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(73) Patenthaver: **Impossible Foods Inc., 400 Saginaw Drive, Redwood City, CA 94063, USA**

(72) Opfinder: **VRLJIC, Marija, 461 West 25th Avenue, San Mateo, California 94403, USA**
SOLOMATIN, Sergey, 422 Hillwood Court, Mountain View, California 94040, USA
FRASER, Rachel, 3653 24th Street Apt. 2, San Fransisco, California 94110, USA
BROWN, Patrick O'Reilly, 76 Peter Coutts Circle, Stanford, California 94305, USA
KARR, Jessica, 456 Kansas Street, San Fransisco, California 94107, USA
HOLZ-SCHIETINGER, Celeste, 1159 Sage Street, East Palo Alto, California 94303, USA
EISEN, Michael, 2408 McGee Avenue, Berkeley, California 94703, USA
VARADAN, Ranjani, 1580 Skelton Court, Fremont, CA 94536, USA

(74) Fuldmægtig i Danmark: **Novitas Patent AB, P.O. Box 55557, 102 04 Stockholm, Sverige**

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DESCRIPTION

_TECHNICAL FIELD

[0001] This disclosure relates to consumable products.

BACKGROUND

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

[0003] The consumption of meat has a profound negative impact on human health. The health benefits of a vegetarian diet are well established. If the human population would shift to a more vegetarian diet, there would be a decrease in health care costs.

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

[0005] Plant based meat substitutes have largely failed to cause a shift to a vegetarian diet. The current state of the art for meat substitute compositions involves the extrusion of soy/grain mixture, resulting in products which largely fail to replicate the experience of cooking and eating meat. Common limitations of these products are a texture and mouthfeel that are more homogenous than that of equivalent meat products. Furthermore, as the products must largely be sold pre-cooked, with artificial flavors and aromas built in, they fail to replicate aromas, flavors, and other key features associated with cooking meat. As a result, these products appeal mainly to a limited consumer base that is already committed to vegetarianism/veganism, but have failed to appeal to the larger consumer segment accustomed to eating meat.

[0006] Food is any substance that is either eaten or drunk by any animal, including humans, for nutrition or pleasure. It is usually of plant or animal origin, and contains essential nutrients, such as carbohydrates, fats, proteins, vitamins, or minerals. The substance is ingested by an organism and assimilated by the organism's cells in an effort to produce energy, maintain life,

or stimulate growth.

[0007] Food typically has its origin in a photosynthetic organism, typically from plants. Some food is obtained directly from plants; but even animals that are used as food sources are raised by feeding them food derived from plants. Edible fungi and bacteria are used to transform materials from plants or animals into other food products, mushrooms, bread, yogurt and the like.

[0008] In most cases, the plant or animal is fractionated into a variety of different portions, depending upon the purpose of the food. Often, certain portions of the plant, such as the seeds or fruits, are more highly prized by humans than others and these are selected for human consumption whilst other less desirable portions, such as the stalks of grasses, are typically used for feeding animals.

[0009] Animals are typically butchered into smaller cuts of meat with specific flavor and handling properties before consumption.

[0010] While many foods can be eaten raw, many also undergo some form of preparation for reasons of safety, palatability, texture, or flavor. At the simplest level, this may involve washing, cutting, trimming, or adding other foods or ingredients. It may also involve mixing, heating or cooling or fermentation and individual foods may be combined with other food products to achieve the desired mix of properties.

[0011] In recent years, attempts have been made to bring scientific rigor to the process of food preparation, under the fields of food science and molecular gastronomy. Food science broadly studies the safety, microbiology, preservation, chemistry, engineering and physics of food preparation, whereas molecular gastronomy focuses on the use of scientific tools such as liquid nitrogen, emulsifying agents such as soy lecithin and gelling agents such as calcium alginates to transform food products into unexpected forms.

[0012] However, the raw material is typically an entire organism (plant or animal) or an isolated tissue such as a steak, the fruiting body of a fungus, or the seed of a plant. In some cases, the isolated tissue is modified before food preparation, such as making flour or isolating oils and bulk proteins from seeds.

[0013] Despite that fact that all of these items comprise a mixture of proteins, carbohydrates, fats, vitamins and minerals, the physical arrangement of these materials in the original plant or animal determines the use to which the plant or animal tissue will be put. Disclosed herein are improved methods for the production of consumables.

[0014] US 2009/010042 A1 discloses means for improving the body taste, taste and flavor of foods, comprising a long-chain highly unsaturated fatty acid and/or an ester thereof as a main component.

[0015] Calkins and Hodgen (Meat Science, vol 77 (2007), pages 63-80) describe a review of meat flavor and the numerous factors contributing to it.

SUMMARY

[0016] Aspects of the present invention are set forth in the appended claims. Provided herein is a method for imparting a beef like flavor to a consumable product, comprising injecting to the consumable composition a heme-containing protein, wherein after cooking, a beef-like flavor is imparted to the consumable composition.

[0017] Also provided is a method for making a poultry composition taste like beef, the method comprising adding a heme protein to the poultry composition, respectively.

[0018] In some embodiments, the heme-containing protein has an amino acid sequence with at least 70% homology to any one of the amino acid sequences set forth in SEQ ID NOs: 1-27.

[0019] 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 disclosure pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the disclosure, suitable methods and materials are described below. 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.

[0020] The details of one or more embodiments of the disclosure are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the disclosure will be apparent from the description and drawings, and from the claims. The word "comprising" in the claims may be replaced by "consisting essentially of' or with "consisting of," according to standard practice in patent law.

DESCRIPTION OF THE DRAWING

[0021] FIG. 1 contains amino acid sequences of exemplary heme-containing proteins.

DETAILED DESCRIPTION

[0022] A group of people can be asked to rate a certain food product, for instance ground beef, according to properties which describe the food product. A consumable described herein can be rated by the same people to determine equivalence.

[0023] Flavor of the food product can also be assessed. Flavors can be rated according to

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

[0024] In some compositions, subjects asked to identify the consumable identify it as a form of a food product, or as a particular food product, e.g., a subject will identify the consumable as meat. For example, in some compositions a human will identify the consumable as having properties equivalent to meat. In some cases one or more properties of the consumable are equivalent to the corresponding properties of meat according to a human's perception. Such properties include the properties that can be tested.

[0025] Experiments can demonstrate that a consumable is acceptable to consumers. A panel can be used to screen a variety of consumables described herein. A number of human panelists can test multiple consumable samples, namely, natural meats vs. the consumable compositions described herein, or a meat substitute vs. a consumable composition described herein. Variables such as fat content can be standardized, for example to 20% fat using lean and fat meat mixes. Fat content can be determined using the Babcock for meat method (S. S. Nielson, Introduction to the Chemical Analysis of Foods (Jones & Bartlett Publishers, Boston, 1994)). Mixtures of ground beef and consumables of the disclosure prepared according to the procedure described herein can be formulated.

[0026] Panelists can be served samples (e.g., in booths), under red lights or under white light, in an open consumer panel. Samples can be assigned random three-digit numbers and rotated in ballot position to prevent bias. Panelists can be asked to evaluate samples for tenderness, juiciness, texture, flavor, and overall acceptability using a hedonic scale from 1=dislike extremely, to 9=like extremely, with a median of 5=neither like nor dislike. Panelists can be encouraged to rinse their mouths with water between samples, and given opportunity to comment on each sample.

[0027] The results of this experiment can indicate significant differences or similarities between the traditional meats and the compositions of the disclosure.

[0028] These results can demonstrate that the compositions described herein are judged as acceptably equivalent to real meat products. Additionally, these results can demonstrate that compositions described herein are preferred by panelist over other commercially available meat substitutes. Thus, in some cases the present disclosure describes for consumables that are similar to traditional meats and are more meat like than previously known meat alternatives.

[0029] 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 cases, be used to evaluate the properties of a consumable. For example volatile chemicals can be isolated from the head space around meat. These chemicals can be identified using GCMS. A profile of the volatile chemicals in the headspace around meat is thereby created. In some instances each peak of the GCMS can be further evaluated. For instance, a human could rate the experience of smelling the chemical responsible for a certain peak. This information could be used to further refine the profile. GCMS could then be used to evaluate the properties of the consumable. The GCMS profile can be used to refine the consumable.

[0030] 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 cases, the consumable is tested for similarity to meat during or after cooking. In some cases human ratings, human evaluation, olfactometer readings, or GCMS measurements, or combinations thereof, are used to create an olfactory map of cooked meat. Similarly, an olfactory map of the consumable, for instance a meat replica, can be created. These maps can be compared to assess how similar the cooked consumable is to meat. In some cases the olfactory map of the consumable during or after cooking is similar to or indistinguishable from that of cooked or cooking meat. In some cases the similarity is sufficient to be beyond the detection threshold of human perception. The consumable may be created so its characteristics are similar to a food product after cooking, but the uncooked consumable may be have properties that are different from the predicate food product prior to cooking.

[0031] The main determinant of the color of meat is the concentration of iron carrying proteins in the meat. In the skeletal muscle component of meat products, one of the main iron-carrying proteins is myoglobin. It is estimated that the white meat of chicken has under 0.05% myoglobin; pork and veal have 0.1-0.3% myoglobin; young beef has 0.4-1.0% myoglobin; and old beef has 1.5-2.0% myoglobin. Normally, myoglobin in meat exists in three states: Oxymyoglobin (Fe^{2+}) (oxygenated = bright red); myoglobin (Fe^{2+}) (non-oxygenated = purplish/magenta); and metmyoglobin (Fe^{3+}) (oxidized = brown). The transition of oxymyoglobin to metmyoglobin in the presence of oxygen is thought to be the cause of the color change of ground meat from red to brown. Meat shelf life extenders have been developed to extend the lifetime of the red color of meat products including but not limited to carbon monoxide, nitrites, sodium metabisulfite, Bombal, vitamin E, rosemary extract, green

tea extract, catechins and other anti-oxidants.

[0032] However an intrinsically more stable heme protein such a hemoglobin isolated from *Aquifex aeolicus* (SEQ ID NO:3) or *Methylacidiphilum infernorum* (SEQ ID NO: 2) will oxidize more slowly than a mesophilic hemoglobin such as myoglobin. The heme proteins described herein (see, e.g., FIG. 1) also may have the lifetime of the reduced heme-Fe^{2+} state extended by meat shelf life extenders such as carbon monoxide and sodium nitrite. Heme proteins may be selected for the desired color retention properties. For example for low temperature *sous-vide* cooking, a relatively unstable heme protein such as one from *Hordeum vulgare* may provide a brown product that appears cooked under conditions wherein myoglobin would retain its red, uncooked appearance. In some cases the heme protein may be selected to have increased stability where for example the meat replica may retain an attractive medium rare appearance despite being thoroughly cooked for food safety.

