

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

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is related to the following co-pending patent applications:
application Serial No. PCT/US12/46560; application Serial No PCT/US12/46552;
application Serial No. 61/751,816, filed January 11, 2013; application Serial No.
5 61,876,676, filed September 11, 2013; and application Serial No. 61/751,818, filed
January 11, 2013, all of which are incorporated herein by reference.

TECHNICAL FIELD

This invention relates to food products and more particularly, to food products
that include heme-containing proteins and one or more flavor precursor molecules.

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BACKGROUND

Food is any substance that is either eaten or drunk by any animal, including
humans, for nutrition or pleasure. It is usually of plant or animal origin, and can contain
essential nutrients, such as carbohydrates, fats, proteins, vitamins, or minerals. The
15 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.

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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.

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Current plant-based meat substitutes have largely failed to cause a shift to a
vegetarian diet. Meat substitute compositions are typically extruded soy/grain mixtures

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

SUMMARY

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

As provided herein, and without being bound by theory, certain characteristic meaty flavors and/or aromas (e.g., beefy, bacony, umami, savory, bloody, brothy, gravy, metallic, bouillon-like; see Tables 2 and 7), including one or more specific chemical compounds associated with the same (see Tables 3, 8, and 9), 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 heme cofactor (e.g., an isolated heme moiety; a heme moiety bound to a protein) in the food product. In some embodiments, the flavors and/or aromas are not generated in the absence of the heme (e.g., in the absence of a heme-containing protein). Accordingly, as

described herein, 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 heme proteins, 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) can generate or provide a range of savory and meaty aromas and tastes (see, e.g., Tables 2 and 7) 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, sunflower oil, coconut oil, and mango 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 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 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 or bacon. 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 beef-like.

Food products for use in the present disclosure include those that have a heme protein and one or more flavor precursor molecules included therein. The heme protein and the one or more flavor precursor molecules can be homogenously or heterogeneously included in the food products. The heme protein can be isolated and purified prior to inclusion in the food product. Non-limiting examples of consumable food products which can include a heme protein and one or more flavor precursor molecules include

animal-based or non-animal (e.g., plant-based), or combinations of animal-based and non-animal-based, food products in the form of hot dogs, burgers, ground meat, sausages, steaks, filets, roasts, breasts, thighs, wings, meatballs, meatloaf, bacon, strips, fingers, nuggets, cutlets, or cubes.

5 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 a heme 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
10 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
15 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
20 consumable food product before, during, or after cooking of the consumable food product.

In some embodiments, a flavor additive composition including 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
25 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 heme-protein and 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
30 flavors.

In one aspect, this document features a food product that includes a heme-containing protein (e.g., a plant, mammalian, a yeast or filamentous fungi, or bacterial heme-containing protein); 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, sunflower oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a hydrolyzed protein, a malt extract, a yeast extract, and a peptone. The food product can include 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, 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:1-20. 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, 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 a heme-containing protein, cysteine, and ribose, and one

or more optional flavor precursor molecules to form a mixture, the one or more optional flavor precursor molecules selected from the group consisting of glucose, fructose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, sunflower oil, coconut oil, mango oil, a free fatty acid, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a hydrolyzed protein, 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 2-ethyl furan, 2-Pentanone, 2-pentyl-thiophene, 2-Propenal, 2-Tridecen-1-ol, 4-Octene, 1-Penten-3-one, 2-methyl thiazole, methyl-pyrazine, trans-2-(2-Pentenyl)furan, 3-Ethylcyclopentanone, 2-Thiophenecarboxaldehyde, and 3-Thiophenecarboxaldehyde. The method can include combining lactic acid, lysine, and/or thiamine with the heme-containing protein, cysteine, and ribose.

This document also features a method for producing a flavor compound. The method includes combining a heme-containing protein, cysteine, and glucose, and one or more optional flavor precursor molecules to form a mixture, the one or more optional flavor precursor molecules selected from the group consisting of 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, mango oil, a free fatty acid, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a hydrolyzed protein, 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

benzaldehyde, 2-butanone, dimethyl trisulfide, 2-pentyl-furan, 2-methyl-propanal, thiazole, butyrolactone, 2-acetylthiazole, pentanal, 3-methyl-butanal, methyl-thiirane, nonanal, heptanal, 2,3-butanedione, 1,3,5-cycloheptatriene, propyl-cyclopropane, 2-hexyl-furan, butanal, 2-methyl-butanal, 2-octanone, propanal, trichloromethane, 2-methyl-furan, furan, pyrazine, thiophene, 1,3-dimethyl-benzene, octanal, thiazole, furfural, 2-nonanone, 2-Heptenal, 2-Heptenal, 1-Octene, heptyl ester formic acid, 1-octen-3-one, 3-pentyl-furan, 4-penten-2-one, and pyrrole. The method can include combining lactic acid, lysine, and/or thiamine with the heme-containing protein, cysteine, and glucose.

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

This document also features a method for imparting a beef like flavor to a food product. The method includes contacting the food product with a flavoring composition, the flavoring composition comprising i) a heme-containing protein; and ii) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, sunflower oil, coconut oil, mango oil, a

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

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are
25 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
30 accompanying drawings and the description below. Other features, objects, and

advantages of the invention will be apparent from the description and drawings, and from the claims. The word “comprising” in the claims may be replaced by “consisting essentially of” or with “consisting of,” according to standard practice in patent law.

DESCRIPTION OF THE DRAWINGS

FIG. 1 is a bar graph of the amount of 4-methyl-5-Thiazoleethanol, 3,3'-dithiobis[2-methyl-furan, and 4-Methylthiazole compounds that were created by heating a mixture of LegH, thiamine, a sugar (either glucose or ribose), and cysteine at 150 C for 3 minutes. The reactions shown in bars 1-4 contained LegH in the mixture and the reactions shown in bars 5-8 did not contain LegH.

FIG. 2 is a bar graph of flavor compounds created with Heme protein (1%) in a reaction at pH 6, with cysteine (10 mM), and glucose (20 mM), IMP (1 mM) and guanosine monophosphate (GMP) (1 mM).

FIG. 3 contains the amino acid sequences of exemplary heme-containing proteins.

