin Lys Thr Gly Gly Tyr Lys Val
 405 410 415
 Asn Asp Thr Val Leu Tyr Gin Leu Gin Gin Ser Cys Ala Val Leu Glu
 420 425 430
 Lys Leu Pro Asn Ala Pro Leu Val Val Thr Ala Met Val Arg Asp Ala
 435 440 445
 Arg Ala Lys Asp Ala Leu Thr Leu Arg Val Ala His Lys Lys Pro Val
 450 455 460
 Lys Gly Ser He Val Pro Arg Phe Gin Thr Ala He Thr Asn Phe Lys
 465 470 475 480
 Ala Thr Gly Lys Lys Ser Ser Gly Tyr Thr Gly Phe Gin Ala Lys Thr
 485 490 495
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 Cys Ser
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 <212> PR
 <213> Fusarium-graminearum
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 <400> 8
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 35 40 45
 He Trp Leu Thr Gly Ala Ala Val Ala Gly Gly Ala Gly Tyr Tyr Phe
 50 55 60
 Tyr Gly Asn Ser Ala Ser Ser Ala Thr Ala Lys Val Phe Asn Pro Ser
 65 70 75 80

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Lys Glu Asp Tyr Gin Lys Val Tyr Asn Glu He Ala Ala Arg Leu Glu
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 Glu Lys Asp Asp Tyr Asp Asp Gly Ser Tyr Gly Pro Val Leu Val Arg
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 Leu Ala Trp His Ala Ser Gly Thr Tyr Asp Lys Glu Thr Gly Thr Gly
 115 120 125
 Gly Ser Asn Gly Ala Thr Met Arg Phe Ala Pro Glu Ser Asp His Gly
 130 135 140
 Ala Asn Ala Gly Leu Ala Ala Ala Arg Asp Phe Leu Gin Pro Val Lys
 145 150 155 160
 Glu Lys Phe Pro Trp He Thr Tyr Ser Asp Leu Trp He Leu Ala Gly
 165 170 175
 Val Cys Ala He Gin Glu Met Leu Gly Pro Ala He Pro Tyr Arg Pro
 180 185 190
 Gly Ser Asp Arg Asp Val Ser Gly Cys Thr Pro Asp Gly Arg Leu
 ^{Arg} 195 200 205
 Pro Asp Ala Ser Lys Arg Gin Asp His Leu Arg Gly He Phe Gly Arg
 210 215 220
 Me Gly Phe Asn Asp Gin Glu He Val Ala Leu Ser Gly Ala His Ala
 ^t 225 230 235 240
 Leu Gly Arg Cys His Thr Asp Arg Ser Gly Tyr Ser Gly Pro Trp Thr
 245 250 255
 Phe Ser Pro Thr Val Leu Thr Asn Asp Tyr Phe Arg Leu Leu Val Glu
 260 265 270
 Glu Lys Trp Gin Trp Lys Lys Trp Asn Gly Pro Ala Gin Tyr Glu Asp
 275 280 285
 Lys Ser Thr Lys Ser Leu Met Met Leu Pro Ser Asp He Ala Leu He
 290 295 300
 Glu Asp Lys Lys Phe Lys Pro Trp Val Glu Lys Tyr Ala Lys Asp Asn
 305 310 315 320
 As Ala Phe Phe Lys Asp Phe Ser Asn Val Val Leu Arg Leu Phe Glu
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 Lys Pro Thr His Gin Glu

355

~~<210> SEQ ID NO 9 <211> LENGTH:~~
~~122 <212> TYPE: PRT~~

~~<213> ORGANISM: Chlamydomonas eugametos <400>~~

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 Tyr Phe Ser Asn Thr Asp Met Lys Val Gin Arg Ser Lys Gin Phe Ala
 35 40 45
 Phe Leu Ala Tyr Ala Leu Gly Gly Ala Ser Glu Trp Lys Gly Lys ^{As}_n
 50 55 60
 Me Arg Thr Ala His Lys Asp Leu Val Pro His Leu Ser Asp Val His
 ^t 65 70 75 80