[0033] As used herein, the term "heme containing protein" can be used interchangeably with "heme containing polypeptide" or "heme protein" or "heme polypeptide" and includes any polypeptide that can covalently or noncovalently bind a heme moiety. In some embodiments, the heme-containing polypeptide is a globin and can include a globin fold, which comprises a series of seven to nine alpha helices. Globin type proteins can be of any class (e.g., class I, class II, or class III), and in some embodiments, can transport or store oxygen. For example, a heme-containing protein can be a non-symbiotic type of hemoglobin or a leghemoglobin. A heme-containing polypeptide can be a monomer, i.e., a single polypeptide chain, or can be a dimer, a trimer, tetramer, and/or higher order oligomers. The life-time of the oxygenated Fe^{2+} state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption. The life-time of the unoxygenated Fe^{2+} state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption

[0034] Non-limiting examples of heme-containing polypeptides can include an androglobin, a cytoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a leghemoglobin, a flavohemoglobin, Hell's gate globin I, a myoglobin, an erythrocruorin, a beta hemoglobin, an alpha hemoglobin, a protoglobin, a cyanoglobin, a cytoglobin, a histoglobin, a neuroglobins, a chlorocruorin, a truncated hemoglobin (e.g., HbN or HbO), a truncated 2/2 globin, a hemoglobin 3 (e.g., Glb3), a cytochrome, or a peroxidase.

[0035] Heme-containing proteins that can be used in the consumables described herein can be from mammals (e.g., farms animals such as cows, goats, sheep, horses, pigs, ox, or rabbits), birds, plants, algae, fungi (e.g., yeast or filamentous fungi), ciliates, or bacteria. For example, a heme-containing protein can be from a mammal such as a farm animal (e.g., a cow, goat, sheep, pig, ox, or rabbit) or a bird such as a turkey or chicken. Heme-containing proteins can be from a plant such as *Nicotiana tabacum* or *Nicotiana sylvestris* (tobacco); *Zea*

mays (corn), *Arabidopsis thaliana,* a legume such as *Glycine max* (soybean), *Cicer arietinum* (garbanzo or chick pea), *Pisum sativum* (pea) varieties such as garden peas or sugar snap peas, *Phaseolus vulgaris* varieties of common beans such as green beans, black beans, navy beans, northern beans, or pinto beans, *Vigna unguiculata* varieties (cow peas), *Vigna radiata* (Mung beans), *Lupinus albus* (lupin), or *Medicago sativa* (alfalfa); *Brassica napus* (canola); *Triticum sps.* (wheat, including wheat berries, and spelt); *Gossypium hirsutum* (cotton); *Oryza sativa* (rice); *Zizania sps.* (wild rice); *Helianthus annuus* (sunflower); *Beta vulgaris* (sugarbeet); *Pennisetum glaucum* (pearl millet); *Chenopodium sp.* (quinoa); *Sesamum sp.* (sesame); *Linum usitatissimum* (flax); or *Hordeum vulgare* (barley). Heme-containing proteins can be isolated from fungi such as *Saccharomyces cerevisiae, Pichia pastoris, Magnaporthe oryzae, Fusarium graminearum,* or *Fusarium oxysporum.* Heme-containing proteins can be isolated from bacteria such as *Escherichia coli, Bacillus subtilis, Bacillus megaterium, Synechocistis sp., Aquifex aeolicus, Methylacidiphilum infernorum,* or thermophilic bacteria (e.g, that grow at temperatures greater than 45°C) such as *Thermophilus.* Heme-containing proteins can be isolated from algae such as *Chlamydomonas eugametos.* Heme-containing proteins can be isolated from protozoans such as *Paramecium caudatum* or *Tetrahymena pyriformis.* In some embodiments, the bacterial hemoglobins are selected from the group consisting of *Aquifex aeolicus, Thermobifida fusca, Methylacidiphilum infernorum* (Hells Gate), *Synechocystis SP,* or *Bacillus subtilis.* The sequences and structure of numerous heme-containing proteins are known. See for example, Reedy, et al., Nucleic Acids Research, 2008, Vol. 36, Database issue D307-D313 and the Heme Protein Database available on the world wide web at http://hemeprotein.info/heme.php.

[0036] For example, a non-symbiotic hemoglobin can be from a plant selected from the group consisting of soybean, sprouted soybean, alfalfa, golden flax, black bean, black eyed pea, northern, garbanzo, moong bean, cowpeas, pinto beans, pod peas, dried peas, quinoa, sesame, sunflower, wheat berries, spelt, barley, wild rice, or rice.

[0037] Any of the heme-containing proteins described herein that can be used for producing consumables can have at least 70% (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%) sequence identity to the amino acid sequence of the corresponding wild-type heme-containing protein or fragments thereof that contain a heme-binding motif. For example, a heme-containing protein can have at least 70% sequence identity to an amino acid sequence set forth in FIG. 1, including a non-symbiotic hemoglobin such as that from *Vigna radiata* (SEQ ID NO: 1), *Hordeum vulgare* (SEQ ID NO:5), *Zea mays* (SEQ ID NO: 13), *Oryza sativa subsp. japonica (rice)* (SEQ ID NO:14), or *Arabidopsis thaliana* (SEQ ID NO:15), a Hell's gate globin I such as that from *Methylacidiphilum infernorum* (SEQ ID NO:2), a flavohemoprotein such as that from *Aquifex aeolicus* (SEQ ID NO:3), a leghemoglobin such as that from *Glycine max* (SEQ ID NO:4), *Pisum sativum* (SEQ ID NO: 16), or *Vigna unguiculata* (SEQ ID NO: 17), a heme-dependent peroxidase such as from *Magnaporthe oryzae,* (SEQ ID NO:6) or *Fusarium oxysporum* (SEQ ID NO:7), a cytochrome c peroxidase from *Fusarium graminearum* (SEQ ID NO:8), a truncated hemoglobin from *Chlamydomonas moewusii* (SEQ ID NO:9), *Tetrahymena pyriformis* (SEQ ID NO:10, group I truncated), *Paramecium caudatum* (SEQ ID NO: 11, group I truncated), a hemoglobin from *Aspergillus niger* (SEQ ID NO: 12), or a mammalian myoglobin

protein such as the *Bos taurus* (SEQ ID NO:18) myoglobin, *Sus scrofa* (SEQ ID NO:19) myoglobin, or *Equus caballus* (SEQ ID NO:20) myoglobin, a heme-protein from *Nicotiana benthamiana* (SEQ ID NO:21), *Bacillus subtilis* (SEQ ID NO:22), *Corynebacterium glutamicum* (SEQ ID NO:23), Synechocystis PCC6803 (SEQ ID NO:24), Synechococcus sp. PCC 7335 (SEQ ID NO:25), *Nostoc commune* (SEQ ID NO:26), or *Bacillus megaterium* (SEQ ID NO:27). See FIG. 1.

[0038] 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 Bl2seq program can be found in the readme file accompanying BLASTZ. Bl2seq performs a comparison between two amino acid sequences using the BLASTP algorithm. To compare two amino acid sequences, the options of Bl2seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two amino acid sequences: C:\Bl2seq-i c:\seq1.txt -j c:\seq2.txt -p blastp -o c:\output.txt. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology, then the designated output file will not present aligned sequences. Similar procedures can be following for nucleic acid sequences except that blastn is used.

[0039] 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.

[0040] 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.

[0041] 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.

[0042] 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).

[0043] In some embodiments, heme-containing proteins are isolated and purified from other components of the source material (e.g., other animal, plant, fungal, algal, or bacterial proteins) using techniques described above. As used herein, the term "isolated and purified" indicates that the preparation of heme-containing protein is at least 60% pure, e.g., greater than 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% pure.

[0044] Heme-containing proteins also can be recombinantly produced using polypeptide expression techniques (e.g., heterologous expression techniques using bacterial cells, insect cells, algal cells, fungal cells such as yeast cells, plant cells, or mammalian cells). For example, the heme-containing protein can be expressed in *E. coli* cells. The heme-containing proteins can be tagged with a heterologous amino acid sequence such as FLAG, polyhistidine (e.g., hexahistidine, HIS tag), hemagluttinin (HA), glutathione-S-transferase (GST), or maltose-binding protein (MBP) to aid in purifying the protein. In some embodiments, a recombinant heme containing protein including a HIS-tag and a protease (e.g., TEV) site to allow cleavage of the HIS-tag, can be expressed in *E.coli* and purified using His-tag affinity chromatography (Talon resin, CloneTech). In some cases, standard polypeptide synthesis techniques (e.g., liquid-phase polypeptide synthesis techniques or solid-phase polypeptide synthesis techniques) can be used to produce heme-containing proteins synthetically. In some cases, cell-free translation techniques can be used to produce heme-containing proteins synthetically.

[0045] 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.

[0046] The proteins used in the consumable can be altered (e.g., hydrolyzed, cleaved, crosslinked, denatured, polymerized, extruded, electrospun, spray dried or lyophilized, or derivatized or chemically modified). For example, the proteins can be modified by covalent attaching sugars, lipids, cofactors, peptides, or other chemical groups including phosphate, acetate, methyl, and other natural or unnatural molecule. For example, the peptide backbones of the proteins can be cleaved by exposure to acid or proteases or other means. For example, the proteins can be denatured, i.e., their secondary, tertiary, or quaternary structure can be altered, by exposure to heat or cold, changes in pH, exposure to denaturing agents such as detergents, urea, or other chaotropic agents, or mechanical stress including shear. The

alignment of proteins in a solution, colloid, or a solid assembly can be controlled to affect the mechanical properties including tensile strength, elasticity, deformability, hardness, or hydrophobicity.

[0047] The consumable can include compositions which can indicate that the consumable is cooking or has cooked. The release of odorants upon cooking is an important aspect of meat consumption. Some reactions that generate odorants released during cooking of meat can be catalyzed by iron, in particular the heme iron of myoglobin. Thus, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by iron. In some cases, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by heme. In some cases, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by the heme iron in leghemoglobin. In some cases, some of the characteristic flavor and fragrance components are produced during the cooking process by chemical reactions catalyzed by the heme iron in a heme protein. For example, hemeproteins (e.g., from *Aquifex aeolicusm, Methylacidiphilum infernorum, Glycine max, Hordeum vulgare,* or *Vigna radiate*) provide a significantly different profile of volatile odorants when heated in the presence of cysteine and glucose than any subset of the three components when analysed by GC-MS. 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. Under these conditions, cysteine and glucose alone or in the presence of iron salts such as ferrous glucanate produced a sulfurous, odor but addition of heme proteins reduced the sulfurous odor and replaced it with flavors including but not limited to chicken broth, burnt mushroom, molasses, and bread.

[0048] Additionally, a hemeprotein (e.g., from *Aquifex aeolicus, Methylacidiphilum infernorum, Glycine max, Hordeum vulgare,* or *Vigna radiata*) when heated in the presence of ground chicken increased specific volatile odorants that are elevated in beef compared to chicken when analyzed by GC-MS. Volatile flavor components that are increased under these conditions include but are not limited to propanal, butanal, 2-ethyl-furan, heptanal, octanal, trans-2-(2-pentenyl)furan, (Z)-2-heptenal (E)-2-octenal pyrrole, 2,4-dodecadienal, 1-octanal, or (Z)-2-decenal 2-undecenal.

[0049] The color of meat is an important part the experience of cooking and eating meat. For instance, cuts of beef are of a characteristic red color in a raw state and gradually transition to a brown color during cooking. As another example, white meats such as chicken or pork have a characteristic pink color in their raw state and gradually transition to a white or brownish color during cooking. The amount of the color transition is used to indicate the cooking progression of beef and titrate the cooking time and temperature to produce the desired state of done-ness. The main determinant of the nutritional definition of the color of meat is the concentration of iron carrying proteins in the meat. In the skeletal muscle component of meat products, one of the main iron-carrying proteins is myoglobin. As described above, the myoglobin content of varies from under 0.05% in the white meat of chicken to 1.5-2.0% in old beef. Leghemoglobin,

similar in structure and physical properties to myoglobin, is readily available as an unused by-product of commodity legume crops (e.g., soybean or 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.