DETAILED DESCRIPTION

This document is based on methods and materials for modulating the taste and/or aroma profile of food products. As described herein, compositions containing one or more flavor precursors and one or more heme-containing proteins can be used to modulate the taste and/or aroma profile of food products.

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, hydrolyzed proteins, extracts, phospholipids, lecithin, and organic molecules. Non-limiting examples of such flavor precursors are provided in Table 1.

TABLE 1

Flavor Precursor Molecules

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

lactose, ribitol, gluconic acid and glucuronic acid, amylose, amylopectin, or xylose
Oils: coconut oil, mango oil, sunflower oil, cocoa butter, palm fruit oil, palm oil, soybean oil, canola oil, corn oil, sesame oil, walnut oil, grapeseed oil, peanut oil, or olive oil
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, allicin, selenocysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, 5-hydroxytryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, or tyrosine
Nucleosides and Nucleotides: inosine, inosine monophosphate (IMP), guanosine, guanoside monophosphate (GMP), adenosine, adenosine monophosphate (AMP)
Vitamins: thiamine, vitamin C
Misc: phospholipid, lecithin, pyrazine, creatine, pyrophosphate
Acids: alpha hydroxy acids such as lactic acid or glycolic acid, citric acid, acetic acid, dicarboxylic acid such as succinic acid or tartaric acid
Peptides and hydrolyzed proteins: glutathione, vegetable hydrolyzed proteins, soy hydrolyzed proteins, yeast hydrolyzed proteins, algal hydrolyzed proteins, meat hydrolyzed proteins
Extracts: a malt extract, a yeast extract, and a peptone

In some embodiments, combinations of 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 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).

5 As used herein, the term “heme containing proteins” includes any polypeptide that can covalently or noncovalently bind to 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
10 example, a heme-containing protein can be a non-symbiotic type of hemoglobin or a leghemoglobin. A heme-containing polypeptide can be a monomer, i.e., a single polypeptide chain, or can be a dimer, a trimer, tetramer, and/or higher order oligomers. The life-time of the oxygenated Fe^{2+} state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or more.

15 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 erythrocrucorin, a beta hemoglobin, an alpha hemoglobin, a protoglobin, a cyanoglobin, a cytoglobin, a histoglobin, a neuroglobins, a chlorocruorin, a truncated hemoglobin (e.g., HbN or HbO), a truncated 2/2 globin, a hemoglobin 3 (e.g., Glb3), a
20 cytochrome, and 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
25 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
30 beans such as green beans, black beans, navy beans, northern beans, or pinto beans,

Vigna unguiculata varieties (cow peas), *Vigna radiate* (Mung beans), *Lupinus albus* (lupin), or *Medicago sativa* (alfalfa); *Brassica napus* (canola); *Triticum* *sps.* (wheat, including wheat berries, and spelt); *Gossypium hirsutum* (cotton); *Oryza sativa* (rice); *Zizania* *sps.* (wild rice); *Helianthus annuus* (sunflower); *Beta vulgaris* (sugarbeet);
5 *Pennisetum glaucum* (pearl millet); *Chenopodium* *sp.* (quinoa); *Sesamum* *sp.* (sesame); *Linum usitatissimum* (flax); or *Hordeum vulgare* (barley). Heme-containing proteins can be isolated from fungi such as *Saccharomyces cerevisiae*, *Pichia pastoris*, *Magnaporthe oryzae*, *Fusarium graminearum*, or *Fusarium oxysporum*. Heme-containing proteins can be isolated from bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Synechocystis* *sp.*,
10 *Aquifex aeolicus*, *Methylobacterium inferorum*, or thermophilic bacteria such as *Thermophilus*. The sequences and structure of numerous heme-containing proteins are known. See for example, Reedy, *et al.*, *Nucleic Acids Research*, 2008, Vol. 36, Database issue D307–D313 and the Heme Protein Database available on the world wide web at <http://hemeprotein.info/heme.php>.

15 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
20 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. 3, including a non-
25 symbiotic hemoglobin such as that from *Vigna radiata* (SEQ ID NO:1), *Hordeum vulgare* (SEQ ID NO:5), *Zea mays* (SEQ ID NO:13), *Oryza sativa subsp. japonica* (rice) (SEQ ID NO:14), or *Arabidopsis thaliana* (SEQ ID NO:15), a Hell's gate globin I such as that from *Methylobacterium inferorum* (SEQ ID NO:2), a flavohemoprotein such as that from *Aquifex aeolicus* (SEQ ID NO:3), a leghemoglobin such as that from *Glycine max*
30 (SEQ ID NO:4), *Pisum sativum* (SEQ ID NO:16), or *Vigna unguiculata* (SEQ ID

NO:17), a heme-dependent peroxidase such as from *Magnaporthe oryzae*, (SEQ ID NO:6) or *Fusarium oxysporum* (SEQ ID NO:7), a cytochrome c peroxidase from *Fusarium graminearum* (SEQ ID NO:8), a truncated hemoglobin from *Chlamydomonas moewusii* (SEQ ID NO:9), *Tetrahymena pyriformis* (SEQ ID NO:10, group I truncated),
5 *Paramecium caudatum* (SEQ ID NO:11, group I truncated), a hemoglobin from *Aspergillus niger* (SEQ ID NO:12), or a mammalian myoglobin protein such as the *Bos taurus* (SEQ ID NO:18) myoglobin, *Sus scrofa* (SEQ ID NO:19) myoglobin, or *Equus caballus* (SEQ ID NO:20) myoglobin.

The percent identity between two amino acid sequences can be determined as follows. First, the amino acid sequences are aligned using the BLAST 2 Sequences (Bl2seq) program from the stand-alone version of BLASTZ containing BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from Fish & Richardson's web site (e.g., www.fr.com/blast/) or the U.S. government's National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov). Instructions explaining
15 how to use the Bl2seq program can be found in the readme file accompanying BLASTZ. Bl2seq performs a comparison between two amino acid sequences using the BLASTP algorithm. To compare two amino acid sequences, the options of Bl2seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second amino acid sequence to be compared
20 (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two amino acid sequences: C:\Bl2seq -i c:\seq1.txt -j c:\seq2.txt -p blastp -o c:\output.txt. If the two compared sequences share homology, then the designated output file will present
25 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
30 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
5 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
10 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.,
15 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
20 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
25 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.
30 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, antibody to the 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, 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.

