produce flavorants having more intensity and an organoleptic profile more closely resembling that of cooked meat. These oxidation products provide the broad spectrum of precursor compounds -- unsaturated aldehydes and ketones -- needed to provide the desirable flavorants of the present invention. Hence, instead of providing a limited number of aldehydes and ketones from other sources, as may be taught by the prior art and which are inherently restricted in their scope and range, applicants are able to provide, in situ, a much broader range of such precursor compounds.

The process of the present invention is carried out in such a manner that at the end of the process, the reaction product has a peroxide value of substantially zero. In other words, essentially all of the oxidation products are utilized in the process to produce a flavorant of exceptional intensity and character.

The meat flavorants of the present invention offer the additional advantages of exhibiting shelf stability for over one year at room temperature and do not require refrigeration. Still further, the intensity of the flavorants are four to five times greater than freshly cooked meat on a dry solids basis.

DETAILED DESCRIPTION OF THE INVENTION

The process for preparing the flavorants of the present invention for imparting a cooked meat flavor to foodstuffs comprises combining a lipid material and a sulfur-containing compound to form a mixture and then reacting the mixture to form the flavorant wherein either before or during the reaction step, the lipid material is oxidized by being contacted with an oxidizing agent. In a preferred embodiment, the reaction mixture also contains a source of amino acid.

The lipid material that is employed in the present invention includes triglycerides, unsaturated fatty acids or a combination thereof and may be obtained from a variety of different sources. For example, the triglycerides include, but are not limited to, animal fats such as chicken fat, lard, butter fat, beef tallow and the like; and hydrogenated animal oils prepared from, for example, oils derived from fowl, beef, pork, fish and the like. These triglycerides may be employed alone or in combination with one another. The unsaturated fatty acids that may be employed may be derived from the triglycerides themselves by means of enzymatic breakdown employing lipases which are well known in the art. The formation of free fatty acids derived from triglycerides offers a wide range of precursor compounds for subsequent oxidation which in turn will react with the sulfur-containing compound to form the ultimate flavorant product.

The fatty acids are desirably straight or branch chained acids having from 11 to 24 carbon atoms and at least one unsaturated linkage in the chain. Such fatty acids include, but are not limited to, oleic acid, linoleic acid, linolenic acid, arachidonic acid and the like.

The lipid material is preferably derived from a source which is most closely related to the type of flavorant desired. Thus, where it is desired to produce a chicken flavorant, the triglyceride source that is most preferred is chicken fat. Similarly, in the preparation of a pork flavorant, it will be most desirable to employ pork fat as the source of the lipid material.

The lipid material, be it a triglyceride, an unsaturated fatty acid, or a combination thereof, may then be oxidized and combined with a sulfur-containing compound or, alternatively, may first be combined with the sulfur-containing compound and then be oxidized during the reaction step itself.

The lipid material may be oxidized by being contacted with an oxidizing agent which includes, but is not limited to, contact with gaseous materials such as oxygen, air, ozone, and the like, or solid and liquid oxidizing materials such as hydrogen peroxide, ferrous sulfate, and the like. Oxidizing gases are generally employed in a manner such that the amount of oxygen available is in the range of from about .015 to .15 lbs/hour and preferably from about .02 to .06 lbs/hour per

kilogram of unoxidized lipid material. Non-gaseous oxidizing agents are generally employed in an amount from about 0.1 to 1.0% by weight based on the weight of the lipid material. The oxidation of the lipid material is carried out at a temperature of about 50° to 120°C for a period of from about 0.5 to 6.0 hours. Preferably, it is carried out at a temperature of 80° to 100°C and for a period of from about 1.0 to 2.0 hours.