[0050] Heme proteins described herein may be added to meat to enhance the properties of the meat. A heme protein containing solution can be injected into raw (e.g., raw white meat) or cooked meat to improve the organoleptic properties of the meat during cooking adding a "beefy" flavor (e.g., to white meats such as chicken).

[0051] Leghemoglobin can be obtained from a variety of plants. Various legumes species and their varieties (e.g., soybean, fava bean, lima bean, cowpeas, English peas, yellow peas, Lupine, kidney beans, garbanzo beans, peanuts, Alfalfa, Vetch hay, clover, Lespedeza, or pinto bean) contain nitrogen-fixing root nodules in which leghemoglobin has a key role in controlling oxygen concentrations (for example root nodules from a pea plant). In one case leghemoglobin protein is purified from root nodules of legume plants (e.g., soybeans, favabeans, or peas) using ion-exchange chromatography. In an case, leghemoglobin is purified from soybean, favabean or sweet pea root nodules.

[0052] Plants can be grown using standard agricultural methods, with the exception that, in some instances, fertilizer is not applied and soil is enriched in natural nitrogen-fixing bacteria from the *Rhizobium* genus. Either whole roots or root nodules can be harvested and lysed, for example in 20mM potassium phosphate pH 7.4, 100mM potassium chloride and 5mM EDTA using grinder-blender. During this process, leghemoglobin is released into the buffer. Root-nodule lysate containing leghemoglobin can be cleared from cell debris by filtration through 5µm filter. In some cases, filtration is followed by centrifugation (7000g, 20min). Clarified lysate containing leghemoglobin is then filtered through 200nm filter and applied onto anion-exchange chromatography column (High Prep Q; High Prep DEAE, GE Healthtcare) on fast protein liquid chromatography machine (GE Healthcare). Leghemoglobin is collected in the flowthrough fraction and concentrated over 3kDa filtration membrane to a desired concentration. Purity (partial abundance) of purified leghemoglobin is analyzed by SDS-PAGE gel: in lysate leghemoglobin is present at 20-40%, while after anion-exchange purification it is present at 70-80%. In another case, soybean leghemoglobin flowthrough from anion-exchange chromatography is applied onto size-exclusion chromatography (Sephacryl S-100 HR, GE Healthcare). Soybean leghemoglobin is eluted as two fractions corresponding to dimer and monomer species. Purity (partial abundance) of leghemoglobin was analyzed by SDS-PAGE and determined to be ~ 90-100%.

[0053] Proteins in legume root-nodule lysate can be transferred into 10 mM sodium carbonate pH 9.5, 50mM sodium chloride buffer, filtered through 200nm filter and applied onto anion-exchange chromatography column on fast protein liquid chromatography instrument (GE Healthcare). Leghemoglobin can be bound to anion-exchange chromatography matrix and eluted using sodium chloride gradient. Purity (partial abundance) of leghemoglobin can be analyzed by SDS-PAGE and determined to be ~ 60-80%.

[0054] Undesired small molecules from legume roots can be removed from purified leghemoglobin by passing leghemoglobin in solution over anion-exchange resin. These small molecules imbue varying shades of brown color to root-root nodule lysates, thus decreasing the color quality of leghemoglobin solution. In one case, anion-exchange resin is FFQ, DEAE, Amberlite IRA900, Dowex 22, or Dowex 1x4. Leghemoglobin purified either by ammonium sulfate fractionation (60% wt/v and 90% wt/v ammonium sulfate) or by anion-exchange chromatography was buffer exchanged into 20mM potassium phosphate pH 7.4, 100mM sodium chloride and solution passed over one of the above mentioned anion-exchange resins. Flowthrough can be collected and its colored compared to the color of the solution before passage over anion-exchange resins. Color improvement to purified leghemoglobin solution as evaluated by visual inspection can be observed (from yellow/brown to more apparent red), however to different extent of removal of yellow-brown tinge.

[0055] Alternatively, the heme-containing protein can be recombinantly produced as described in section III B. For example, a non-symbiotic hemoglobin from moong bean can be recombinantly expressed in *E.coli* and purified using anion-exchange chromatography or cation-exchange chromatography. A cell lysate can be loaded over FF-Q resin on fast protein liquid chromatography instrument (GE Healthcare). Moong bean non-symbiotic hemoglobin eluted in the flowthrough fractions. Purity (partial abundance) of Moong bean non-symbiotic hemoglobin was analyzed by SDS-PAGE and determined to be as a fraction of total protein: 12 % in *E.coli* lysate, and 31% after purification on FFQ. UV-Vis analysis of purified protein showed spectra characteristic of heme bound protein.

[0056] Alternatively, the cell lysate can be loaded over a FF-S resin on a fast protein liquid chromatography instrument (GE Healthcare). Moong bean non-symbiotic hemoglobin can be bound to FF-S column and eluted using sodium chloride gradient (50mM- 1000mM). Purity (partial abundance) of Moong bean non-symbiotic hemoglobin can be analyzed by SDS-PAGE and determined to be: *E.coli* lysate 13 %, after purification on FFQ 35%. UV-Vis analysis of purified protein can show spectra characteristic of heme bound protein.

[0057] Given the usefulness of heme proteins for coloring consumables, it is useful to detect whether a product contains a particular heme protein. For example, an ELISA, a proximity-ligation assay, a luminex assay, or western blot analysis can be performed to determine whether leghemoglobin or other heme-containing protein is present in a food product such as meat or a meat replica. In one case the detection methods are performed to determine whether meat has been altered with leghemoglobin or other heme-containing protein.

EXAMPLES

REFERENCE EXAMPLE 1: PROTEIN ISOLATION.

[0058] All steps were carried out at 4°C or room temperature. Centrifugation steps were at 8000 g for 20 mins, 4°C or room temperature. The flour is suspended in a specific buffer, the suspension is centrifuged and the supernatant is microfiltered through a 0.2 micron PES membrane and then concentrated by ultrafiltration on a 3 kDa, 5 kDa, or 10 kDa molecular weight cutoff PES membrane on a Spectrum Labs KrosFlo hollow fiber tangential flow filtration system.

[0059] Once fractionated, all ammonium sulfate precipitate fractions of interest were stored at -20°C until further use. Prior to their use in experiments, the precipitates were resuspended in 10 volumes of 50 mM K Phosphate buffer, pH 7.4, + 0.5 M NaCl. The suspensions were centrifuged and the supernatants microfiltered through a 0.2 micron PES membrane and then concentrated by ultrafiltration on a 3 kDa, 5 kDa, or 10 kDa molecular weight cutoff PES membrane on a Spectrum Labs KrosFlo hollow fiber tangential flow filtration system. Protein composition at individual fractionation steps was monitored by SDS-PAGE and protein concentrations were measured by standard UV-Vis methods.

(i) Pea-albumins: Dry green or yellow pea flour was used as a source of pea albumins. The flour was suspended in 10 volumes of 50 mM sodium acetate buffer pH 5 and stirred for 1 hr. Soluble protein was separated from un-extracted protein and pea seed debris by either centrifugation (8000 g, 20 minutes) or filtration through a 5 micron filter. Supernatant or filtrate, respectively, was collected. To this crude protein extract, solid ammonium sulfate was added to 50% wt/v saturation. The solution was stirred for 1 hour and then centrifuged. To the supernatant from this step, ammonium sulfate was added to bring to 90% wt/v saturation. The solution was stirred for 1 hour, and then centrifuged to collect the pea albumin proteins in the pellet. The pellet was stored at -20°C until further use. Protein was recovered from the pellet and prepared for use as described above, with the exception that final buffer can contain 0-500 mM sodium chloride.
In some cases, the flour was suspended in 10 volumes of 50 mM NaCl, pH 3.8 and stirred for 1 hour. Soluble protein was separated from un-extracted protein and pea seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 Kda cutoff PES membrane.

(ii) Pea-globulins: Dry green pea flour was used to extract pea globulin proteins. The flour was suspended in 10 volumes of 50 mM potassium phosphate buffer pH 8 and 0.4M sodium chloride and stirred for 1hr. Soluble protein was separated from pea seed debris by centrifugation. The supernatant was subjected to ammonium sulfate fractionation in two steps at 50% and 80% saturation. The 80% pellet containing globulins of interest was stored at -20°C until further use. Protein was recovered from the pellet and prepared for use as described above.

iii) Soybean 7S and 11S globulins: Globulins from soybean flour were isolated by first suspending lowfat/defatted soy flour in 4-15 volumes of 10 (or 20) mM potassium phosphate pH 7.4. The slurry was centrifuged at 8000 rcf for 20 mins or clarified by 5 micron filtration and the supernatant was collected. The crude protein extract contained both the 7S and 11S globulins. The solution then was 0.2 micron filtered and concentrated using a 10 kDa molecular

weight cutoff PES membrane on a Spectrum Labs KrosFlo hollow fiber tangential flow filtration system or by passing over anion-exchange resin prior to use in experiments. The 11S globulins were separated from the 7S proteins by isoelectric precipitation. The pH of the crude protein extract was adjusted to 6.4 with dilute HCl, stirred for 30 min-1 hr and then centrifuged to collect the 11S precipitate and 7S proteins in the supernatant. The 11S fraction was resuspended with 10mM Potassium phosphate pH 7.4 and the protein fractions were microfiltered and concentrated prior to use.

Soybean proteins also can be extracted by suspending the defatted soy flour in 4-15 volumes (e.g., 5 volumes) of 20 mM sodium carbonate, pH 9 (or water, pH adjusted to 9 after addition of the flour) or 20 mM potassium phosphate buffer pH 7.4 and 100 mM sodium chloride to decrease off-flavors in the purified protein. The slurry is stirred for one hour and centrifuged at 8000 xg for 20 minutes. The extracted proteins are ultrafiltered and then processed as above or alternatively, the supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(iv) Moong bean 8S globulins: Moong bean flour was used to extract 8S globulins by first suspending the flour in 4 volumes of 50 mM KPhosphate buffer pH 7 (+ 0.5M NaCl for lab scale purifications). After centrifugation, proteins in the supernatant were fractionated by addition of ammonium sulfate in 2 steps at 50% and 90% saturation respectively. The precipitate from the 90% fraction contained the 8S globulins and was saved at -20°C until further use. Protein was recovered from the pellet and prepared for use as described above.

Moong bean globulins also can be extracted by suspending the flour in 4 volumes of 20 mM sodium carbonate buffer, pH 9 (or water adjusted to pH 9 after addition of the moong flour) to reduce off-flavors in the purified protein fractions. The slurry is centrifuged (or filtered) to remove solids, ultrafiltered and then processed as described above.

(v) Late embryogenesis abundant proteins: Flour (including but not limited to moong bean and soy flour) was suspended in 20 mM Tris-HCl, pH 8.0, 10 mM NaCl, and stirred at room temperature for 1 hour then centrifuged. Acid (HCl or acetic acid) was added to the supernatant to a 5% concentration (v/v), stirred at room temperature then centrifuged. The supernatant was heated to 95°C for 15 minutes, and then centrifuged. The supernatant was precipitated by adding Trichoroacetic acid to 25%, centrifuged, then washed with acetone. Heating and acid wash steps can be carried out in the reverse direction as well.