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 10, 15, 20, 25, 50, 100, 150, 200, or 250g/L.

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

Modulating Flavor and/or Aroma Profiles

As described herein, different combinations of flavor precursors can be used with one or more heme-containing proteins to produce different flavor and aroma profiles when the flavor precursors and heme proteins 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 heme-containing protein, the temperature of the reaction, and the amount of water activity in the product, among other factors.

5 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-containing proteins. See also Table 7.

TABLE 2

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

yeasty	goaty
vegetable broth	

Flavor and aroma profiles are created by different chemical compounds formed by chemical reactions between the heme-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 the heme-containing protein and one or more flavor precursors.

Table 3 provides non-limiting examples of compounds that can be produced.

TABLE 3

Compounds Produced

Tridecane	Butanal, 3-methyl-	2-Nonenal, (E)-
trans-2-(2-Pentenyl)furan	Butanal, 2-methyl-	2-Nonanone
Thiophosphordiamide, S-methyl ester	Butanal	2-n-Octylfuran
Thiophene, 2-pentyl-	Benzene, 1,3-dimethyl-	2-n-Heptylfuran
Thiophene	Benzene, 1,3-bis(1,1-dimethylethyl)-	2-Methyl-3-furanthiol
Thiirane, methyl-	Benzene	2-Hexenal, (E)-
Thiirane	Benzaldehyde	2-Heptenal, (Z)-
Thiazole, 2-methyl-	Acetaldehyde	2-Heptenal, (E)-
Thiazole	5-Thiazoleethanol, 4-methyl-	2-Heptanone
Silanol, trimethyl-	5-Hepten-2-one, 6-methyl-	2-Decanone
Pyrrole	4-Penten-2-one	2-Butanone
Pyridine	4-Octene, (E)-	2-Acetylthiazole
Pyrazine, methyl-	4-Methylthiazole	1,3,5-Cycloheptatriene
Pyrazine	3-Thiophenecarboxaldehyde	1,2,5-Oxadiazole-3-carbonitrile, 4-amino-

Propanenitrile, 3-(1-methylethoxy)-	3-Pentanone, 2-methyl-	1,2-Benzisothiazol-3(2H)-one
Propanal, 2-methyl-	3-Ethylcyclopentanone	1-Undecene, 8-methyl-
Propanal	2,4-Dimethyl-1-heptene	1-Propen-2-ol, acetate
Phenylacetaldehyde	2,3-Pentanedione	1-Penten-3-one
Phenol, 2,4-bis(1,1-dimethylethyl)-	2,3-Butanedione	1-Pentanol
Pentane, 3-ethyl-2,2-dimethyl-	2-Tridecen-1-ol, (E)-	1-Octene
Pentane, 3-ethyl-	2-Thiophenemethanamine	1-Octen-3-one
Pentane, 2,3,4-trimethyl-	2-Thiophenecarboxaldehyde	1-Octanol
Pentanal	2-Propenal	1-Hexanol, 2-ethyl-
Octane, 2,4,6-trimethyl-	2-Pentanone	1-Heptene, 2-methyl-
Octane	2-Octenal, (E)-	1-Decen-3-one
Octanal	2-Octanone	1-(Ethylthio)-2-(methylthio)-buta-1,3-diene
Nonane, 2,6-dimethyl-	2-Nonenal, (Z)-	Carbon disulfide
Nonanal	Methyl thiolacetate	Butyrolactone
Furan, 2-propyl-	Methyl ethanoate	Dimethyl trisulfide
Furan, 2-pentyl-	Methanesulfonyl chloride	Dimethyl sulfide
Furan, 2-methyl-	Isothiazole	Dihydro-3-(2H)-thiophenone
Furan, 2-hexyl-	Isopropyl Alcohol	Decane, 2,2,6-trimethyl-
Furan, 2-ethyl-	Hexane, 3,3-dimethyl-	Decanal
Furan	Heptane, 4-methyl-	Cyclopropane, propyl-
Formic acid, heptyl ester	Heptane, 2,4-dimethyl-	Heptanal
Ethanol	Heptane, 2,3,4-trimethyl-	Furfural

Ethanethiol	Heptane, 2-methyl-	Furan, 3,3'-dithiobis[2-methyl-
Furan, 3-pentyl-		

In some embodiments, a heme-containing protein described herein is heated in the presence of ground chicken, to increase specific volatile flavor and odorant components typically elevated in beef. For example, propanal, butanal, furan 2-ethyl, heptanal, 5 octanal, trans-2-(2-pentenyl)furan, 2-heptenal (Z), 2-octenal (E), pyrrole, 2,4-dodecadienal, 1-octanal, 2-decenal (Z), 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, a heme-containing protein described herein is heated in the presence of cysteine and glucose to provide a different profile of volatile odorants than 10 when any subset of the three components are used individually. Volatile flavor components that are increased under these conditions include but are not limited to furan, acetone, thiazole, furfural, benzaldehyde, 2-pyridinecarboxaldehyde, 5-methyl-2-thiophenecarboxaldehyde, 3-methyl-2-thiophenecarboxaldehyde, 3-thiophenmethanol and decanol. See, e.g., Tables 8 and 9. Under these conditions, cysteine and glucose 15 alone or in the presence of iron salts such as ferrous glucanate produced a sulfurous, odor, but addition of heme-containing proteins reduced the sulfurous odor and replaced it with flavors including but not limited to chicken broth, burnt mushroom, molasses, and bread.

In some embodiments, a heme-containing protein described herein is heated in the 20 presence of cysteine and ribose to provide a different profile of volatile odorants. Heating in the presence of ribose created some additional compounds as compared to when a heme-containing protein and glucose were heated together. See Tables 8 and 9.

In some embodiments, a heme-containing protein described herein can be heated 25 in the presence of thiamine and a sugar to affect the formation of 5-Thiazoleethanol, 4-methyl-furan, 3,3'-dithiobis[2-methyl-furan, and 4-Methylthiazole. These compounds are known to be present in meat and have beefy, meaty taste notes.