Regardless of how the lipid material is oxidized or what oxidizing agent is employed to do so, and whether the lipid material is oxidized prior to being combined with the sulfur-containing compound or after, the lipid material is oxidized to the extent that it has a peroxide value in the range of from about 5 to 150 milliequivalents per kilogram of lipid material. Preferably, the lipid material is oxidized to a peroxide value of 7 to 10 milliequivalents per kilogram of lipid material. "Peroxide value" is a conventional term which is well known to those skilled in the art for measuring the degree of oxidation of a material, particularly, lipids. The peroxide value may be determined by the American Oil Chemists' Society Method No. 28.023 as set forth at page 489 of the Official Methods of Analyses of the AOAC (1975), published by the Association of Official Analytical Chemists. Oxidation of the lipid material to the extent recited in the foregoing ranges is required in order to provide the necessary precursors which facilitate the production of the flavorants of the present invention which have increased intensity and a broader organoleptic profile.

As a result of the oxidation of the lipid material, hydroperoxides are formed. These hydroperoxides are generally unstable and decompose to form a wide range of fatty, unsaturated aldehydes and ketones.

From about 1 to about 10 parts by weight and preferably, from about 2 to about 5 parts of a sulfur-containing compound are employed per part by weight of lipid material regardless of whether the lipid material is initially oxidized or oxidized as part of the reaction step.

Suitable sulfur-containing compounds include, but are not limited to, sulfur-containing amino acids, which liberate hydrogen sulfide, lower alkyl mercaptans, or lower alkyl sulfides or disulfides, or may include inorganic sulfur compounds, or hydrogen sulfide itself. Such inorganic sulfur compounds may include the sulfides or hydrosulfides of alkali or alkaline earth metals or ammonium. In the context of the present invention, where a sulfur-containing compound is referred to, it is understood that any one or more of the above-noted sulfur-containing compounds may be employed at any one time to react with the oxidized lipid material.

Specific examples of some sulfur-containing compounds are cysteine, cystine, thiamine, methionine, glutathione, 2-amino-ethane sulfonic acid or their salts, and the like. Sulfur compounds may also be derived from plants of the Allium or Cruciferae species.

The mixture of the sulfur-containing compound and the lipid material is then reacted to form the flavorants of the present invention. The reaction is carried out at elevated temperatures, normally, at a temperature in the range of from about 80° to about 120°C and, more preferably, in the range of from about 85° to about 100°C for a period of about .5 to 2.5 hours and preferably, from about 1.0 to 2.0 hours. The reaction is carried out until the peroxide value of the reacted mixture is essentially zero milliequivalents per kilogram of lipid material.

Provided that an oxidizing agent is brought into contact with the reaction mixture, the reaction conditions are such that in the embodiment where an unoxidized lipid material is combined with a sulfur-containing compound, the lipid material is oxidized during the reaction to form the required oxidation products. As the lipid material is oxidized and is forming oxidation products in situ, such oxidation products are immediately and simultaneously reacted with the sulfur-containing compound to form the desired flavorants. The lipid material may be oxidized in situ by simply bubbling an oxidizing gas such as air or oxygen through the reaction system as it is being heated. Alternatively, an edible liquid or solid oxidizing agent such as hydrogen peroxide or ferrous sulfate may be added to the mixture of unoxidized lipid material and the sulfur-containing compound and then the mixture heated to the reaction conditions, which conditions are essentially the same for oxidizing the lipid material.

The essential difference between a reaction system containing an unoxidized lipid material and one containing a lipid material that has been pre-oxidized is the presence of an oxidizing agent during the reaction of the mixture for the former embodiment. Either way, the sulfur-containing compound reacts with the oxidation products of the lipid material to form heterocyclic ring compounds such as thiophenes, thiazoles, thiazolines, thialdine, trithianes and the like which are primarily responsible for the great enhancement and intensification of the characteristic flavor of cooked meat found in the flavorants of the present invention.

In addition to the two main ingredients of a sulfur-containing compound and an oxidized lipid material (be it formed prior to combination with the sulfur compound or formed in situ during reaction therewith), in order to even further enhance and intensify the flavorants produced in the present invention, it is most desirable to add an amino acid source to the reaction mixture. As a source of amino acid, one may employ a single amino acid which is specifically associated with the desired flavorant or a mixture of various amino acids. Protein hydrolysates may also be employed and animal protein hydrolysates are especially preferred. Hydrolysates are well known and readily obtainable from a variety of proteinaceous materials. Vegetable protein hydrolysates, for example, may be obtained from wheat germ, corn gluten, soy protein, linseed protein, peanut press cake, yeast and the like.