(vi) Pea-Prolamins: Dry green pea flour was suspended in 5x (w/v) 60% ethanol, stirred at room temperature for one hour, then centrifuged (7000g, 20min) and the supernatant collected. The ethanol in the supernatant was evaporated by heating the solution to 85°C and then cooling to room temperature. Ice-cold acetone was added (1: 4 v/v) to precipitate the proteins. The solution then was centrifuged (4000g, 20min), and protein recovered as the light-beige colored pellet.

(vii) Zein-Prolamins: Corn protein concentration or flour was suspended in 5x (w/v) 60% ethanol, stirred at room temperature for one hour, then centrifuged. Ethanol in supernatant was evaporated with heat, and then the solution is centrifuged, and the protein recovered as the pellet.

(viii) RuBisCO was fractionated from alfalfa greens by first grinding leaves with 4 volumes of cold 50 mM potassium phosphate buffer pH 7.4 buffer (0.5M NaCl + 2mM DTT + 1mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (crude lysate) was used in further purification steps. Proteins in the crude lysate were fractionated by addition of ammonium sulfate to 30% (wt/v) saturation. The solution was stirred for 1hr and then centrifuged. The pellet from this step was discarded and additional ammonium sulfate was added to the supernatant to 50 % (wt/v) ammonium sulfate saturation. The solution was centrifuged again after stirring for 1hr. The pellet from this step contains RuBisCO, and was kept at -20°°C until used. Protein was recovered from the pellet and prepared for use as described above.

RuBisCO also can be purified by adjusting the crude lysate to 0.1M NaCl and applying to an anion exchange resin. The weakly bound protein contaminants are washed with 50 mM KPhosphate buffer pH 7.4 buffer + 0.1M NaCl. RuBisCO was then eluted with high ionic strength buffer (0.5M NaCl).

RuBisCO solutions were decolorized (pH 7-9) by passing over columns packed with activated carbon. The colorants bound to the column while Rubisco was isolated in the filtrate.

RuBisCO solutions were also alternatively decolorized by incubating the solution with FPX66 (Dow Chemicals) resin packed in a column (or batch mode). The slurry is incubated for 30mins and then the liquid is separated from the resin. The colorants bind to the resin and RuBisCO was collected in the column flow-through.

In some cases, RuBisCO was isolated from spinach leaves by first grinding the leaves with 4 volumes of 20mM potassium Phosphate buffer pH 7.4 buffer + 150 mM NaCl + 0.5 mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (crude lysate) was filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

In some cases, RuBisCO was extracted from alfalfa or wheatgrass juice powder by mixing the powder with 4 volumes of 20mM potassium Phosphate buffer pH 7.4 buffer + 150 mM NaCl + 0.5 mM EDTA) in a blender. The resulting slurry was centrifuged to remove debris, and the supernatant (crude lysate) was filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(ix) Leghemoglobin. Soy root nodules were suspended and lysed in 20 mM potassium phosphate pH 7.4, 100mM potassium chloride and 5mM EDTA using grinder-blender. During this process leghemoglobin is released into the buffer. Root-nodule lysate containing leghemoglobin was cleared from cell debris by filtration through 5 micron filter. In some cases, filtration was followed by centrifugation (7000g, 20min). Clarified lysate containing leghemoglobin was then filtered through 0.2 micron filter and applied onto anion-exchange chromatography column (High Prep Q; High Prep DEAE, GE Healthtcare) on fast protein liquid chromatography instrument (GE Healthcare). Leghemoglobin was collected in flowthrough fraction and concentrated over 3kDa molecular weight cutoff PES membrane on a Spectrum Labs KrosFlo hollow fiber tangential flow filtration system to a desired concentration. Purity (partial abundance) of purified leghemoglobin was analyzed by SDS-PAGE gel: in lysate leghemoglobin is present at 20-40%, while after anion-exchange purification it is present at 70-80%. In another case, soybean leghemoglobin flow through from anion-exchange

chromatography was applied onto size-exclusion chromatography (Sephacryl S-100 HR, GE Healthcare). Soybean leghemoglobin eluted as two fractions corresponding to dimer and monomer species. Purity (partial abundance) of leghemoglobin was analyzed by SDS-PAGE and determined to be ~ 90-100%. Analysis of UV-VIS spectra (250-700nm) revealed spectral signature consistent with heme loaded leghemoglobin.

(x) Non-symbiotic hemoglobin from moong bean was cloned into pJexpress401 vector (DNA2.0) and transformed into *E. coli* BL21. Cells were grown in LB media containing soytone instead of tryptone, kanamycin, 0.1mM ferric chloride and 10 µg/ml 5-aminolevulinic acid. Expression was induced by 0.2mM IPTG and cells grown at 30°C for 20hr. *E.coli* cells expressing moong bean non-symbiotic hemoglobin were collected and resuspended in 20mM MES buffer pH 6.5, 50mM NaCL, 1mmM $MgCl_2$, 1mM $CaCl_2$. Add a bit of DNAaseI, and protease inhibitors. Cells were lysed by sonication. Lysate was cleared from cell debris by centrifugation at 16 000g for 20 min, followed by filtration over 200nm filter. Cell lysate was then loaded over FF-S resin on fast protein liquid chromatography instrument (GE Healthcare). Moong bean non-symbiotic hemoglobin bound to FF-S column and was eluted using sodium chloride gradient (50mM- 1000mM). Purity (partial abundance) of moong bean non-symbiotic hemoglobin was analyzed by SDS-PAGE and determined to be: *E.coli* lysate 13 %, after purification on FFQ 35%. UV-Vis analysis of purified protein showed spectra characteristic of heme bound protein.

(xi) Hemeproteins were synthesized with an N-terminal His6 epitope tag and a TEV cleavage site, cloned into pJexpress401 vector (DNA2.0), and transformed into E. *coli* BL21. Transformed cells were grown in LB media containing soytone instead of tryptone, kanamycin, 0.1 mM ferric chloride and 10 µg/ml 5-aminolevulinic acid. Expression was induced by 0.2mM IPTG and cells grown at 30°C for 20hr. *E.coli* cells expressing heme proteins were collected and resuspended in 50 mM potassium phosphate pH 8, 150 mM NaCl, 10 mM imidazole, 1 mM $MgCl_2$, 1 mM $CaCl_2$, DNAaseI, and protease inhibitors. Cells were lysed by sonication and clarified by centrifugation at 9000 x g. Lysate was incubated with NiNTA resin (MCLAB), washed with 5 column volumes (CV) of 50 mM potassium phosphate pH 8, 150 mM NaCl, 10 mM imidazole, and eluted with 50 mM potassium phosphate pH 8, 150 mM NaCl, 500 mM imidazole. SDS-PAGE and UV-vis spectra confirmed expected molecular weights and complete heme-loading, respectively.

In some cases, transformed cells were grown in seed media comprised of 10g/L glucose monohydrate, 8g/L Monopotassium Phosphate, 2.5g/L Sensient Amberferm 6400, 2.5g/L Sensient Tastone 154, 2g/L Diammonium Phosphate, 1mL/L Trace Metals Mixture (Teknova 1000x Trace Metals Mixture Cat. No. T1001), 1g/L Magnesium Sulfate, 0.25mL 0.1M solution Ferric Chloride, 0.5mL/L Sigma Anti-foam 204, 1mL/L Kanamycin Sulfate 1000x solution. 250mL of media was used in a (4)-1L baffeled shakeflasks, innoculated with 0.25mL each from a single vial of glycerol stock culture. Shakeflasks were grown for 5.5 hours, with 250RPM agitation at 37°C. 40L of seed media was steam-sterlized in a 100L bioreactor, cooled to 37°C, pH-adjusted to 7.0 and innoculated with 800mL of shakeflask culture once a shakeflask OD of 2.5 was achieved. Aeration to the bioreactor was supplied at 40L/m and agitation was 250RPM. After 2.2 hours of growth, an OD of 2.20 was reached and 22L of culture was

transferred to the final 4m^3 bioreactor. The starting media for the final bioreactor comprised of the following components steamed-in-place: 1775L deionised water, 21.75kg Monopotassium Phosphate, 2.175kg Diammonium Phosphate, 4.35kg Ammonium Ferric Citrate, 8.7kg Ammonium Sulfate, 10.875kg Sensient Amberferm 6400, 10.875kg Sensient Tastone 154. After 30 minutes of steaming, the media components were cooled to 37°C and post-sterilzation additions were made: 2.145L of 0.1M Ferric Chloride solution, 59.32kg 55%w/w Glucose Monohydrate, 3.9L of Trace Metals Mixture (Teknova 1000x Trace Metals Mixture Cat. No. T1001), 10.88L of 200g/L Diammonium Phosphate, 36.14L 1M Magnesium Sulfate, and 2.175L Sigma Anti-foam 204, 2.175L Kanamycin Sulfate 1000x solution. pH was controlled at 7.0 via the addition of 30% Ammonium Hydroxide. Aeration was supplied at 2.175m3/min, dissolved oxygen was controlled at 25% by varying agitation between 60-150RPM. At two timepoints (EFT=4 and EFT8), bolus additions of additional nutrients were supplied. Each addition added 5.5kg of Sensient Amberferm 6400, 5.5kg of Sensient Tastone 154 and 4.4kg of Diammonium Phosphate, in autoclaved solutions (100g/L solution for Amberferm and Tastetone, 200g/L for Diammonium Phosphate). A sterile glucose solution of 55%w/w Glucose Monohydrate was fed into the bioreactor to maintain a level of residudual glucose of 2-5g/L. Once an OD of 25 was reached, the temperature was reduced to 25°C and the culture was induced with 0.648L of 1M Isopropyl β-D-1-thiogalactopyranoside. The culture was allowed to grow for a total time of 25 hours, at which point the culture was diluted 1:1 with deionized water, then centrifuged, concentrating the centrate to 50%v/v solids content. Cell centrate was frozen at -20°C. Centrate was thawed to 4°C and diluted in 20 mM potassium phosphate pH 7.8, 100 mM NaCl, 10 mM imidazole, and homogenized at 15,000 PSI. Homogenized cells were 0.2 um filtered by tangential flow filtration (TFF) and filtered lysate was loaded directly onto a zinc-charged IMAC column (GE). Bound proteins were washed with 10 column volumes (CV) 20 mM potassium phosphate pH 7.4, 100 mM NaCl, 5 mM histidine and eluted with 10 CV 500 mM potassium phosphate monobasic, 100 mM NaCl. Eluted leghemoglobin was concentrated and diafiltered using a 3kDa molecular weight cutoff PES membrane and TFF. The concentrated sample was reduced with 20 mM sodium dithionite and desalted using G-20 resin (GE). Desalted leghemoglobin samples were frozen in liquid nitrogen and stored at -20 C. Leghemoglobin concentration and purity were determined by SDS-PAGE and UV-vis analysis.

(xi) Oleosin. Sunflower oil bodies were purified from sunflower seeds. Sunflower seeds were blended in 100 mM sodium phosphate buffer pH 7.4, 50mM sodium chloride, 1 mMEDTA at 1:3 wt/v. Oil-bodies were collected by centrifugation (5000g, 20min), and resuspended at 1:5 (wt/v) in 50 mM sodium chloride, 2M urea and stir for 30min, 4°C. 2M urea wash and centrifugation steps were repeated. Oil-bodies collected by centrifugation were resuspended in 100 mM sodium phosphate buffer pH 7.4, 50mM sodium chloride. Centrifugation and washing steps were repeated once more, and the final washed oil-bodies fraction was obtained from a last centrifugation step. Oil-bodies were resuspended at 10% wt/w in 100 mM sodium phosphate buffer pH 7.4, 50mM sodium chloride, 2% wt/v vegetable oil fatty acid salts, homogenized at 5000 psi and incubated at 4°C for 12 hr. Solution was centrifuged (8000g, 30min), top layer removed and soluble fraction collected. SDS-PAGE analysis suggested that oleosins are a major protein present in the soluble fraction. Oleosin concentration was 2.8 mg/ml.