In some embodiments, 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 4-octene (E), Furan, 2-ethyl, 2-pentanone, 2,3-butanedione, thiazole, 2-methyl, pyrazine, methyl-,
5 tridecane, 2-octenal (E), 2-thiopenecarboxaldehyde, and 3-thiopenecarboxaldehyde. These compounds are known to be present in meat and have a beefy, meaty, buttery, and or savory flavor notes.

In some embodiments, a heme-containing protein can be heated in the presence of lysine, a sugar such as ribose, and cysteine to control the formation of flavor compounds
10 such as dimethyl trisulfide, nonanal, thiophene, 2-pentyl, furfural, 2-nonenal, 1-octanol, 2-nonenal, thiazole, 2-acetylthiazole, phenylacetaldehyde, and 2-acetylthiazole. These compounds are known to be present in meat and some have a beefy, meaty, and or savory flavor.

In some embodiments, a heme-containing protein can be heated in the presence of
15 lactic acid, a sugar such as ribose, and cysteine to control the formation of the flavor compounds nonanal, thiazole, 2-acetylthiazole, and 1-undecene, 8-methyl. These compounds are known to be present in meat and have beefy, savory, browned, bready, and malty notes.

In some embodiments, a heme protein and one or more flavor precursors can be
20 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, specific flavor compounds, such as those described in
25 Tables 3, 8, or 9, 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
30 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

5 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, 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,
10 thighs, wings, meatballs, meatloaf, bacon, strips, fingers, nuggets, cutlets, or cubes.

In addition, food products described herein can be used to modulate the taste and/or aroma profile of other food products (e.g., meat replicas, meat substitutes, tofu, mock duck or other gluten based vegetable product, textured vegetable protein such as textured soy protein, or poultry products such as chicken or turkey products) and can be
15 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
20 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
25 gluten, pea vicilin, and/or pea legumin), protein concentrates (e.g., soy protein concentrate), emulsifiers (e.g., lecithin), gelling agents (e.g., k-carrageenan or gelatin), fibers (e.g., bamboo 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,
30 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, rosemary extract, green tea extract, catechins and other anti-oxidants.

Food products described herein can be free of animal products (e.g., animal heme-
5 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.

10 *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-
15 digit numbers and rotated in ballot position to prevent bias. Sensory judgments can be scaled for “acceptance” or “likeability” or use special terminology. For example, letter scales (A for excellent, B for good, C for poor) or number scales may be used (1 = dislike, 2 = fair, 3 = good; 4 = very good; 5=excellent). A scale can be used to rate the overall acceptability or quality of the food product or specific quality attributes such
20 beefiness, texture, and flavor. Panelists can be encouraged to rinse their mouths with water between samples, and given opportunity to comment on each sample.

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

In some embodiments, an olfactometer allows the training and automatic evaluation of expert panels. In some embodiments, a food product described herein causes similar or identical olfactometer readings. In some embodiments, the differences
30 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
5 more flavor precursors.

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

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

20 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
25 meat like than previously known meat alternatives.

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

EXAMPLES

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

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

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

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

TABLE 4
Replica Burger Ingredients

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

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

TABLE 5**Sensory evaluation of replica burger with Heme**

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

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

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

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

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

	with precursors	without precursors
Average	3.5	1.8
STDV	0.6	0.5

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

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

TABLE 7**Flavors generated by addition of precursors to LegH (1 %)**

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

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

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

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

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

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

TABLE 8

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

LegH 1%		
Compounds created	cysteine (10 mM), glucose (20 mM)	cysteine (10 mM), ribose (20 mM)
Benzaldehyde	X	X
2-Butanone	X	X
Dimethyl trisulfide	X	X
Furan, 2-pentyl-	X	X
Propanal, 2-methyl-	X	X
Thiazole	X	X
Butyrolactone	X	X
2-Acetylthiazole	X	X
Pentanal	X	X
Butanal, 3-methyl-	X	X
Thiirane, methyl-	X	X
Nonanal	X	X
Heptanal	X	X
2,3-Butanedione	X	X
1,3,5-Cycloheptatriene	X	X
Cyclopropane, propyl-	X	X
Furan, 2-hexyl-	X	X
Butanal	X	X
Butanal, 2-methyl-	X	X
Furan, 2-ethyl-		X
2-Octanone	X	X
Propanal	X	X

Trichloromethane	X	
Furan, 2-methyl-	X	X
Furan	X	X
Pyrazine	X	X
Thiophene	X	X
Benzene, 1,3-dimethyl-	X	X
Octane		X
Octanal	X	X
Thiazole	X	X
2-Pentanone		X
Furfural	X	X
2-Nonanone	X	X
2-Heptenal, (Z)-	X	X
2-Heptenal, (E)-	X	X
1-Octene	X	X
Formic acid, heptyl ester	X	X
Thiophene, 2-pentyl-		X
1-Octen-3-one	X	X
Furan, 3-pentyl-	X	X
2-Propenal		X
2-Tridecen-1-ol, (E)-		X
Benzene		X
4-Octene, (E)-		X
1-Penten-3-one		X
4-Penten-2-one	X	X
Thiazole, 2-methyl-		X
Pyrazine, methyl-		X
trans-2-(2-Pentenyl)furan		X
3-Ethylcyclopentanone		X

Pyrrole	X	X
2-Thiophenecarboxaldehyde		X
3-Thiophenecarboxaldehyde		X

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

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

Flavor reaction mixtures at pH 6 containing LegH (1%), cysteine (10 mM), thiamine (1 mM), either glucose or ribose (20 mM), and with or without glutamic acid (10 mM) were prepared and subsequently heated to 150 C for 3 minutes. These flavor reaction samples then were evaluated by GCMS for the flavor compounds generated and evaluated by a trained panel for their sensory profiles. Volatile chemicals were isolated from the head space around the flavor reactions. GCMS showed 5-Thiazoleethanol, 4-methyl-, Furan, 3,3'-dithiobis[2-methyl-, 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. See, FIG. 1.