~~Phe-Gln-Ala-Val-Ala-Arg-His-Leu-Ser-Asp-Thr-Leu-Thr-Glu-Leu-Gly-85-90~~
~~95~~

~~-continued~~

~~Val-Pro-Pro-Glu-Asp-Ile-Thr-Asp-Ala-Met-Ala-Val-Val-Ala-Ser-Thr-100-105~~
~~110~~

~~Arg-Thr-Glu-Val-Leu-Asn-Met-Pro-Gln-Gln-115~~
~~120~~

~~<210> SEQ ID NO 10 <211> LENGTH:~~
~~121 <212> TYPE: PRT~~

~~<213> ORGANISM: Tetrahymena pyriformis <400>~~

~~SEQUENCE: 10~~

~~Me Asn Lys Pro Gln Thr He Tyr Glu Lys Leu Gly Gly Glu Asn Ala~~
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~~Me Lys Ala Ala Val Pro Leu Phe Tyr Lys Lys Val Leu Ala Asp Glu~~
~~20 25 30~~
~~Arg Val Lys His Phe Phe Lys Asn Thr Asp Met Asp His Gln Thr Lys~~
~~35 40 45~~
~~Gln Gln Thr Asp Phe Leu Thr Met Leu Leu Gly Gly Pro Asn His Tyr~~
~~50 55 60~~
~~Lys Gly Lys Asn Met Thr Glu Ala His Lys Gly Met Asn Leu Gln Asn~~
~~65 70 75 80~~
~~Leu His Phe Asp Ala He He Glu Asn Leu Ala Ala Thr Leu Lys Glu~~
~~85 90 95~~
~~Leu Gly Val Thr Asp Ala Val He Asn Glu Ala Ala Lys Val He Glu~~
~~100 105 110~~
~~His Thr Arg Lys Asp Met Leu Gly Lys~~

~~115 120~~

~~<210> SEQ ID NO 11 <211> LENGTH:~~
~~117 <212> TYPE: PRT~~

~~<213> ORGANISM: Paramecium caudatum <400>~~

~~SEQUENCE: 11~~

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

~~-continued~~

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<400> SEQUENCE: 12
Me Pro Leu Thr Pro Glu Gin He Lys He He Lys Ala Thr Val Pro
1 5 10 15
Val Leu Gin Glu Tyr Gly Thr Lys He Thr Thr Ala Phe Tyr Met Asn
20 25 30
Me Ser Thr Val His Pro Glu Leu Asn Ala Val Phe Asn Thr Ala Asn
35 40 45
Gin Val Lys Gly His Gin Ala Arg Ala Leu Ala Gly Ala Leu Phe Ala
50 55 60
Tyr Ala Ser His He Asp Asp Leu Gly Ala Leu Gly Pro Ala Val Glu
65 70 75 80
Leu He Cys Asn Lys His Ala Ser Leu Tyr He Gin Ala Asp Glu Tyr
85 90 95
Lys He Val Gly Lys Tyr Leu Leu Glu Ala Met Lys Glu Val Leu Gly
100 105 110
Asn Ala Cys Thr Asp Asp He Leu Asp Ala Trp Gly Ala Ala Tyr Trp
115 120 125
Ala Leu Ala Asp He Met He Asn Arg Glu Ala Ala Leu Tyr Lys Gin
130 135 140
Ser Gin Gly

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145

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<210> SEQ ID NO 13
<211> LENGTH: 165
<212> TYPE: PRF
<213> ORGANISM: Zea mays