(xii) Pea total proteins: Dry green or yellow pea flour was used to extract total pea proteins. The flour was suspended in 10 volumes of 20mM potassium phosphate buffer pH 8 and 100 mM sodium chloride and stirred for 1hr. Soluble protein was separated from pea seed debris by centrifugation. The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 Kda cutoff PES membrane.

(xiii) Pea vicilin and Pea legumin: Dry green or yellow pea flour was used to extract total pea proteins as described above. The crude pea mixture obtained thereof was fractionated into pea vicilin and pea legumin using ion-exchange chromatography. Material was loaded on Q Sepharose FastFlow resin and fractions were collected as salt concentration was varied from 100 mM to 500 mM NaCl. Pea vicilin was collected at 350 mM sodium chloride while pea legumin was collected at 460 mM sodium chloride. The collected fractions were concentrated using a 10 KDa cutoff PES membrane.

(xv) Lentil total proteins: Air classified lentil flour was used to extract crude mixture of lentil proteins. Flour was suspended in 5 volumes of 20 mM potassium phosphate buffer pH 7.4 and 0.5 M sodium chloride and stirred for 1 hr. Soluble protein was separated from un-extracted protein and lentil seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(xvi) Lentil albumins: Air classified lentil flour was suspended in 5 volumes of 50 mM sodium chloride, pH 3.8 and stirred for 1 hr. Soluble protein was separated from un-extracted protein and lentil seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(xvii) Chickpea / Garbanzo bean total proteins: Garbanzo bean flour was suspended in 5 volumes of 20 mM potassium phosphate buffer pH 7.4 and 0.5 M sodium chloride and stirred for 1 hr. Soluble protein was separated from un-extracted protein and chickpea seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(xviii) Chickpea/ Garbanzo bean albumins: Garbanzo bean flour was suspended in 5 volumes of 50 mM sodium chloride, pH 3.8 and stirred for 1 hr. Soluble protein was separated from un-extracted protein and lentil seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 10 KDa cutoff PES membrane.

(xix) Amaranth flour dehydrins: Amaranth flour was suspended in 5 volumes of 0.5 M sodium chloride, pH 4.0 and stirred for 1 hr. Soluble protein was separated from un-extracted protein and lentil seed debris by centrifugation (8000 g, 20 minutes). The supernatant was collected and filtered through a 0.2 micron membrane and concentrated using a 3 KDa cutoff PES membrane. Further enrichment of dehydrins from this fraction was obtained by boiling the concentrated protein material, spinning at 8000 g for 10 minutes and collecting the supernatant.

REFERENCE EXAMPLE 2: CONSTRUCTING A MUSCLE TISSUE ANALOG

[0060] To prepare a muscle tissue replica, 8 ml of moong bean protein solution (114 mg/ml in 20 mM phosphate buffer (pH 7.4) and 400 mM sodium chloride) were mixed with 16 ml of leghemoglobin solution (6 mg/ml leghemoglobin in 20 mM potassium phosphate, 400 mM NaCl, pH 7.3). The resulting mixture was concentrated using Amicon spin concentrators (10 kDa cut-off) to a final concentration of moong bean 8S globulin 61 mg/ml, and of leghemoglobin 6.5 mg/ml. Approximately 400 mg of transglutaminase powder were added to the solution, which were thoroughly mixed, and divided into two 50 ml Falcon tubes and incubated overnight at room temperature. Final total protein concentrations was 67.5 mg/ml total protein. The muscle tissue replica formed an opaque gel of reddish-brown color, with small amounts (< 1 ml) of inclusions of dark red, venous blood colored liquid.

REFERENCE EXAMPLE 3: ADIPOSE REPLICA OF BEEF FAT

[0061] An adipose tissue replica was made by gelling a solution of purified moong bean 8S protein emulsified with equal amounts of cocoa butter, coconut butter, olive oil and palm oil. Moong bean 8S protein was purified as described in Reference Example 1, and had a concentration of 140 mg/ml in 20mM K-phosphate pH 7.4, 400mM NaCl. A fat mixture was prepared by melting individual fats from solid to liquid state at 45°C for 30 min. Individual fats (cocoa butter, coconut butter, olive oil and palm oil) in liquid states were then mixed at a 1:1:1:1 (v/v) ratio. A protein-fat emulsion was formed by mixing a 70% v/v liquid fat mixture with 4.2% wt/v moong bean 8S protein, 0.4% wt/v soybean lecithin and emulsified by vortexing for 30 sec followed by sonication for 1 min. After homogenization, the fat-protein emulsion was in a single liquid phase as judged by visual observation.

[0062] One adipose tissue replica emulsion was stabilized by cross-linking with 0.2% wt/v transglutaminase enzyme at 37°C for 12hr. Another fat tissue replica was stabilized by gelling of proteins by heating to 100°C in a water bath followed by cooling to ambient room temperature. The resulting adipose tissue replicas were in a single phase. The adipose tissue replica matrix formed by the transglutaminase was a softer solid than the adipose tissue replica matrix formed by heat/cool induced gelling.

REFERENCE EXAMPLE 4: CONNECTIVE TISSUE ANALOG

[0063] Connective tissue fiber replicas were manufactured by electrospinning a solution of moong bean globulin (22.5 mg/ml) containing 400 mM sodium chloride, 6.75% w/v of poly(vinyl

alcohol) and trace amounts of sodium azide (0.007% w/v). The resulting solution was pumped at 3 µl/min using a syringe pump, from a 5 ml syringe through a Teflon tube and a blunted 21 gauge needle. The needle was connected to a positive terminal of a Spellman CZE 30 kV high voltage supply set at 17kV and fixed 12 cm from a an aluminum drum (ca. 12 cm long, 5 cm in diameter) that was wrapped in aluminum foil. The drum was attached to a spindle that is rotated by an IKA RW20 motor at about 220 rpm. The spindle was connected to a ground terminal of the high voltage supply. The protein/polymer fibers that accumulated on the foil were scraped off and used as the connective tissue replicas.

REFERENCE EXAMPLE 5: EXTENDING THE LIFETIME OF REDUCED (HEME-FE2+) LEGHEMOGLOBIN

[0064] Equine myoglobin was purchased from Sigma. Myoglobin was resuspended at 10 mg/ml in 20 mM potassium phosphate, pH 8.0, 100 mM NaCl. SDS-PAGE analysis suggested that protein purity was ~ 90%.

[0065] Soy leghemoglobin was purified from *Glycine max* root nodules via ammonium sulfate precipitation (60%/90% fractionation) as detailed in Reference Example 1. Resuspended 90% ammonium sulfate leghemoglobin was further purified by anion-exchange chromatography (HiTrap Q FF 5mL FPLC column) in 20 mM potassium phosphate, pH 8.0, 100 mM NaCl. The leghemoglobin eluted in flow through fractions. SDS-PAGE analysis suggested that protein purity was ~ 70%. Leghemoglobin was buffer exchanged into 20 mM potassium phosphate, pH 7.4, 100 mM NaCl and concentrated to 10 mg/ml on 3.5kDa membrane concentrators.

[0066] Carbon monoxide treatment: Myoglobin at 10 mg/ml in 20 mM potassium phosphate, pH 8.0, 100 mM NaCl and leghemoglobin at 10 mg/ml in 20 mM potassium phosphate, pH 7.4, 100 mM NaCl were first degassed under vacuum for 1 hour at 4°C then perfused with carbon monoxide gas for 2 minutes. Globins were then reduced from heme-Fe^{3+} to heme-Fe^{2+} state by adding 10 mM sodium dithionite, 0.1mM sodium hydroxide for 2 minutes. Sodium dithionite and sodium hydroxide were removed from the protein solution by using size-exclusion chromatography (PD-10 desalting column) in 20 mM potassium phosphate, pH 8.0, 100 mM NaCl and 20 mM potassium phosphate, pH 7.4, 100 mM NaCl respectively. Globins fractions were collected as a peak red colored fractions as evaluated by visual estimation. UV-VIS spectra confirmed the presence of heme-Fe^{2+} state for both proteins. After desalting, the solution was again perfused with gas for another 2 minutes. The color of the solutions was evaluated by taking UV-Vis spectra (250nm- 700nm) every 20 minutes using the nanodrop spectrophotometer. Control samples were not treated with carbon monoxide.

[0067] Sodium nitrite treatment: Myoglobin at 10 mg/ml in 20 mM potassium phosphate, pH 8.0, 100 mM NaCl and leghemoglobin at 10 mg/ml in 20 mM potassium phosphate, pH 7.4, 100mM NaCl were reduced from heme-Fe^{3+} to heme -Fe^{2+} by adding 10 mM sodium dithionite, 0.1mM sodium hydroxide for 2 minutes. Sodium dithionite and sodium hydroxide

were removed from the protein solution by using size-exclusion chromatography (PD-10 desalting column) in 20 mM potassium phosphate, pH 8.0, 100 mM NaCl and 20 mM potassium phosphate, pH 7.4, 100 mM NaCl, respectively. Globins fractions were collected as peak red colored fractions as evaluated by visual estimation. UV-VIS spectra confirmed the presence of heme-Fe^{2+} state for both proteins. Sodium nitrite was then added to a final concentration of 1 mM from 100 mM nitrite in phosphate buffer pH 7.4. The lifetime of heme-Fe^{2+} state was followed by recording UV-VIS spectra (250-700nm) using spectrophotometer as a function of time. Control samples were not treated with sodium nitrite.

[0068] Data analysis of heme-Fe^{2+} life-times for myoglobin and leghemoglobin samples treated with carbon monoxide and sodium nitrite was performed in Microsoft Excel by plotting the amplitude of the absorbance peak at the 540nm wavelength. The "baseline" of the 540 nm absorbance was determined by the state of the UV-vis spectrum of the globin solutions prior to the addition of any additives, dithionite reduction, or desalting. The built-in curve fit function was used to produce an exponential line-of-best-fit, the exponent of which directly relates to the half life of the peak amplitude.

[0069] The life-time of the heme-Fe^{2+} state and accompanying red color of myoglobin and leghemoglobin solutions in the absence of carbon monoxide and sodium nitrite were ~ 6hr and ~ 4hr respectively. Addition of sodium nitrite extended the life-time of the heme-Fe^{2+} state and accompanying red color to more than seven days. Addition of carbon monoxide extended the life-time of heme-Fe^{2+} state and accompanying red color to more than two weeks.

REFERENCE EXAMPLE 6: PREPARATION OF MEAT REPLICAS IN WHICH THE PARTICLE SIZE OF INDIVIDUAL TISSUE REPLICA UNITS IS VARIED TO CONTROL AROMA GENERATION DURING COOKING.

[0070] Muscle tissue replica and adipose tissue replica were prepared separately and then combined into a meat tissue replica such that the size of individual tissue replica units was varied to control aroma generation during cooking. Individual fat, muscle and connective tissue replicas were constructed in the following manner.