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 4-octene (E), Furan, 2-ethyl, 2-pentanone, 2,3-butanedione, thiazole, 2-methyl, pyrazine,

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

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

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

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

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

process where precursors reacting Heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction and identified using GCMS, creating a profile of the generated compounds. Nonanal, thiazole, 2-acetylthiazole, and 1-undecene,8-methyl 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, thiophene,2-pentyl, furfural, 2-nonenal, 1-octanol, 2-nonenal, thiazole, 2-acetylthiazole, phenylacetaldehyde, 2-acetylthiazole. These compounds are known to be present in meat and some have a beefy, meaty, and or savory flavor.

Reactions containing Heme protein at 1%, cysteine (10 mM), and ribose (20 mM), and lysine (1mM), at pH 6.0, were prepared and heated to 150C for 3 minutes. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors could react with the Heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction. Dimethyl trisulfide, nonanal, thiophene,2-pentyl, furfural, 2-nonenal, 1-octanol, 2-nonenal, thiazole, 2-acetylthiazole, phenylacetaldehyde, 2-acetylthiazole compounds were created by a mixture of Hemeprotein 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 Furan, 2-pentyl-, 2,3-Butanedione, Thiophene, Thiazole, 2-methyl-, Pyrazine, Furan, Pyrrole, Furan, 2-methyl- and distinct differences in the flavor compounds, including but not limited to Thiophene, 2-pentyl-, Nonanal, 2-Nonanone, and 1-Octen-3-one. These differences in flavor compounds can change the overall taste profile. The different types of Heme-protein were LegH, Barley, *B. myoglobin*, or *A. aeolicus* used at 1% w/w in a reaction mixed with cysteine (10 mM) and ribose (10 mM) at pH 6. The pre- reaction mixture was heated to 150 C for 3 minutes; this reaction created flavor compounds known to be present in meat; see Table 9. The characteristic flavor and fragrance components are mostly produced during the cooking process where the flavor precursor molecules react with the Heme-protein. Samples were evaluated by GCMS to identify the flavor compounds generated after heating and also evaluated for their sensory profiles. Volatile chemicals were isolated from the head space around the flavor reactions. Table 9 shows the similarity and differences in volatile flavor compounds created by the different types of Heme-proteins.

TABLE 9

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

Name	LegH	Barley	<i>B. myoglobin</i>	<i>A. aeolicus</i>
Furan	x	x	x	x
Thiazole	x	x	x	x

Attorney Docket No. 38767-0010P02

Benzaldehyde	x	x	x	x
2-Acetylthiazole	x	x	x	x
Propanal, 2-methyl-	x	x	x	x
Furfural	x	x	x	x
2,3-Butanedione	x	x	x	x
Furan, 2-pentyl-	x	x	x	x
2-Pentanone	x	x		
Pyrazine	x	x	x	x
Dimethyl trisulfide	x	x	x	x
Butanal, 3-methyl-	x	x		x
Thiazole, 2-methyl-	x	x	x	x
Pentanal	x	x	x	x
1,3,5-Cycloheptatriene	x	x	x	x
Methacrolein	x	x	x	x
Heptanal	x	x	x	x
Butanal, 2-methyl-	x	x		x
Isothiazole	x	x	x	x
Thiophene	x	x	x	x
Propanal	x	x	x	x
2-Heptenal	x		x	x
Pyrazine, methyl-	x	x	x	x
1-Octene	x		x	x
Butanal	x	x	x	x
1-Propen-2-ol, acetate	x	x	x	x
Pyrrole	x	x	x	x
Furan, 2-methyl-	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
2-Heptanone, 4-methyl-			x	x
Octanal			x	x
2-Undecenal				x
Butyrolactone				x
1-Octen-3-one				x
3-Methylheptyl acetate				x
Thiophene, 2-pentyl-				x

OTHER EMBODIMENTS

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

5

WHAT IS CLAIMED IS:

1. A food product comprising:
 - a) a heme-containing protein; and
 - b) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, sunflower oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a hydrolyzed protein, a malt extract, a yeast extract, and a peptone.
2. The food product of claim 1, wherein said food product comprises two to fifty flavor precursors.
3. The food product of claim 1, wherein said food product comprises two to forty flavor precursors.
4. The food product of claim 1, wherein said food product comprises two to thirty-five flavor precursors.
5. The food product of claim 1, wherein said food product comprises two to ten flavor precursors.
6. The food product of claim 1, wherein said food product comprises two to six flavor precursors.

29 7. The food product of any one of claims 1-6, wherein the one or more flavor
30 precursor molecules are selected from the group consisting of glucose, ribose, cysteine, a
31 cysteine derivative, thiamine, lysine, a lysine derivative, glutamic acid, a glutamic acid
32 derivative, IMP, GMP, lactic acid, and mixtures thereof.

33
34 8. The food product of any one of claims 1-7, wherein the heme-containing protein
35 is a plant heme-containing protein.

36
37 9. The food product of any one of claims 1-7, wherein the heme-containing protein
38 is a mammalian heme-containing protein.

39
40 10. The food product of any one of claims 1-7, wherein the heme-containing protein
41 is a yeast or filamentous fungi heme-containing protein.

42
43 11. The food product of any one of claims 1-7, wherein the heme-containing protein
44 is a bacterial heme-containing protein.

45
46 12. The food product of any one of claims 1-8, wherein the heme-containing protein
47 is a non-symbiotic hemoglobin.

48
49 13. The food product of any one of claims 1-8, wherein the heme-containing protein
50 is a leghemoglobin.

51
52 14. The food product of claim 13, wherein the leghemoglobin is a plant
53 leghemoglobin.

54
55 15. The food product of claim 14, wherein the plant leghemoglobin is from soybean,
56 alfalfa, lupin, pea, cow pea, or lupine.

16. The food product of any one of claims 1-15, 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:1-20.

17. The food product of any one of claims 1-16, wherein said heme-containing protein is isolated and purified.

18. The food product of any one of claim 1-17, further comprising a food-grade oil, a seasoning agent, a flavoring agent, a protein, a protein concentrate, an emulsifier, a gelling agent, or a fiber.