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<400> SEQUENCE: 13
Me Ala Leu Ala Glu Ala Asp Asp Gly Ala Val Val Phe Gly Glu Glu
1 5 10 15
Gin Glu Ala Leu Val Leu Lys Ser Trp Ala Val Met Lys Lys Asp Ala
20 25 30
Ala Asn Leu Gly Leu Arg Phe Phe Leu Lys Val Phe Glu He Ala Pro
35 40 45
Ser Ala Glu Gin Met Phe Ser Phe Leu Arg Asp Ser Asp Val Pro Leu
50 55 60
Glu Lys Asn Pro Lys Leu Lys Thr His Ala Met Ser Val Phe Val Me
65 70 75 80
Thr Cys Glu Ala Ala Ala Gin Leu Arg Lys Ala Gly Lys Val Thr Val
85 90 95
Arg Glu Thr Thr Leu Lys Arg Leu Gly Ala Thr His Leu Arg Tyr Gly
100 105 110
Val Ala Asp Gly His Phe Glu Val Thr Gly Phe Ala Leu Leu Glu Thr
115 120 125
He Lys Glu Ala Leu Pro Ala Asp Met Trp Ser Leu Glu Met Lys Lys
130 135 140
Ala Trp Ala Glu Ala Tyr Ser Gin Leu Val Ala Ala He Lys Arg Glu
145 150 155 160
Me
1 Lys Pro Asp Ala

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165

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Me Ala Leu Val Glu Gly Asn Asn Gly Val Ser Gly Gly Ala Val Ser
1      5      10      15
Phe Ser Glu Glu Gin Glu Ala Leu Val Leu Lys Ser Trp Ala He Me
20      25      30      35
Lys Lys Asp Ser Ala Asn He Gly Leu Arg Phe Phe Leu Lys He Phe
35      40      45
Glu Val Ala Pro Ser Ala Ser Gin Met Phe Ser Phe Leu Arg Asn Ser
50      55      60
As Val Pro Leu Glu Lys Asn Pro Lys Leu Lys Thr His Ala Met Ser
65      70      75      80
Val Phe Val Met Thr Cys Glu Ala Ala Ala Gin Leu Arg Lys Ala Gly
85      90      95
Lys Val Thr Val Arg Asp Thr Thr Leu Lys Arg Leu Gly Ala Thr His
100     105     110
Phe Lys Tyr Gly Val Gly Asp Ala His Phe Glu Val Thr Arg Phe Ala
115     120     125
Leu Leu Glu Thr He Lys Glu Ala Val Pro Val Asp Met Trp Ser Pro
130     135     140
Ala Met Lys Ser Ala Trp Ser Glu Ala Tyr Asn Gin Leu Val Ala Ala
145     150     155     160
He Lys Gin Glu Met Lys Pro Ala Glu

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165

<210> SEQ ID NO 15 <211> LENGTH:

160 <212> TYPE: PRT

<213> ORGANISM: Arabidopsis thaliana <400>

SEQUENCE: 15

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Me Glu Ser Glu Gly Lys He Val Phe Thr Glu Glu Gin Glu Ala Leu
1      5      10      15
Val Val Lys Ser Trp Ser Val Met Lys Lys Asn Ser Ala Glu Leu Gly
20      25      30
Leu Lys Leu Phe He Lys He Phe Glu He Ala Pro Thr Thr Lys Lys
35      40      45
Me Phe Ser Phe Leu Arg Asp Ser Pro He Pro Ala Glu Gin Asn Pro
50      55      60
Lys Leu Lys Pro His Ala Met Ser Val Phe Val Met Cys Cys Glu Ser
65      70      75      80
Ala Val Gin Leu Arg Lys Thr Gly Lys Val Thr Val Arg Glu Thr Thr
85      90      95
Leu Lys Arg Leu Gly Ala Ser His Ser Lys Tyr Gly Val Val Asp Glu
100     105     110
His Phe Glu Val Ala Lys Tyr Ala Leu Leu Glu Thr He Lys Glu Ala
115     120     125
Val Pro Glu Met Trp Ser Pro Glu Met Lys Val Ala Trp Gly Gin Ala
130     135     140
Tyr Asp His Leu Val Ala Ala He Lys Ala Glu Met Asn Leu Ser As
145     150     155     160