As with the lipid material, the preferred meat protein hydrolysate is advantageously also derived from the animal associated with the desired flavorant. Thus, hydrolyzed chicken meat, for example, would desirably be employed to produce a chicken flavorant. The utilization of fish protein to form fish protein hydrolysates for the production of fish flavorants is also contemplated.

The meat is preferably proteolytically hydrolyzed with any protein-splitting enzyme including exopeptidases, endopeptidases, or enzymes which exhibit both of these types of activity. In addition to proteases, i.e., protein-splitting enzymes, lipases, enzymes which split lipids, may also be employed. As meats contain some fat at least, the fat present may be employed as a source of the required lipid material which will be oxidized during the reaction step after being combined with the sulfur-containing compound. Protein hydrolysis of meat by means of proteases does not affect the fat content thereof. The nature of the fat contained in the meat may be left unchanged and employed as such with the hydrolyzed meat in conjunction with the sulfur-containing compound to be subsequently oxidized. As an alternative, however, the fat may be cleaved by means of lipases to form unsaturated fatty acids which may also serve as the required lipid material.

The amount of proteolytic enzyme material employed is typically in the range of from about 0.001% to 2.0% by weight of the meat employed, with 0.05 to 1.5% being preferred.

The meat is hydrolyzed for a period which is generally in the range of from about 1 to 8 hours and preferably from about 2 to 6 hours. The temperature employed for hydrolysis depends upon the particular enzyme employed but is usually in the range of from about 45° to 65°C. At the end of the hydrolysis period, the enzymes are deactivated by heating the hydrolysis mixture at a temperature of approximately 100°C for 5 to 15 minutes.

The addition to the reaction mixture of a protease treated meat and, desirably, a meat that has been treated with lipase as well, greatly enhances the flavors that are produced. In this preferred embodiment, where a source of amino acid is added to the reaction mixture, the fatty aldehydes and ketones resulting from the oxidized lipid material, in addition to reacting with the sulfur-containing compounds, also undergo non-enzymatic browning reactions with the amino acids and peptides derived from the hydrolyzed meat. These additional reactions provide for an even broader organoleptic profile of the meat flavorants produced.

The amount of amino acid source employed is in the range of from about 0 to about 90 parts by weight per part of oxidized lipid material employed and is preferably in the range of from about 50 to 70 parts by weight.

It is noted that no water is added to the reaction mixture of the present invention. In the embodiment in which a protein hydrolysate is employed some water may be present,

originating from the hydrolysis of the meat. Accordingly, the reaction is essentially carried out in a fatty medium. Additionally, it is also noted that in the process of the present invention, no saccharides are added to the reaction mixture. Applicants have found that the presence of mono-, di-, or polysaccharides in the reaction mixture can detract from the organoleptic quality of the resulting flavor. Hence, saccharides should not be added to the reaction mixture of this invention, although small amounts may be present in the ingredients used themselves.

The reaction is carried out until the peroxide value of the mixture is substantially near zero which is an indication that essentially all of the lipid oxidation products have reacted with the sulfur-containing compound(s), alone or in combination with the optional amino acid source. As is readily apparent, the amount of sulfur-containing compound and optional amino acid source present in the reaction mixture should be at least enough to chemically react with essentially all of the oxidation products of the oxidized lipid materials.

After the reaction has taken place, the flavorant may be used as is, or alternatively, be blended with suitable solvents, thickeners, extenders or carriers such as hydrolyzed plant proteins, malto-dextrins, starches, etc. If desired, flavor potentiators or enhancers such as spices, condiments, monosodium glutamate, 5'-nucleotides and the like may also be combined with

the resulting flavorant. Alternatively, some of these additives may also be combined with the mixture prior to the reaction step.