[0071] A muscle tissue replica was prepared as in Reference Example 2. The muscle tissue replica formed an opaque gel of reddish-brown color, with small amounts (< 1 ml) of inclusions of dark red, venous blood colored liquid. A connective tissue replica was prepared as in Reference Example 4. An adipose tissue replica was prepared as in Reference Example 3.

[0072] Meat replicas with a lean-to-fat ratio 85/15 were prepared by combining individual muscle, connective and adipose tissues such that particle size of individual tissues replicas varied. (a) 2.1 g of muscle replica with 0.9 g of chunks of fat replica 5-10 mm in size ("coarse mix"); (b) 2.1 g of muscle replica with 0.9 g of fat replica chopped to 2-3 mm in size ("fine mix");

and (c) 2.1 g of muscle replica with 0.9 g of fat replica thoroughly blended to <1 mm in size ("blend"). "Muscle only" control sample contained 3 g of muscle replica alone. "Fat only" control sample contained 3 g of fat replica alone as 5-10 mm sized particle. Meat, muscle and fat tissue samples were cooked in sealed glass vials at 150°C for 10 min. Aroma profiles of the samples were analyzed by a panel of testers, and by GC-MS.

[0073] Sensory olfactory analysis of meat replica samples performed by a panel of testers, suggested that the size of individual tissue units and the extent of their mixing within meat tissue replicas correlated with generation of different aromas. Muscle tissue replica cooked by itself generated aromas associated with store-bought gravy, faint citrus and star anise. Adipose tissue replica cooked by itself generated aromas associated with musty, rancid and sweet aromas. Cooked meat tissue replica (coarse particle size) generated aromas of store-bought gravy, sweet, slightly musty and star-anise. Cooked meat tissue replica (fine particle size) generated aromas associated with soy sauce, musty, slightly rancid and beef bouillon. Cooked meat tissue replica (very fine particle size) generated aromas associated with sweet, musty and soy-sauce. All samples with the exception of adipose tissue replica generated aromas associated with burnt meat smell, however to varying intensities.

[0074] Analysis of GCMS data indicated that the size of individual tissue units and the extent of their mixing within meat tissue replicas had profound effects on the generation of aromatic compounds upon cooking. In particular, multiple aromatic compounds associated with fruity/grean bean/metallic (2-pentyl-furan); nutty/green (4-methylthiazole); peanut butter/musty (pyrazine, ethyl); raw potato/roasted/earthy (Pyrazine, 2,3-dimethyl); vinegary (acetic acid); spicy/caramel/almond (5-methyl-2-furancarboxaldehyde); creamy (butyrolactone); sweet (2,5-dimethyl-3-(3-methyl butyl) pyrazine); fruity/stale beer (2-cyclopentene-1-one, 2-hydroxy-3-methyl); musty/nutty/coumarin/licorice/walnut/bread (3-acetyl-1H-pyrolline); coconut/woody/sweet (pantolactone); penetrating (1-H-pyrrole-2-2carboxaldehyde, 1-methyl); minty (caprolactam); toasty caramel (4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl) aromas appeared only in mixed meat replicas, but not in individual tissue replicas. Some other aromatic compounds, for example associated with gasoline-like (nonane, 2,6-dimethyl), petroleum-like (3-hexene, 3-methyl); sour/putrid/fish-like (pyridine); bland/woody/yogurt (acetoin); fatty/honey/citrus (octanal); pungent/sweet/caramelic (2-propanone, 1-hydroxy) and nutty/burnt green (ethenyl pyrazine) aromas appeared only in individual tissue replicas, but did not accumulate in mixed meat replicas. Furthermore, the levels to which all of the above compounds accumulated during cooking depended on the sizes of tissue units and how they are mixed (coarse particle size, fine particle size, or very fine particle size (blended)).

[0075] Similar to meat tissue replica, it was found that structural organization and particle size of beef tissues modify response of beef tissues to cooking. For example, the flavor of meat is modified by the size of the particles. Beef samples were prepared as following: samples of beef muscle and beef fat were cut separately with a knife and: (a) "ground", where knife-cut tissue cubes were passed through standard meat grinder. 80/20 (wt/wt) lean/fat ground beef sample was prepared by mixing muscle and fat tissue cubes at appropriate ratio prior to grinding. This sample preparation is referred to as a "fine size particle mix", (b) Ground tissue particle size

was further reduced by freezing ground tissue in liquid nitrogen and crushing it using mortar and pestle to a very fine powder (particle size <1mm). This sample preparation is referred to as a "very fine size particle mix". All samples were cooked in sealed glass vials at 150 °C for 10 min. Aroma profiles of the samples were analyzed by a panel of testers, and by GC-MS, as described in Reference Example 1. "Muscle only" control sample contained 3 g of muscle tissue alone. "Fat only" control sample contained 3 g of fat tissue alone. Ground beef sample contained 3 g of a 80/20 (wt/wt) muscle/fat mixture.

[0076] Sensory olfactory analysis of beef samples performed by a panel of testers, suggested that the size of individual tissue units and the extent of their mixing within the samples correlated with generation of different aromas. Beef muscle cooked by itself generated typical aromas associated with cooked ground beef. Fat tissue replica cooked by itself generated slightly sweet aromas, and aromas associated with burnt mushrooms. Cooked ground beef with "fine size particle mix" generated typical aromas associated with cooked ground beef, with presence of slightly sweet aromas characteristic of cooked fat. Cooked ground beef with "very fine size particle mix" generated aromas associated with cooked ground beef, but no slightly sweet aroma characteristic of cooked fat was detected.

[0077] Analysis of GCMS data indicated that the particle size of the individual tissue units has effect on generation of aromatic compounds upon cooking. In particular generation and/or amount of multiple aromatic compounds by individual tissue samples or ground beef sample varied in correlation with particle size of the tissue. Some of the aromatic compounds that differed between fine and very fine particle size of muscle tissue: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 3-Acetyl-1H-pyrolline, 1-(6-methyl-2-pyrazinyl)-1-ethanone, 2,5-dimethyl-3-(3-methyl butyl) pyrazine, 2-furancarboxyaldehyde, 5-methyl, Acetic acid, Ethenyl pyrazine, Pyrazine, 2,3-dimethyl , 2-Propanone, 1-hydroxy, Octanal, Acetoin, 4-Methylthiazole, Pseudo-2-pentyl-furan, 2-pentyl-furan. Some of the aromatic compounds that differed between fine and very fine particle size of fat tissue: triethylene glycol: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, Caprolactam, 1-(6-methyl-2-pyrazinyl)-1-ethanone, 2-Cyclopentene-1-one, 2-hydroxy-3-methyl, Butyrolactone, 2-furancarboxyaldehyde, 5-methyl, Ethanone, 1(2 furanyl), Acetic acid, 2-ethyl-5-methyl pyrazine, Pyrazine, 2,3-dimethyl, Pyrazine, ethyl, Octanal, Acetoin, 4-Methylthiazole, Pseudo-2-pentyl-furan, Pyridine, Nonane, 2,6-dimethyl. Some of the aromatic compounds that differed between fine and very fine particle size of 80/20 muscle/fat sample: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, Caprolactam, 1H-1Pyridine, 3-carbonitrile, 4-ethyl-2-oxo-2,5, 1-H-Pyrrole-2-2carboxaldehyde, 1-methyl, 2-Cyclopentene-1-one, 2-hydroxy-3-methyl, 2,5-dimethyl-3-(3-methyl butyl) pyrazine, Butyrolactone, 2-furancarboxyaldehyde, 5-methyl, Ethanone, 1(2 furanyl), Acetic acid, Ethenyl pyrazine, 2-ethyl-5-methyl pyrazine, Pyrazine, 2,3-dimethyl; 2-Propanone, 1-hydroxy, Octanal, Acetoin, 2-pentyl-furan.

EXAMPLE 7: LEGHEMOGLOBIN CONTRIBUTION TO FLAVOR

[0078] Beef flavors and aromas can be created in non beef consumables by addition of heme

proteins. Ground chicken (90% lean, 10% fat) was strained with cheesecloth and mixed with recombinant soy leghemoglobin or recombinant bovine myoglobin to a final concentration of 0.5-1.0% wt/wt. The recombinant heme proteins were expressed in *E. coli* and purified by nickel affinity purification as described in Reference Example 1. Prior to being mixed with chicken, the heme proteins were reduced with 20 mM Na Dithionite. Na dithionite was removed from the sample with a Zeba desalting column (Thermo Scientific). Leghemoglobin was desalted into 20 mM potassium phosphate pH 7.4, 100 mM NaCl. Myoglobin was desalted into either 20 mM potassium phosphate pH 7.4, 100 mM NaCl or 20 mM Na citrate pH 6.0, 100 mM NaCl. The reduced heme protein samples were divided in two, and half the sample was bubbled with carbon monoxide (CO) for 2 minutes. After mixing the heme protein samples with ground chicken, the mixture was poured into nugget-shaped molds and incubated overnight at 4°C. The nuggets were oven baked or pan fried at 165°C until each nugget reached an internal temperature of 165°C. A panel of judges tasted nuggets containing chicken alone, chicken mixed with buffer, chicken mixed with either leghemoglobin or myoglobin +/-CO, or beef (90% lean, 10% fat). Judges filled out a survey to evaluate the aroma and flavor of each nugget. Judges rated the aroma and flavor of each nugget as follows: 1 = chicken, 2 = chicken + faint beef, 3 = 50/50 chicken + beef, 4 = beef + faint chicken, 5 = beef. Shown in Table 2 are the average scores received for each nugget. Percentages indicate the final concentration of heme protein wt/wt (abbreviations: KP = 20 mM potassium phosphate pH 7.4, 100 mM NaCl buffer. NC = 20 mM Na citrate pH 6.0, 100 mM NaCl buffer. n/d = not determined). Adding recombinant leghemoglobin or myoglobin to chicken resulted in an increased beef aroma and flavor. The perceived levels of beef flavor and aroma increased with the myoglobin and leghemoglobin content. Leghemoglobin and myoglobin provide the same benefit to the flavor and aroma.

TABLE 2

	Oven Baked		Pan Fried	
	Aroma	Flavor	Aroma	Flavor
Chicken	1	1	1	1
Chicken KP	1	1	2.5	1.2
Chicken NC	1.5	1.5	1.5	1
Chicken 0.5% legH KP	1.5	2.5	3.67	3.2
Chicken 0.5% legH+CO KP	2.5	2.5	2.67	2.2
Chicken 0.5% Myo NC	2	2	1.5	2.4
Chicken 0.5% Myo+CO NC	2	2	2.5	3
Chicken 0.5% Myo+CO KP	2.5	2.5	2.33	2
Chicken 0.8% Myo+CO NC	2	3	4	2.6
Chicken 1% Myo NC	4.5	4	n/d	n/d
Chicken 1% legH NC	4	4	n/d	n/d
Beef	5	5	5	5

REFERENCE EXAMPLE 8: Generation of beef flavor in replica burgers by the addition of Heme and flavor precursors.