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-continued

Me Gly Phe Thr Asp Lys Gin Glu Ala Leu Val Asn Ser Ser Trp Glu
 1 5 10 15
 Ser Phe Lys Gin Asn Leu Ser Gly Asn Ser He Leu Phe Tyr Thr He
 20 25 30
 He Leu Glu Lys Ala Pro Ala Ala Lys Gly Leu Phe Ser Phe Leu Lys
 35 40 45
 As Thr Ala Gly Val Glu Asp Ser Pro Lys Leu Gin Ala His Ala Glu
 50 55 60
 Gin Val Phe Gly Leu Val Arg Asp Ser Ala Ala Gin Leu Arg Thr Lys
 65 70 75 80
 Gly Glu Val Val Leu Gly Asn Ala Thr Leu Gly Ala He His Val Gin
 85 90 95
 Arg Gly Val Thr Asp Pro His Phe Val Val Val Lys Glu Ala Leu Leu
 100 105 110
 Gin Thr He Lys Lys Ala Ser Gly Asn Asn Trp Ser Glu Glu Leu As
 115 120 125
 Thr Ala Trp Glu Val Ala Tyr Asp Gly Leu Ala Thr Ala He Lys Lys
 130 135 140
 Ala Met Thr

145

<210> SEQ ID NO 17 <211> LENGTH:

145 <212> TYPE: PRT

<213> ORGANISM: Vigna unguiculata <400>

SEQUENCE: 17

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

Ala Glu Leu Ala Tyr Asp Glu Leu Ala Ala Ala lie Lys Lys Ala 135 140
 Val
 130 Tyr
 145

-continued

Me Gly Leu Ser AspGly Glu Trp Gin Leu Val Leu AsnAla Trp Gly
 1 5 10 15
 LysVal Glu Ala AspVal Ala Gly His Gly Gin Glu Val Leu He Arg
 20 25 30
 LeuPhe Thr GlyHis Pro Glu Thr Leu Glu Lys Phe AspLys Phe Lys
 35 40 45
 His LeuLys Thr Glu Ala Glu MetLys Ala Ser Glu AspLeu Lys Lys
 50 55 60
 His Gly Asn Thr Val Leu Thr Ala Leu Gly Gly He Leu Lys Lys Lys
 65 70 75 80
 GlyHis His GluAla Glu Val Lys His LeuAla Glu Ser His Ala Asn
 85 90 95
 LysHis Lys He Pro Val Lys Tyr Leu Glu Phe He Ser AspAla He
 100 105 110
 He His Val LeuHis Ala Lys His Pro Ser AspPhe Gly Ala AspAla
 115 120 125
 GinAla Ala Me Ser Lys Ala Leu Glu Leu Phe Arg AsnAspMetAla
 130 135 140
 Ala Gin Tyr LysVal Leu Gly Phe His Gly
 145 150

<210> SEQ ID
 NO 19
 <211> LENGTH:
 <212> PR
 <213> Sus serofa
 ORGANISM:
 <400>
 SEQUENCE: 19

Me Gly Leu Ser AspGly Glu Trp Gin Leu Val Leu AsnVal Trp Gly
 1 5 10 15
 LysVal Glu Ala AspVal Ala Gly His Gly Gin Glu Val Leu He Arg
 20 25 30
 LeuPhe Lys GlyHis Pro Glu Thr Leu Glu Lys Phe AspLys Phe Lys
 35 40 45
 His LeuLys Ser Glu AspGlu MetLys Ala Ser Glu AspLeu Lys Lys
 50 55 60
 His Gly Asn Thr Val Leu Thr Ala Leu Gly Gly He Leu Lys Lys Lys
 65 70 75 80
 GlyHis His GluAla Glu Leu Thr Pro LeuAla Gin Ser His Ala Thr
 85 90 95
 LysHis Lys He Pro Val Lys Tyr Leu Glu Phe He Ser Glu Ala He
 100 105 110
 He GinVal LeuGin Ser Lys His Pro Gly Asn Phe Gly Ala AspAla
 115 120 125
 GinGly Ala Me Ser Lys Ala Leu Glu Leu Phe Arg AsnAspMetAla
 130 135 140
 Ala Lys Tyr LysGlu Leu Gly Phe Gin Gly