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

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

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

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

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

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

a) combining a heme-containing protein, cysteine, and ribose, and one or more optional flavor precursor molecules to form a mixture, said one or more optional flavor precursor molecules selected from the group consisting of glucose, fructose, arabinose,

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

b) heating the mixture to form one or more flavor compounds selected from the group consisting of 2-ethyl furan, 2-Pentanone, 2-pentyl-thiophene, 2-Propenal, 2-Tridecen-1-ol, 4-Octene, 1-Penten-3-one, 2-methyl thiazole, methyl-pyrazine, trans-2-(2-Pentenyl)furan, 3-Ethylcyclopentanone, 2-Thiophenecarboxaldehyde, and 3-Thiophenecarboxaldehyde.

25. The method of claim 24, said method comprising combining lactic acid, lysine, and/or thiamine with the heme-containing protein, cysteine, and ribose.

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

a) combining a heme-containing protein, cysteine, and glucose, and one or more optional flavor precursor molecules to form a mixture, said one or more optional flavor precursor molecules selected from the group consisting of 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, mango oil, a free fatty acid, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a hydrolyzed protein, a malt extract, a yeast extract, and a peptone; and

b) heating the mixture to form one or more flavor compounds selected from the group consisting of benzaldehyde, 2-butanone, dimethyl trisulfide, 2-pentyl-furan, 2-methyl-propanal, thiazole, butyrolactone, 2-acetylthiazole, pentanal, 3-methyl-butanal, methyl-thiirane, nonanal, heptanal, 2,3-butanedione, 1,3,5-cycloheptatriene, propyl-cyclopropane, 2-hexyl-furan, butanal, 2-methyl-butanal, 2-octanone, propanal, trichloromethane, 2-methyl-furan, furan, pyrazine, thiophene, 1,3-dimethyl-benzene, octanal, thiazole, furfural, 2-nonanone, 2-Heptenal, 2-Heptenal, 1-Octene, heptyl ester formic acid, 1-octen-3-one, 3-pentyl-furan, 4-penten-2-one, and pyrrole.

27. The method of claim 26, said method comprising combining lactic acid, lysine, and/or thiamine with the heme-containing protein, cysteine, and glucose.

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

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

b) heating the mixture to form one or more flavor compounds set forth in Table 3.

29. A method for imparting a beef like flavor to a food product comprising contacting said food product with a flavoring composition, said flavoring composition comprising i) a heme-containing protein; and ii) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose

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

30. The method of claim 29, wherein said flavoring composition further comprises a seasoning agent, a flavoring agent, a protein, a protein concentrate, or an emulsifier.

31. The method of claim 29 or claim 30, wherein said flavoring composition is sealed within a packet or shaker.

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

ABSTRACT

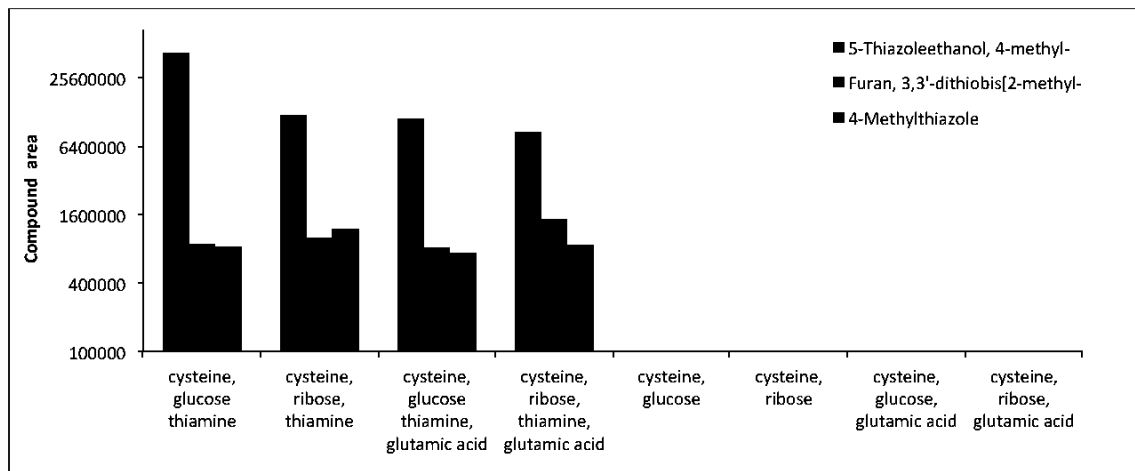
This document relates to food products containing heme proteins and one or more flavor precursors and using such food products to modulate the flavor and/or aroma profile of other foods. The food products described herein can be prepared in various ways and can be formulated to be free of animal products.