If there is any water present in the reaction product, it may be removed by drying the flavorant material by any conventional means such as by spray-drying, vacuum-drying, or freeze-drying.

Factors which may affect the nature and quality of the flavorants produced include the nature and relative amounts of the lipid material, sulfur-containing compounds and, when employed, the amino acid source, as well as the reaction time and the temperature of the reaction mixture.

The flavorants of the present invention may be incorporated in soups and soup mixes, casserole dishes, canned and frozen vegetables, animal or pet foods, sauces, gravies, stews, simulated meat products, meat spreads and dips, bakery products, replacements for beef, pork, fish, and chicken extracts, and the like.

The amount of a particular flavorant employed will be dependent, of course, upon the specific application. Generally, however, an amount of 0.1 to 4.0% by weight of a flavorant produced by the present invention and preferably, about 0.5 to 1.0% by weight is usually enough to impart a cooked meat flavor to the foodstuff having the intensity, flavor and aroma characteristics that would normally be associated with freshly cooked meat. For example, the addition of about 0.5 to 1.0 parts

by weight of a chicken flavorant prepared by the process of the present invention added to a vegetable protein material such as texturized soy protein would impart an organoleptic profile to the soy protein material of freshly cooked chicken.

Having described the basic concepts of this invention the following Examples are set forth to illustrate the same in which the parts and percentages are by weight. These Examples are not, however, to be construed as limiting the invention in any manner.

EXAMPLE 1

This Example is illustrative of an embodiment of the present invention in which the lipid material is oxidized prior to being combined with the sulfur-containing compound.

A flavoring agent having the characteristic flavor and aroma of cooked chicken is prepared from the following constituents:

Chicken fat	30.00 parts
Hydrolyzed chicken meat	64.75
L-Cysteine HCl	5.00
Linoleic acid	0.25

The hydrolyzed chicken meat is prepared by blending 99.0 parts of chicken meat with 1 part of protease and heating the mixture at 50°C for 5 hours. After hydrolysis is complete, the enzyme is inactivated by heating the mixture at 100°C for 10 minutes.

The chicken fat is first oxidized by heating the fat to 100°C and contacting it with 0.2 standard cubic meter/hr of oxygen until a peroxide value of 10 meq/kg is reached. The remaining ingredients are then added to the oxidized fat and reacted at 100°C for 2 hours. The reaction is complete and the flavorant produced at the end of 2 hours at which time the peroxide value is zero.

25 parts of the flavorant is then blended with 64.5 parts of hydrolyzed corn protein, 10 parts of malto-dextrin and 0.5 parts of a 50/50% mixture of sodium inosinate and sodium guanylate. The resulting paste is then vacuum-dried to a solids content of 98%.

The resulting flavorant has the characteristic flavor and aroma of boiled chicken. Compared to a similar flavorant prepared without the use of oxidized fat, the oxidized flavorant of this Example has a richer, more full-bodied mouthfeel. The flavorant of this Example also has a stronger, more intense brothy profile, similar to stewed chicken or chicken stock and a noticeable increase in fat flavor.

EXAMPLE 2

This Example illustrates the embodiment of the present invention wherein the lipid material employed is endogenous to the animal protein hydrolysate employed and is oxidized during the reaction step.

A flavorant having the characteristic flavor and aroma of cooked pork is prepared employing the following constituents:

Hydrolyzed pork meat	92.25 parts
L-Cysteine HCl	4.00
Thiamine HCl	2.00
Onion concentrate (65% total solids)	1.50
Linoleic acid	0.25

The pork meat, containing 80% fat, is first hydrolyzed with 0.5% protease by weight for 8 hours at 50°C. At the end of the hydrolysis reaction, the enzyme is inactivated by heating the mixture for 15 minutes at 100°C.

The hydrolyzed pork meat containing the fat is then combined with the remaining ingredients to form a mixture. The mixture is then brought to a temperature of 100°C and maintained at that temperature for 2 hours while simultaneously, 0.2 standard cubic meter/hr of air is bubbled through the medium in order to oxidize the fat contained within the hydrolyzed pork

meat. At the end of this time period, the flavorant is produced having a peroxide value of zero.