145 150

-continued

Me Gly Leu Ser Asp Gly Glu Trp Gin Gin Val Leu Asn Val Trp Gly
 1 5 10 15
 Lys Val Glu Ala Asp He Ala Gly His Gly Gin Glu Val Leu He Arg
 20 25 30
 Leu Phe Thr Gly His Pro Glu Thr Leu Glu Lys Phe Asp Lys Phe Lys
 35 40 45
 His Leu Lys Thr Glu Ala Glu Met Lys Ala Ser Glu Asp Leu Lys Lys
 50 55 60
 His Gly Thr Val Val Leu Thr Ala Leu Gly Gly He Leu Lys Lys Lys
 65 70 75 80
 Gly His His Glu Ala Glu Leu Lys Pro Leu Ala Gin Ser His Ala Thr
 85 90 95
 Lys His Lys He Pro He Lys Tyr Leu Glu Phe He Ser Asp Ala He
 100 105 110
 He His Val Leu His Ser Lys His Pro Gly Phe Gly Ala Asp Ala
 115 120 125
 Gin Gly Ala Met Thr Lys Ala Leu Glu Leu Phe Arg Asn Asp He Ala
 130 135 140
 Ala Lys Tyr Lys Glu Leu Gly Phe Gin Gly

145 150

<210> SEQ ID NO: 21 <211> LENGTH: 152 <212> TYPE: PRT

<213> ORGANISM: Nicotiana benthamiana

<400> SEQUENCE: 21

Me Ser Ser Phe Thr Glu Glu Gin Glu Ala Leu Val Val Lys Ser Trp
 1 5 10 15
 Asn Ser Met Lys Lys Asn Ala Gly Glu Trp Gly Leu Lys Leu Phe Leu
 20 25 30
 Lys He Phe Glu He Ala Pro Ser Ala Lys Lys Leu Phe Ser Phe Leu
 35 40 45
 Lys Asp Ser Asn Val Pro Leu Glu Gin Asn Ala Lys Leu Lys Pro His
 50 55 60
 Ser Lys Ser Val Phe Val Met Thr Cys Glu Ala Ala Val Gin Leu Arg
 65 70 75 80
 Lys Ala Gly Lys Val Val Val Arg Asp Ser Thr Leu Lys Lys Leu Gly
 85 90 95
 Ala Thr His Phe Lys Tyr Gly Val Ala Asp Glu His Phe Glu Val Thr
 100 105 110
 Lys Phe Ala Leu Leu Glu Thr He Lys Glu Ala Val Pro Glu Met Trp
 115 120 125
 Ser Val Asp Met Lys Asn Ala Trp Gly Glu Ala Phe Asp Gin Leu Val
 130 135 140
 Asn Ala He Lys Thr Glu Met Lys

145 150

Me Gly Gin Ser Phe Asn Ala Pro Tyr Glu Ala He Gly Glu Glu Leu
 1 5 10 15
 Leu Ser Gin Leu Val Asp Thr Phe Tyr Glu Arg Val Ala Ser His Pro
 20 25 30
 Leu Leu Lys Pro He Phe Pro Ser Asp Leu Thr Glu Thr Ala Arg Lys
 35 40 45
 Gin Lys Gin Phe Leu Thr Gin Tyr Leu Gly Gly Pro Pro Leu Tyr Thr
 50 55 60
 Glu Glu His Gly His Pro Met Leu Arg Ala Arg His Leu Pro Phe Pro
 65 70 75 80
 He Thr Asn Glu Arg Ala Asp Ala Trp Leu Ser Cys Met Lys Asp Ala
 85 90 95
 Me Asp His Val Gly Leu Glu Gly Glu He Arg Glu Phe Leu Phe Gly
 100 105 110
 Arg Leu Glu Leu Thr Ala Arg His Met Val Asn Gin Thr Glu Ala Glu
 115 120 125
 Asn Arg Ser Ser