10

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38767-0010P02
Page 1 of 6

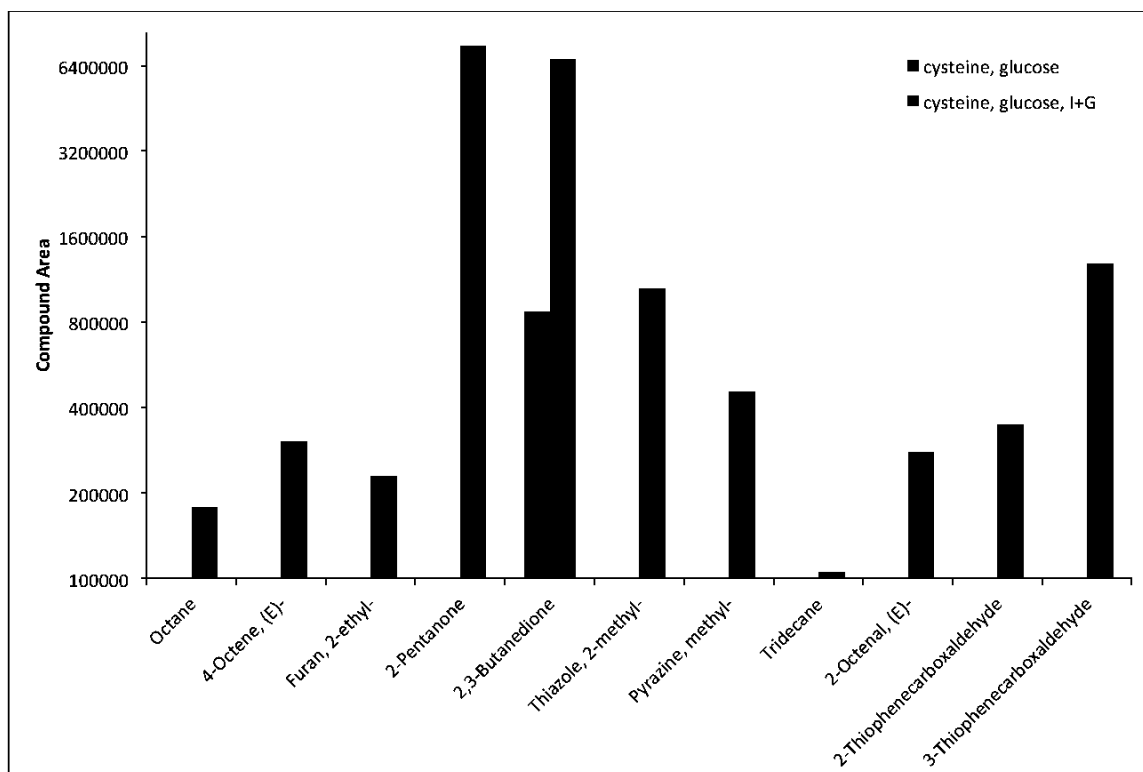
FIGURE 1



38767-0010P02

Page 2 of 6

FIGURE 2



38767-0010P02

Page 3 of 6

FIGURE 3

SEQ ID NO:1 *Vigna radiata*

MTTTLERGFTEEQEALVVKSWNVMMKNSGELGLKFFLKIFEIAPSAQKLFSFLRDSTVP
LEQNPCLKPHAVSVFVMTCDASVQLRKAGKVTVRESNLKKLGATHFRTGVANEHFEVTK
FALLETIKEAVPEMWSPAMKNAWGEAYDQLVDAIKYEMKPPSS

SEQ ID NO:2 *Methylobacterium radiatum*

MIDQKEKELIKESWKRIEPNKNEIGLLFYANLFKEEPTVSVLFQNPISQSRKLMQVLG
ILVQGIDNLEGLIPTLQDLGRRHKQYGVVDSHYPLVGDCLLKSIEYLGQGFTEEAkaa
WTKVYGIAAQVMTAE

SEQ ID NO:3 *Aquifex aeolicus*

MLSEETIRVIKSTVPLLKEHGTEITARMYELLFSKYPKTKELFAGASEEQPKLANAI I
AYATYIDRLEELDNAISTIRSHVRRNVKPEHYPLVKECLLQAIEEVLPNGEEVLKAW
EAYDFLAKTLITLKKLYSQP

SEQ ID NO:4 *Glycine max*

MGAFTKQEALVSSSFEEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLSNGVDPSNP
TGHAEKLFGLVRDSAGQLKANGTVVADAALGSIHAQKAITDPQFVVVKEALLKTIKEAV
GDKWSDELSSAWEVAYDELAAAIKKA

SEQ ID NO:5 *Hordeum vulgare*

MSAAEGAVVFSEEKEALVLKSWAIMKKDSANLGLRFFLKIFEIAPSARQMFPFLRDS
PLETNPKLKTHAVSVFVMTCEAAAQLRKAGKITVRETTKRLGGTHLKYGVADGHFEV
RFALLETIKEALPADMWGPEMRNAWGEAYDQLVAAIKQEMKPAE

Magnaporthe oryzae, (SEQ ID NO:6)

1 mdgavrlldwt gldltgheih dgvpasrvq vmvsfplfk qhiimsskes psrksstigg
61 strngscqad tqkgqlppvg ekpkpvkenp mkklkemsqr plptqhgdt yptekklgti
121 gedlkhirgy dvktllamvk sklkgeklkd dktmlmervm qlvarlptes kkraeltdsl
181 inelwesldh pplnylgpeh syrtpdgsyn hpfnpqlgaa gsryarsvip tvtpggalpd
241 pglifdsimg rtpnsyrkhp nnvssilwyw atiihdifw tdprdtntk sssyldlapl
301 ygnsqemqds irtfkdgrmk pdcyadkrla gmpgqvsvll imfnrfhnhv aenlalineg
361 grfnkpsdll egeareaawk kynddlfqa rlvtsglyin itlvdyvrni vnlrvdttw
421 tldprqdaga hvgtadgaer gtgnavsae nlcyrwhsci sekdskfvea qfqnfqkpa
481 sevrpdemwk gfakmeqntp adpggrtfgg fkrpgdgkfd dddlvrcise avedvagafg
541 arnvpqamkv vetmgliqgr kwnvaglnef rkhfhlpys tfedinsdpg vaealrrlyd
601 hpdnvelypg lvaeedkqpm vpgvgipty tisrvvlsda vclvrgdrfy ttdftprnlt
661 nwgykevdtd lsvnhgcvfy klfirafpnh fkgnsvyahy pmvvpsekr ilealgradl

38767-0010P02

Page 4 of 6

721 fdfeapkyip prvnitsygg aeyiletqek ykvtwheglg flmgeggklf mlsyddplha
781 qqrkcmaaql ykdgwteavk afyagmmeel lvksyflgn nkhrhvdiir dvgnmvhvhf
841 asqvfglplk taknptgvft eqemygilaa ifttiffdld psksfplrtk trevcqklak
901 lveanvclin kipwsrgmfv gkpakdepls iygktmikgl kahglscydi awshvvpstg
961 amvpnqaqvf aqavdyylsp agmhyipeih mvalqpstpe tdalllgyam egirlagtfq
1021 syreaavddv vkedngrqvp vkagdrvfvs fvdaardpkh fpdpevvnpv rpakkyihyg
1081 vgphaclgrd asqiaitemf rclfrrrnvr rvpgpqgelk kvprpggfyv ymredwgglf
1141 pfpvtmrvmw dde

Fusarium oxysporum (SEQ ID NO:7)