After cooling, the flavorant is blended with the same carriers and additives as set forth in Example 1 and then vacuum-dried to a 98% total solids content.

The resulting flavorant has the flavor and aroma of freshly cooked pork meat. The flavorant has little roast character and instead exhibits a well-cooked meaty character. The flavorant exhibits a brothy character, a meaty, full-bodied profile and a volatile pleasant aroma.

EXAMPLE 3
(COMPARISON EXAMPLE)

The process of Example 1 is repeated with the only exception being that the chicken fat is not oxidized at all. Thus, the peroxide value of the unoxidized chicken fat is only 1 meq/kg. The remaining constituents and process steps set forth in Example 1 are repeated in this Example.

The resulting flavorant produced in this Example, which is not in accordance with this invention, does not have the intensity, aroma and flavor characteristics as that of the flavorant produced in Example 1. The flavorant of this comparison Example exhibits a much weaker profile, a lesser intensity and lacks the meaty, brothy, fatty full-bodied flavor of the flavorant produced in Example 1.

EXAMPLE 4
(COMPARISON EXAMPLE)

The process of Example 2 is repeated with the only exception being that the fat contained within the hydrolyzed pork meat is not subjected to oxidation conditions during the reaction step such that the fat is not oxidized. The remaining constituents and process steps are employed as discussed in Example 2.

The resulting pork flavorant produced in this Example which is not in accordance with this invention lacks the intensity and specificity of the flavorant produced in Example 2. Moreover, the flavorant also lacks the brothy, meaty character and the fatty flavor of the flavorant of Example 2. A taste panel comparing the flavorants of Example 2 and this Example indicated a preference for the Example 2 flavorant, the flavorant produced in accordance with the present invention, at a 95% confidence level.

EXAMPLE 5

A flavorant having the flavor and aroma of cooked liver is prepared employing the following constituents:

Hydrolyzed beef liver	72.0 parts
L-Cysteine HCl	3.5
Thiamine HCl	1.5
Onion concentrate (65% total solids)	3.0
Beef fat	20.0

The beef liver is first hydrolyzed in the same manner as set forth in Example 1. The hydrolyzed beef liver is then combined with the remaining ingredients and the mixture brought to a temperature of 100°C and maintained at that temperature for 2 hours while 0.2 standard cubic meter/hour of ozone is bubbled through the reaction medium. At the end of the reaction period, the flavorant is blended with the same carriers and additives as set forth in Example 1.

The flavorant produced has the characteristic flavor and aroma of cooked liver. The flavorant has an earthy, fatty profile which is both intense and aromatic. 1.7 to 1.8 grams of the flavorant produced in this Example can replace at least 2.0 grams of a flavorant prepared without oxidation of the lipids and still achieve the same impact and intensity.

EXAMPLE 6

Example 5 is repeated with the exception that instead of employing beef liver, ground beef is substituted therefor.

The resulting flavorant has an excellent cooked beef, brothy and fatty flavor and an intense aroma.

A taste panel, when comparing the flavorant of this Example with that prepared without oxidation of the lipid materials, found the present flavorant to be more meaty, broth-like and flavorful and to have an increased aroma intensity.

EXAMPLE 7

Example 6 is repeated with the only exception being that the beef fat is oxidized prior to being combined with the remaining ingredients. Thus, the beef fat is contacted with 0.2 standard cubic meter/hr of oxygen for 4 hours at 100°C to oxidize it to have a peroxide value of 10 meq/kg. Inasmuch as the fat is already oxidized, no further air or oxygen is bubbled through the mixture during the reaction.

When the peroxide value of the reacted mixture is near zero, the reaction is complete and the flavorant produced.

The flavorant produced in this Example and that of Example 6 are indistinguishable from one another. This points out the interchangeability of oxidizing the lipid material either before or during the reaction step.

EXAMPLE 8

This Example is illustrative of the embodiment in which no amino acid source (meat hydrolysate) is employed. It also demonstrates the preparation of the flavorant without the presence of any water.