130

~~<210> SEQ ID NO 23 <211> LENGTH: 131 <212>~~
~~TYPE: PRT~~

~~<213> ORGANISM: Corynebacterium glutarnicum <400>~~

~~SEQUENCE: 23~~

Me Thr Thr Ser Glu Asn Phe Tyr Asp Ser Val Gly Gly Glu Glu Thr
 1 5 10 15
 Phe Ser Leu He Val His Arg Phe Tyr Glu Gin Val Pro Asn Asn
 20 25 30
 He Leu Gly Pro Met Tyr Pro Pro Asp Asp Phe Glu Gly Ala Glu Gin
 35 40 45
 Arg Leu Lys Met Phe Leu Ser Gin Tyr Trp Gly Gly Pro Lys Asp Tyr
 50 55 60
 Gin Glu Gin Arg Gly His Pro Arg Leu Arg Met Arg His Val Asn Tyr
 65 70 75 80
 Pro He Gly Val Thr Ala Ala Glu Arg Trp Leu Gin Leu Met Ser
 85 90 95
 Ala Leu Asp Gly Val Asp Leu Thr Ala Glu Gin Arg Glu Ala He Trp
 100 105 110
 Glu His Met Val Arg Ala Ala Asp Met Leu He Asn Ser Asn Pro
 115 120 125
 Pro His Ala
 130

~~<210> SEQ ID NO 24 <211> LENGTH: 124 <212> TYPE: PRT~~

~~<213> ORGANISM: Synechocystis sp.~~

~~<400> SEQUENCE: 24~~

Met Ser Thr Leu Tyr Glu Lys Leu Gly Gly Thr Thr Ala Val Asp Leu 15 10
 15

Ala Val Asp Lys Phe Tyr Glu Arg Val Leu Gin Asp Asp Arg Ile Lys 20 25
 30

-continued

His Phe Phe Ala Asp Val Asp Met Ala Lys Gin Arg Ala His Gin Lys
 35 40 45
 Ala Phe Leu Thr Tyr Ala Phe Gly Gly Thr Asp Lys Tyr Asp Gly
 50 55 60
 Tyr Met Arg Glu Ala His Lys Glu Leu Val Glu Asn His Gly Leu As
 65 70 75 80
 Gly Glu His Phe Asp Ala Val Ala Glu Asp Leu Leu Ala Thr Leu Lys
 85 90 95
 Glu Met Gly Val Pro Glu Asp Leu He Ala Glu Val Ala Ala Val Ala
 100 105 110
 Gly Ala Pro Ala His Lys Arg Asp Val Leu Asn Gin
 115 120

<210> SEQ ID NO 25 <211> LENGTH: 183 <212>

TYPE: PRT

<213> ORGANISM: Synechococcus sp.

<400> SEQUENCE: 25

Me Asp Val Ala Leu Leu Glu Lys Ser Phe Glu Gin He Ser Pro Arg
 1 5 10 15
 Ala He Glu Phe Ser Ala Ser Phe Tyr Gin Asn Leu Phe His His His
 20 25 30
 Pro Glu Leu Lys Pro Leu Phe Ala Glu Thr Ser Gin Thr He Gin Glu
 35 40 45
 Lys Lys Leu He Phe Ser Leu Ala Ala He He Glu Asn Leu Arg As
 50 55 60
 Pro Asp He Leu Gin Pro Ala Leu Lys Ser Leu Gly Ala Arg His Ala
 65 70 75 80
 Glu Val Gly Thr He Lys Ser His Tyr Pro Leu Val Gly Gin Ala Leu
 85 90 95
 He Glu Thr Phe Ala Glu Tyr Leu Ala Ala Asp Trp Thr Glu Gin Leu
 100 105 110
 Ala Thr Ala Trp Val Glu Ala Tyr Asp Val He Ala Ser Thr Met He
 115 120 125
 Glu Gly Ala Asp Asn Pro Ala Ala Tyr Leu Glu Pro Glu Leu Thr Phe
 130 135 140
 Tyr Glu Trp Leu Asp Leu Tyr Gly Glu Glu Ser Pro Lys Val Arg As
 145 150 155 160
 Ala He Ala Thr Leu Thr His Phe His Tyr Gly Glu Asp Pro Gin As
 165 170 175
 Val Gin Arg Asp Ser Arg Gly

180

<210> SEQ ID NO 26 <211> LENGTH: 118 <212>

TYPE: PRT

<213> ORGANISM: Nostoc commune

<400> SEQUENCE: 26

Me Ser Thr Leu Tyr Asp Asn He Gly Gly Gin Pro Ala He Glu Gin
 1 5 10 15
 Val Val Asp Glu Leu His Lys Arg He Ala Thr Asp Ser Leu Leu Ala