1 mkgSATlafa lvqfsaasql vwpskwdeve dllymqggfn krgfadalt cefgsnvpqt
61 qntaewlrta fhdaithdak agtgglDasi ywessrpenp gkafnntfgf fsgfhnprat
121 asdltalgtv lavgaengpr ipfragrida ykagpagvpe pstnlkdtfa aftkagftke
181 emtamvacgh aiggvhsvdf peivgikadp nndtnvpfqk dvssfhngiv teylagtskn
241 plvasknatf hsdkrifdnd katmkkllstk agfnsmcadi ltrmidtvpk svqltpvlea
301 ydvrrpyitel slnnknkihf tgsrvrritn nirdnndlai nliyvgdrgk kvtpvtqqvt
361 fqggtstfgag evfanfefdt tmdakngitk ffiqevkpst katvthdnqk tgggkvdtdv
421 lyqlqqscav leklnaplv vtamvrDara kdaltlrvah kkpvkgsivp rfqtaitnfk
481 atgkkssgyt gfqaktmfee qstyfdivlg gspasgvqfl tsqampsqcs

Fusarium graminearum (SEQ ID NO:8)

1 masatrqfar aatratrngf aiaprqvirq qgrryyssep aqkssawiw ltgaavagga
61 gyyfygnsas satakvnps kedyqkvyne iaarleekdd yddgsygpvl vrlawhasgt
121 ydketgtggs ngatmrfape sdhganagla aardflqpvk ekfpwitysd lwilagvcai
181 qemlgpaipy rpgrsdrdvs gctpdgrlpd askrqdhlrg ifgrmgfndq eivalsgaha
241 lgrchtdrsg ysgpwtfspt vltndyfrll veekwqwkww ngpaqyedks tkslmmlpsd
301 ialiedkkfk pwvekyakdn daffkdfsnv vlrlfelgvp faqgtenqrw tfkpthqe

SEQ ID NO: 9 *Chlamydomonas eugametos*

MSLFAKLGGREAVEAAVDKFYNKIVADPTVSTYFSNTDMKVQRSKQFAFLAYALGGASE
WKGKDMRTAHKDLVPHLSDVHFQAVARHLSDTLTELGVPPEDITDAMAVVASTRTEVLN
MPQQ

SEQ ID NO:10 *Tetrahymena pyriformis*

MNKPQTIYEKLGGENAMKAAVPLFYKKVLADERVKHFFKNTDMDHQTKQQTDFLTMLLG
GPNHYKGNMTEAHKGMNLQNLHFDALIIENLAATLKELGVTDAVINEAAKVIEHTRKDM
LGK

38767-0010P02

Page 5 of 6

SEQ ID NO:11 *Paramecium caudatum*

MSLFEQ LGGQAAVQAVTAQFYANIQADATVATFFNGIDMPNQTNKTAAFLCAALGGPNA
WTGRNLKEVHANMGVSNAQFTTVIGHLR SALTGAGVAAALVEQTVAVAETVRGDVVTV

SEQ ID NO:12 *Aspergillus niger*

MPLTPEQIKI IKATVPVLQEYGTKITTA FYMMSTVHPELNAVFNTANQVKGHQARALA
GALFAYASHIDDLGALGPAVELICNKHASLYIQADEYKIVGKYLLEAMKEVLGDACTDD
ILDAWGAA YWALADIMINREAALYKQSQG

SEQ ID NO:13 *Zea mays*

MALAEADDGAVVFGEEQEALVLKSWAVMKKDAANLGLRFFLKVFEIAPSAEQMFSFLRD
SDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRRETTLKRLGATHLRYGVADGHF
EVTGFALLETIKEALPADMWSLEMKKAWAEAYSQLVAAIKREMKPDA

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

MALVEGNNGVSGGAVSFSEEQEALVLKSWAIMKKDSANIGLRFFLKI FEVAPSASQMF S
FLRNSDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRD TTLLKRLGATHFKYGVG
DAHFEVTRFALLETIKEAVPVD MWS PAMKSAWSEAYNQLVAAIKQEMKPAE

SEQ ID NO:15 *Arabidopsis thaliana*

MESEGKIVFTEEQEALVVKSW SVMKKNSAELGLKLF IKIFEIAPT TTKMF SFLRDSPI P
AEQNPKLKPHAMSVFVMCCESAVQLRKTGKVTVRRETTLKRLGASHSKYGVVDEHFEVAK
YALLETIKEAVPEMWSPEMKVAWGQAYDHLVAAIKAEMNLSN

38767-0010P02

Page 6 of 6

SEQ ID NO:16 *Pisum sativum*

MGFTDKQEALVNSSWESFKQNLSGNSILFYTTILEKAPAAKGLFSFLKDTAGVEDSPKL
QAHAEQVFGLVRDSAAQLRTKGEVVLGNATLGAIHVQRGVTDPHFVVVKEALLQTIKKA
SGNNWSEELNTAWEVAYDGLATAIKKAMT

SEQ ID NO:17 *Vigna unguiculata*

MVAFSDKQEALVNGAYEAFKANIPKYSVVFYTTILEKAPAAKNLFSFLANGVDATNPKL
TGHAEKLFGLVRDSAAQLRASGGVVADAALGAVHSQKAVNDAQFVVVKEALVKTLKEAV
GDKWSDELGTAVELAYDELAIAIKKAY

SEQ ID NO:18 *Bos taurus*

MGLSDGEWQLVLNNAWGKVEADVAGHGQEV LIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGNTVL TALGGILKKKGHHEAEVKHLAESHANKHKIPVKYLEFISDAIIHVLHA
KHPSDFGADAQAAMSKALELFRNDMAAQYKVLGFHG

SEQ ID NO:19 *Sus scrofa*

MGLSDGEWQLVLNVWGKVEADVAGHGQEV LIRLFKGHPETLEKFDKFKHLKSEDEMKA
S EDLKKHGNTVL TALGGILKKKGHHEAELTPLAQSHATKHKIPVKYLEFISEAIIQVLQS
KH PGDFGADAQGAMSKALELFRNDMAAKYKELGFQG

SEQ ID NO: 20 *Equus caballus*

MGLSDGEWQQVLNVWGKVEADIAGHGQEV LIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGTVVL TALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHVLHS
KH PGDFGADAQGAMTKALELFRNDIAAKYKELGFQG