[0079] Characteristic flavor and fragrance components in meat are mostly produced during the cooking process by chemical reactions molecules (precursors) including amino acids, fats and sugars that are found in plants as well as meat. Flavor precursors along with 1% Leghemoglobin were added to the muscle component of the burger replicas as indicated in Table 8. Three replicas, one with no precursors, and two different mixtures of precursors, along with 80:20 beef were cooked then served to a trained sensory panel to describe the flavor attributes shown in Table 9. The addition of precursors increased the beefy flavor, the bloody notes, overall flavor quanity, and decreased the off notes in the replica. The replicas and beef sample also were analyzed by GCMS by adding 3 grams of uncooked replica or beef into a GCMS vial. All samples were cooked at 150°C for 3 mins, cooled to 50°C to extract for 12 minutes using GCMS (SPME fiber sampling of headspace). A search algorithm analyzed the retention time and mass fingerprint information to assign chemical names to peaks. In the replica burger with 1% Leg hemeoglobin, and precursor mixture 2, 136 beef compounds were created. In Table 10, all the compounds created in the replica burger that were also identified by GCMS in the beef samples are indicated.

TABLE 8

Flavor precursors added to the beef replicas before cooking.			
Samples	**767**	**804**	**929**
Additive (mm)	**Precursor Mix 1**	**No Precursors**	**Precursor Mix 2**
Alanine	5.61		5.61
Cysteine	0.83		0.83
Glutamic acid	3.40		3.40
Leucine	0.76		0.76
Lysine	0.68		0.68
Methionine	0.67		0.67
Tryptophan	0.49		0.49
Tyrosine	0.55		0.55
Valine	0.85		0.85
Glucose	5.55		5.55
Ribose	6.66		6.66
Lactic acid	1.00		1.00
creatine	1.00		1.00
Thiamine	0.50		0.50
IMP + GMP	0.40		0.40
Sucrose			2.00

Flavor precursors added to the beef replicas before cooking.			
Samples	**767**	**804**	**929**
Additive (mm)	**Precursor Mix 1**	**No Precursors**	**Precursor Mix 2**
Fructose			2.00
Xylose			2.00
Maltodextrin	0.50%		0.50%

TABLE 9

The sensory score determined by the sensory panel for the replica burgers and 80:20 beef sample.

Sample #		Beef	767	804	929
Flavor Quality	mean	7.0	3.8	3.2	4.3
	STDEV	0.0	1.3	1.0	1.2
Flavor Intensity	mean	4.3	4.2	4.3	4.3
	STDEV	0.8	1.2	1.4	1.2
Flavor: Beefy	mean	5.8	3.3	2.3	4.2
	STDEV	1.0	1.2	0.8	1.2
Flavor: bloody/Metallic	mean	4.5	2.0	2.2	3.2
	STDEV	1.2	1.0	0.9	1.4
Flavor: Savory	mean	3.3	3.8	3.7	4.2
	STDEV	1.2	1.2	1.2	1.8
Off Flavors: chemical/oxidized/beany	mean	1.5	2.3	3.5	2.7
	STDEV	0.8	1.2	1.8	1.0

TABLE 10

Beef flavor compounds created in replica burger with 1% LegH and precursor mix 2 as detected by GCMS.		
3-octen-2-one	octanoic acid	(Z)-2-decenal,
1-penten-3-ol	octane	carbon disulfide
n-caproic acid vinyl ester	octanal	butyrolactone
2-acetylthiazole	nonanal	butanoic acid
thiophene	4,7-dimethyl-undecane,	3-methyl-butanal,
methyl-thiirane,	methyl ethanoate	2-methyl-butanal,
thiazole	methional	butanal
styrene	methacrolein	3,6,6-trimethyl-bicyclo[3.1.1]hept-2-ene,
	isovaleric acid	benzyl alcohol

Beef flavor compounds created in replica burger with 1% LegH and precursor mix 2 as detected by GCMS.		
pyrrole	isopropyl alcohol	1,3-dimethyl-benzene
pyridine	hexanoic acid	benzene
trimethyl-pyrazine	2,2,4,6,6-pentamethyl-heptane,	benzaldehyde
tetramethyl-pyrazine,	2-methyl-heptane,	acetophenone
methyl-pyrazine,	heptane	acetonitrile
ethyl-pyrazine	heptanal	acetone
3-ethyl-2,5-dimethyl-pyrazine	furfural	acetoin
2,5-dimethyl-pyrazine	furaneol	acetic acid ethenyl ester
2,3-dimethyl-pyrazine	3-methyl-furan	acetic acid
2-ethyl-5-methyl-pyrazine	2-propyl-furan	acetamide
2-ethenyl-6-methyl-pyrazine,	2-pentyl-furan	acetaldehyde
pyrazine	2-methyl-furan	4-methyl-5-thiazoleethanol
2-methyl-propanal,	2-ethyl-furan	6-methyl-5 -hepten-2-one
propanal	furan	trans-2-(2-pentenyl)furan
phenylacetaldehyde	formamide	(E)-4-octene,
phenol	ethyl acetate	4-cyclopentene-1,3-dione
pentanoic acid	1-(2-furanyl)-ethanone	4-cyanocyclohexene
3-ethyl-2,2-dimethyl-pentane	1-(1H-pyrrol-2-yl)-ethanone	dihydro-2-methyl-3(2H)-furanone,
pentanal	dimethyl trisulfide	(E,E)-3,5-octadien-2-one
p-cresol	dimethyl sulfide	3,5-octadien-2-one
oxalic acid, butyl propyl ester	d-limonene	2,2-dimethyl-undecane
1-heptene	1-octen-3-ol	toluene
1-ethyl-5-methylcyclopentene	1-octanol	1-pentanol
1-butanol	1-hexanol	1-octen-3-one
1H-pyrrole-2-carboxaldehyde	2-methyl-1H-pyrrole	2-butanone
2-nonanone	3-methyl-2-butenal	2-thiophenecarboxaldehyde
2-n-butylacrolein	3 -ethylcyclopentanone	2-pyrrolidinone
2-methyl-2-heptene	2(5H)-furanone	2-propenal

Beef flavor compounds created in replica burger with 1% LegH and precursor mix 2 as detected by GCMS.		
(E)-2-hexenal,	dihydro-5-pentyl-2(3H)-furanone	1 -hydroxy-2-propanone
(E)-2-heptenal,	5-ethyldihydro-2(3H)-furanone	1-(acetyloxy)-2-propanone
6-methyl-2-heptanone	5-acetyldihydro-2(3H)-furanone	2-pentanone
2-heptanone	2,6-dimethylpyrazine	(E)-2-octenal
2-furanmethanol	(E,E)-2,4-nonadienal	2-octanone
3-ethyl-2-1,4-dioxin	(E,E)-2,4-heptadienal	(E)-2-nonenal
3-ethyl-2-methyl-1,3-hexadiene	(E,E)-2,4-decadienal	8-methyl-1-undecene
2- butenal	2,3-dimethyl-5-ethylpyrazine	1-propanol
1-penten-3-one		

OTHER EMBODIMENTS

[0080] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. The following claims define the scope of the invention and methods within the scope of these claims and their equivalents are covered thereby.

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US2009010042A1 [0014]

Non-patent literature cited in the description

- **CALKINSHODGEN**Meat Science, 2007, vol. 77, 63-80 [0015]
- **S. S. NIELSON**Introduction to the Chemical Analysis of FoodsJones & Bartlett Publishers19940000 [0025]
- **REEDY et al.**Nucleic Acids Research, 2008, vol. 36, D307-D313 [0035]

PATENTKRAV

1. Fremgangsmåde til bibringelse af en oksekødslignende smag til et drikke- eller fødevareprodukt, hvilken fremgangsmåde omfatter, at der til drikke- eller fødevareproduktet injiceres en hemholdig proteinopløsning, hvor drikke- eller fødevareproduktet efter tilberedning bibringes en oksekødslignende smag;

hvor drikke- eller fødevareproduktet er lyst kød.

2. Fremgangsmåde ifølge krav 1, hvor drikke- eller fødevareproduktet er fjerkrækød.

3. Fremgangsmåde ifølge krav 2, hvor drikke- eller fødevareproduktet er kylling.

4. Fremgangsmåde ifølge krav 1, hvor drikke- eller fødevareproduktet er svinekød.

5. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor det hemholdige protein er fra et pattedyr, en plante, en svamp, en bakterie, en alge eller en protozo.

6. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor det hemholdige protein er en ikke-symbiotisk type af hæmoglobin eller et leghæmoglobin.

7. Fremgangsmåde ifølge et hvilket som helst af kravene 1-5, hvor det hemholdige protein er et androglobin, et cytoglobin, et globin E, et globin X, et globin Y, et hæmoglobin, et leghæmoglobin, et flavohæmoglobin, “Hell's gate”-globin I, et myoglobin, et erythrocruorin, et beta-hæmoglobin, et alpha-hæmoglobin, et protoglobin, et cyanoglobin, et cytoglobin, et histoglobin, et neuroglobin, et chlorcruorin, et trunkeret hæmoglobin, der indbefatter HbN og HbO, et trunkeret 2/2-globin, et hæmoglobin 3, der indbefatter Glb3, et cytochrom eller en peroxidase.

8. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor det hemholdige protein har en aminosyresekvens med mindst 70 % homologi til en hvilken som af de aminosyresekvenser, som er angivet i SEQ ID NO: 1-27.

9. Anvendelse af et hemprotein til fremstilling af en fjerkrækødsammensætningssmag som oksekød, hvor hemproteinet tilsættes til fjerkrækødsammensætningen.

10. Anvendelse ifølge krav 9, hvor hemproteinet er fra et pattedyr, en plante, en svamp, en bakterie, en alge eller en protozo.

11. Anvendelse ifølge et hvilket som helst af kravene 9-10, hvor hemproteinet er en ikke-symbiotisk type af hæmoglobin eller et leghæmoglobin.

12. Anvendelse ifølge et hvilket som helst af kravene 9-11, hvor hemproteinet er et androglobin, et cytoglobin, et globin E, et globin X, et globin Y, et hæmoglobin, et leghæmoglobin, et flavohæmoglobin, "Hell's gate"-globin I, et myoglobin, et erythrocruorin, et beta-hæmoglobin, et alpha-hæmoglobin, et protoglobin, et cyanoglobin, et cytoglobin, et histoglobin, et neuroglobin, et chlorcruorin, et trunkeret hæmoglobin, der indbefatter HbN og HbO, et trunkeret 2/2-globin, et hæmoglobin 3, der indbefatter Glb3, et cytochrom eller en peroxidase.

13. Anvendelse ifølge et hvilket som helst af kravene 9-12, hvor hemproteinet har en aminosyresekvens med mindst 70 % homologi til en hvilken som helst af de aminosyresekvenser, som er angivet i SEQ ID NO: 1-27.