20 25 30

Pro Val Phe Ala Gly Thr Asp Met Val Lys Gin Arg Asn His Leu Val 35 40
 45

~~<210> SEQ ID NO 27 <211> LENGTH: 136 <212> TYPE: PRT~~

~~<213> ORGANISM: Bacillus megaterium <400> SEQUENCE: 27~~

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Me Arg Glu Lys He His Ser Pro Tyr Glu Leu Leu Gly Gly Glu His
1      5      10      15
Thr He Ser Lys Leu Val Asp Ala Phe Tyr Thr Arg Val Gly Gin His
      20      25      30
Pro Glu Leu Ala Pro He Phe Pro Asp Asn Leu Thr Glu Thr Ala Arg
      35      40      45
Lys Gin Lys Gin Phe Leu Thr Gin Tyr Leu Gly Gly Pro Ser Leu Tyr
      50      55      60
Thr Glu Glu His Gly His Pro Met Leu Arg Ala Arg His Leu Pro Phe
65      70      75      80
Glu He Thr Pro Ser Arg Ala Lys Ala Trp Leu Thr Cys Met His Glu
      85      90      95
Ala Met Asp Glu He Asn Leu Glu Gly Pro Glu Arg Asp Glu Leu Tyr
      100      105      110
His Arg Leu He Leu Thr Ala Gin His Met He Asn Ser Pro Glu Gin
      115      120      125
Thr Asp Glu Lys Gly Phe Ser His
      130      135

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What is claimed is:

45

1. — A food flavor additive composition comprising:
 1. — an isolated heme-containing protein;
2. — a compound selected from glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, maltodextrin, and galactose, and mixtures of two or more thereof; and
3. — a compound selected from cysteine, cystine, thiamine, methionine, and mixtures of two or more thereof; wherein the flavor additive composition contains no animal products; and
 - 55 wherein cooking the food flavor additive composition results in the production of at least two volatile compounds which have a meat-associated aroma.
 4. — The food flavor additive composition of claim 1, wherein the heme-containing protein is selected from the
 - 60 group consisting of a androglobin, a cytoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a myoglobin, a leghemoglobin, an erythrocrucorin, a beta hemoglobin, an alpha hemoglobin, a protoglobin, a cyanoglobin, a cytoglobin, a histoglobin, a neuroglobins, a chlorocruorin, a 65-truncated hemoglobin, a truncated 2/2 globin, and a hemoglobin 3.

5. The food flavor additive composition of claim 1, wherein the heme-containing protein comprises amino acid sequence having at least 80% sequence identity to a polypeptide set forth in SEQ ID NOs: 1-26.

6. The food flavor additive composition of claim 1, wherein the food flavor additive composition is a powder or a liquid composition.

7. The food flavor additive composition of claim 1, wherein the heme-containing protein is a plant, a yeast or filamentous fungi, or bacterial heme-containing protein.

8. The food flavor additive composition of claim 1, further comprising one or more of an extract or hydrolysate selected from a vegetable protein hydrolysate, a soy protein hydrolysate, a yeast extract, a yeast protein hydrolysate, and an algal protein hydrolysate.

9. The food flavor additive composition of claim 1, further comprising one or more of a compound selected from alanine, arginine, asparagine, aspartate, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, maltodextrin, thiamine, IMP, GMP, lactic acid, and creatine.

10. The food flavor additive composition of claim 1, further comprising one or more of an oil selected from coconut oil, mango oil, sunflower oil, cottonseed oil, safflower oil, rice bran oil, cocoa butter, palm fruit oil, palm oil, soybean oil, canola oil, corn oil, sesame oil, walnut oil, flaxseed, jojoba oil, castor, grapeseed oil, peanut oil, olive oil, algal oil, and oil from bacteria or fungi.

11. The food flavor additive composition of claim 1, further comprising one or more of albumin or a globulin.

12. A food flavor additive composition prepared by a process of:

1. combining an isolated heme-containing protein; a compound selected from glucose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose and galactose, and mixtures of two or more thereof and a compound selected from cysteine, cysteine, thiamine, methionine, and mixtures of two or more thereof, to form a heme flavor reaction mixture; and

2. heating the heme flavor reaction mixture to at least 150° C. for at least 1 minute to result in a food flavor additive composition;

wherein the food flavoring additive composition contains no animal products; and

wherein the heme flavor reaction mixture has a meaty flavor and/or meaty aroma during the heating of step b) and the food flavoring additive composition has a meaty flavor and/or meaty aroma after the heating of step b).

3. A food flavor additive composition of claim 10,

wherein the food flavoring wherein the food flavoring additive composition further comprises a compound selected from the group consisting of 2-methyl furan, bis(2-methyl-3-furyl)di-sulfide, 2-pentyl furan, 3,3'-dithiobis-2-methyl-furan, 2,5-dimethyl pyrazine, 2-methyl-3-furanthiol, bis(2-methyl-4,5-dihydro-3-furyl) disulfide, dihydro-3-(2H)-thiophenone, 5-methyl-2-thiophenecarboxaldehyde,

3-methyl-2-thiophenecarboxaldehyde, 2-methyl-thiazole, dimethyl-sulfide, decanal, 5-ethylidihydro-2(3H)-furanone, dihydro-5-pentyl-2(3H)-furanone, 2-octanone, 3,5-octa-dien-2-one, p-Cresol, and hexanoic acid.

4. The food flavor additive composition of claim 11, wherein the compound is detectable in the headspace above the heme flavor reaction mixture during the heating of step b) or in the headspace above the food flavoring additive composition after the heating of step b).

5. The food flavor additive composition of claim 10, where the process further comprises removing the heme-containing protein from the food flavoring additive composition after the heating in step b) of the heme flavor reaction mixture.

6. The food flavor additive composition of claim 10, wherein the heme-containing protein is present in an amount between about 0.01% and about 5% by weight in the heme flavor reaction mixture.

7. The food flavor additive composition of claim 10, wherein the heme-containing protein is selected from the group consisting of an androglobin, a cytoglobin, a globin-E, a globin-X, a globin-Y, a hemoglobin, a myoglobin, a leghemoglobin, an erythrocyruorin, a beta hemoglobin, an alpha hemoglobin, a protoglobin, a cyanoglobin, a cytoglobin, a histoglobin, a neuroglobins, a chlorocruorin, a truncated hemoglobin, a truncated 2/2 globin, and a hemoglobin 3.

8. The food flavor additive composition of claim 10, wherein the heme-containing protein comprises an amino acid sequence having at least 80% sequence identity to a polypeptide set forth in SEQ ID NOs: 1-26.

9. The food flavor additive composition of claim 10, wherein the heme flavor reaction mixture further comprises one or more oils selected from coconut oil, mango oil, sunflower oil, cottonseed oil, safflower oil, rice bran oil, cocoa butter, palm fruit oil, palm oil, soybean oil, canola oil, corn oil, sesame oil, walnut oil, flaxseed, jojoba oil, castor, grapeseed oil, peanut oil, olive oil, algal oil, and oil from bacteria or fungi.

10. The food flavor additive composition of claim 10, further comprising an ingredient selected from an emulsifier and a gelling agent.

11. The food flavor additive composition of claim 10, the process further comprising adding an emulsifier and a gelling agent to the heme flavor reaction mixture prior to the heating of step b).

12. The food flavor additive composition of claim 10, wherein the heme flavor reaction mixture is an aqueous solution.

13. The food flavor additive composition of claim 10, wherein the heme flavor reaction mixture is an oil-based mixture.

14. The food flavor additive composition of claim 10, further comprising one or more of an extract or hydrolysate selected from a vegetable protein hydrolysate, a soy protein hydrolysate, a yeast extract, a yeast protein hydrolysate, and an algal protein hydrolysate, and/or a globulin.

15. A process for imparting a meaty flavor and/or meaty aroma to a consumable food product, comprising: adding to the consumable food product a food flavor additive composition of claim 1; and heating the consumable food product after the adding step.

16. The food flavor additive composition of claim 1, in the form of a soup, stew base, a bouillon powder, bouillon cube, flavor packets or seasoning packet.