DRAWINGS

FIGURE 1

SEQ ID NO:1 *Vigna radiata*

MTTTLERGFTEEQEALVVKSWNVMKKNSGELGLKFFLKIFEIAPSAQKLFSFLRDSTVP
LEQNPKLKPHAVSVFVMTCDSAVQLRKAGKVTVRESNLKKLGATHFRTGVANEHFEVTK
FALLETIKEAVPEMWSPAMKNAWGEAYDQLVDAIKYEMKPPSS

SEQ ID NO:2 *Methylacidiphilum infernorum*

MIDQKEKELIKESWKRIEPNKNEIGLLFYANLFKEEPTVSVLFQNPISSQSRKLMQVLG
ILVQGIDNLEGLIPTLQDLGRRHKQYGVVDSHYPLVGDCLLKSIQEYLGQGFTEEAKAA
WTKVYGIAAQVMTAE

SEQ ID NO:3 *Aquifex aeolicus*

MLSEETIRVIKSTVPLLKEHGTEITARMYELLFSKYPKTKELFAGASEEQPKKLANAII
AYATYIDRLEELDNAISTIARSHVRRNVKPEHYPLVKECLLQAIEEVLNPGEEVLKAWE
EAYDFLAKTLITLEKKLYSQP

SEQ ID NO:4 *Glycine max*

MGAFTEKQEALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLSNGVDPSNPKL
TGHAEKLFGLVRDSAGQLKANGTVVADAALGSIHAQKAITDPQFVVVKEALLKTIKEAV
GDKWSDELSSAWEVAYDELAAAIKKAF

SEQ ID NO:5 *Hordeum vulgare*

MSAAEGAVVFSEEKEALVLKSWAIMKKDSANLGLRFFLKIFEIAPSARQMFPFLRDSDV
PLETNPKLKTHAVSVFVMTCEAAAQLRKAGKITVRETTLKRLGGTHLKYGVADGHFEVT
RFALLETIKEALPADMWGPEMRNAWGEAYDQLVAAIKQEMKPAE

Magnaporthe oryzae, (SEQ ID NO:6)

```
   1 mdgavrldwt gldltgheih dgvpiasrvq vmvsfplfkd qhiimsskes psrksstigq
  61 strngscqad tqkgqlppvg ekpkpvkenp mkklkemsqr plptqhgdgt yptekkltgi
 121 gedlkhirgy dvktllamvk sklkgeklkd dktmlmervm qlvarlptes kkraeltdsl
 181 inelwesldh pplnylgpeh syrtpdgsyn hpfnpqlgaa gsryarsvip tvtppgalpd
 241 pglifdsimg rtpnsyrkhp nnvssilwyw atiiihdifw tdprdintnk sssyldlapl
 301 ygnsqemqds irtfkdgrmk pdcyadkrla gmppgvsvll imfnrfhnhv aenlalineg
 361 grfnkpsdll egeareaawk kydndlfqva rlvtsglyin itlvdyvrni vnlnrvdttw
 421 tldprqdaga hvgtadgaer gtgnavsaef nlcyrwhsci sekdskfvea qfqnifgkpa
 481 sevrpdemwk gfakmeqntp adpgqrtfgg fkrgpdgkfd dddlvrcise avedvagafg
 541 arnvpqamkv vetmgiiqgr kwnvaglnef rkhfhlkpys tfedinsdpg vaealrrlyd
 601 hpdnvelypg lvaeedkqpm vpgvgiapty tisrvvlsda vclvrgdrfy ttdftprnlt
```

```
 661 nwgykevdyd lsvnhgcvfy klfirafpnh fkqnsvyahy pmvvpsenkr ilealgradl
 721 fdfeapkyip prvnitsygg aeyiletqek ykvtwheglg flmgegglkf mlsgddplha
 781 qqrkcmaaql ykdgwteavk afyagmmeel lvsksyflgn nkhrhvdiir dvgnmvhvhf
 841 asqvfglplk taknptgvft eqemygilaa ifttiffdld psksfplrtk trevcqklak
 901 lveanvklin kipwsrgmfv gkpakdepls iygktmikgl kahglsdydi awshvvptsg
 961 amvpnqaqvf aqavdyylsp agmhyipeih mvalqpstpe tdalllgyam egirlagtfg
1021 syreaavddv vkedngrqvp vkagdrvfvs fvdaardpkh fpdpevvnpr rpakkyihyg
1081 vgphaclgrd asqiaitemf rclfrrrnvr rvpgpqqelk kvprpggfyv ymredwgglf
1141 pfpvtmrvmw dde
```

Fusarium oxysporum (SEQ ID NO:7)

```
   1 mkgsatlafa lvqfsaasql vwpskwdeve dllymqggfn krgfadalrt cefgsnvpgt
  61 qntaewlrta fhdaithdak agtggldasi ywessrpenp gkafnntfgf fsgfhnprat
 121 asdltalgtv lavgacngpr ipfragrida ykagpagvpe pstnlkdtfa aftkagftke
 181 emtamvacgh aiggvhsvdf peivgikadp nndtnvpfqk dvssfhngiv teylagtskn
 241 plvasknatf hsdkrifdnd katmkklstk agfnsmcadi lttmidtvpk svqltpvlea
 301 ydvrpyitel slnnknkihf tgsvrvritn nirdnndlai nliyvgrdgk kvtvptqqvt
 361 fqggtsfgag evfanfefdt tmdakngitk ffiqevkpst katvthdnqk tggykvddtv
 421 lyqlqqscav leklpnaplv vtamvrdara kdaltlrvah kkpvkgsivp rfqtaitnfk
 481 atgkkssgyt gfqaktmfee qstyfdivlg gspasgvqfl tsqampsqcs
```

Fusarium graminearum (SEQ ID NO:8)

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

SEQ ID NO: 9 *Chlamydomonas eugametos*

```
MSLFAKLGGREAVEAAVDKFYNKIVADPTVSTYFSNTDMKVQRSKQFAFLAYALGGASE
WKGKDMRTAHKDLVPHLSDVHFQAVARHLSDTLTELGVPPEDITDAMAVVASTRTEVLN
MPQQ
```

SEQ ID NO:10 *Tetrahymena pyriformis*

```
MNKPQTIYEKLGGENAMKAAVPLFYKKVLADERVKHFFKNTDMDHQTKQQTDFLTMLLG
GPNHYKGKNMTEAHKGMNLQNLHFDAIIENLAATLKELGVTDAVINEAAKVIEHTRKDM
LGK
```

SEQ ID NO:11 *Paramecium caudatum*

MSLFEQLGGQAAVQAVTAQFYANIQADATVATFFNGIDMPNQTNKTAAFLCAALGGPNA
WTGRNLKEVHANMGVSNAQFTTVIGHLRSALTGAGVAAALVEQTVAVAETVRGDVVTV

SEQ ID NO:12 *Aspergillus niger*

MPLTPEQIKIIKATVPVLQEYGTKITTAFYMNMSTVHPELNAVFNTANQVKGHQARALA
GALFAYASHIDDLGALGPAVELICNKHASLYIQADEYKIVGKYLLEAMKEVLGDACTDD
ILDAWGAAYWALADIMINREAALYKQSQG

SEQ ID NO:13 *Zea mays*

MALAEADDGAVVFGEEQEALVLKSWAVMKKDAANLGLRFFLKVFEIAPSAEQMFSFLRD
SDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRETTLKRLGATHLRYGVADGHF
EVTGFALLETIKEALPADMWSLEMKKAWAEAYSQLVAAIKREMKPDA

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

MALVEGNNGVSGGAVSFSEEQEALVLKSWAIMKKDSANIGLRFFLKIFEVAPSASQMFS
FLRNSDVPLEKNPKLKTHAMSVFVMTCEAAAQLRKAGKVTVRDTTLKRLGATHFKYGVG
DAHFEVTRFALLETIKEAVPVDMWSPAMKSAWSEAYNQLVAAIKQEMKPAE

SEQ ID NO:15 *Arabidopsis thaliana*

MESEGKIVFTEEQEALVVKSWSVMKKNSAELGLKLFIKIFEIAPTTKKMFSFLRDSPIP
AEQNPKLKPHAMSVFVMCCESAVQLRKTGKVTVRETTLKRLGASHSKYGVVDEHFEVAK
YALLETIKEAVPEMWSPEMKVAWGQAYDHLVAAIKAEMNLSN

SEQ ID NO:16 *Pisum sativum*

MGFTDKQEALVNSSWESFKQNLSGNSILFYTIILEKAPAAKGLFSFLKDTAGVEDSPKL
QAHAEQVFGLVRDSAAQLRTKGEVVLGNATLGAIHVQRGVTDPHFVVVKEALLQTIKKA
SGNNWSEELNTAWEVAYDGLATAIKKAMT

SEQ ID NO:17 *Vigna unguiculata*

MVAFSDKQEALVNGAYEAFKANIPKYSVVFYTTILEKAPAAKNLFSFLANGVDATNPKL
TGHAEKLFGLVRDSAAQLRASGGVVADAALGAVHSQKAVNDAQFVVVKEALVKTLKEAV
GDKWSDELGTAVELAYDELAAAIKKAY

SEQ ID NO:18 Bos taurus

MGLSDGEWQLVLNAWGKVEADVAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGNTVLTALGGILKKKGHHEAEVKHLAESHANKHKIPVKYLEFISDAIIHVLHA
KHPSDFGADAQAAMSKALELFRNDMAAQYKVLGFHG

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

SEQ ID NO: 20 *Equus caballus*

MGLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKAS
EDLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHVLHS
KH PGDFGADAQGAMTKALELFRNDIAAKYKELGFQG

SEQ ID NO: 21 Nicotiana benthamiana
MSSFTEEQEALVVKSWDSMKKNAGEWGLKLFLKIFEIAPSAKKLFSFLKDSNVPL
EQNAKLKPHSKSVFVMTCEAAVQLRKAGKVVVRDSTLKKLGATHFKYGVADE
HFEVTKFALLETIKEAVPEMWSVDMKNAWGEAFDQLVNAIKTEMK

SEQ ID NO: 22 Bacillus subtilis
MGQSFNAPYEAIGEELLSQLVDTFYERVASHPLLKPIFPSDLTETARKQKQFLTQY
LGGPPLYTEEHGHPMLRARHLPFPITNERADAWLSCMKDAMDHVGLEGEIREFL
FGRLELTARHMVNQTEAEDRSS

SEQ ID NO: 23 Corynebacterium glutamicum

MTTSENFYDSVGGEETFSLIVHRFYEQVPNDDILGPMYPPDDFEGAEQRLKMFLS
QYWGGPKDYQEQRGHPRLRMRHVNYPIGVTAAERWLQLMSNALDGVDLTAEQ
REAIWEHMVRAADMLINSNPDPHA

SEQ ID NO: 24 Synechocystis PCC6803
MSTLYEKLGGTTAVDLAVDKFYERVLQDDRIKHFFADVDMAKQRAHQKAFLTY
AFGGTDKYDGRYMREAHKELVENHGLNGEHFDAVAEDLLATLKEMGVPEDLIA
EVAAVAGAPAHKRDVLNQ

SEQ ID NO: 25 Synechococcus sp. PCC 7335
MDVALLEKSFEQISPRAIEFSASFYQNLFHHHPELKPLFAETSQTIQEKKLIFSLAAI
IENLRNPDILQPALKSLGARHAEVGTIKSHYPLVGQALIETFAEYLAADWTEQLA
TAWVEAYDVIASTMIEGADNPAAYLEPELTFYEWLDLYGEESPKVRNAIATLTH
FHYGEDPQDVQRDSRG

SEQ ID NO: 26 Nostoc commune
MSTLYDNIGGQPAIEQVVDELHKRIATDSLLAPVFAGTDMVKQRNHLVAFLAQIF
EGPKQYGGRPMDKTHAGLNLQQPHFDAIAKHLGERMAVRGVSAENTKAALDR
VTNMKGAILNK

SEQ ID NO: 27 Bacillus megaterium
MREKIHSPYELLGGEHTISKLVDAFYTRVGQHPELAPIFPDNLTETARKQKQFLT
QYLGGPSLYTEEHGHPMLRARHLPFEITPSRAKAWLTCMHEAMDEINLEGPER
DELYHRLILTAQHMINSPEQTDEKGFSH