A flavorant is prepared having the characteristic flavor and aroma of cooked chicken employing the following constituents:

Chicken fat	97.0 parts
L-Cysteine HCl	3.0

The chicken fat and L-cysteine HCl are combined and heated to a temperature of 120°C for a period of ½ hour while simultaneously bubbling 0.1 standard cubic meter/hr of air through the medium. At the end of that period, 10 parts of the flavorant produced are blended with 70 parts hydrolyzed corn protein, 19.5 parts malto-dextrin and 0.5 parts of a 50/50% mixture of sodium inosinate and sodium guanylate.

The resulting flavorant has the characteristic flavor and aroma of cooked chicken.

EXAMPLE 9

This Example is illustrative of the embodiment in which a fish flavorant is produced. The particular species of fish employed is dependent upon the particular flavor to be produced.

A flavorant having the characteristic flavor and aroma of fish is prepared employing the following constituents:

Hydrolyzed fish	72.2 parts
Cod liver oil	25.0
L-Cysteine HCl	2.5
Linoleic acid	0.3

The fish is first hydrolyzed by means of a fungal protease which is employed in an amount of 1% by weight of the fish treated. The hydrolysis is carried out for 6 hours at 50°C. At the end of the hydrolysis treatment, the temperature is raised to 100°C and maintained at that temperature for 15 minutes to inactivate the enzyme.

The hydrolyzed fish is then combined with the remaining ingredients and heated to a temperature of 100°C for 1½ hours while simultaneously bubbling air through the system at a rate of 0.15 standard cubic meter/hr. 23 parts by weight of the reaction product is then blended with 60 parts of hydrolyzed corn protein,

16.2 parts malt dextrin and 0.8 parts of a 50/50% mixture of sodium inosinate and sodium guanylate and dried in vacuum to a total solids content of 98% by weight.

The fish flavorant produced has a boiled fish flavor and aroma and is well suited to applications such as chowder where a boiled fish flavor is desired.

Claims

1. A process for preparing a flavorant for imparting a cooked meat or fish flavor to foodstuffs which comprises combining a lipid material being at least one of a tryglyceride and an unsaturated fatty acid with a sulfur-containing compound to form a mixture and reacting the mixture by heating to form the flavorant wherein before or during the reaction step, the lipid material is oxidized by being contacted with an oxidizing agent.

2. The process of claim 1, wherein the lipid material is oxidized to a peroxide value in the range of from about 5 to 150 milliequivalents per kilogram of lipid material.

3. The process of claim 1 or claim 2, wherein from about 1 to about 10 parts by weight of the sulfur-containing compound is mixed with 1 part by weight of the oxidized lipid material.

4. The process of any preceding claim, wherein the lipid material is an animal fat, a hydrogenated animal oil or a straight or branched chain fatty acid having from 11 to 24 carbon atoms and having at least one unsaturated linkage in the chain.

5. The process of any preceding claim, wherein the sulfur-containing compound is at least one of a sulfur-containing amino acid, a lower alkyl mercaptan, a lower alkyl sulfide, a lower alkyl disulfide, hydrogen sulfide and a sulfide or hydrosulfide of an alkali or alkaline earth metal or ammonium.

6. The process of any preceding claim, wherein the reaction mixture contains an amino acid source.

7. The process of claim 6, wherein the reaction mixture contains up to 90 parts by weight of the amino acid source per part by weight of the oxidized lipid material.

8. The process of any preceding claim, wherein the mixture is reacted by heating at a temperature of 80° to 120°C for a period of from 0.5 to 2.5 hours.

9. The process of any preceding claim, wherein the mixture is reacted until the peroxide value is substantially zero.

10. The flavorant obtained by the process of any preceding claim.

Espacenet

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

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

CROSS-REFERENCE TO RELATED APPLICATIONS

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

TECHNICAL FIELD

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

BACKGROUND

Food is any substance that is either eaten or drunk by any animal, including humans,

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

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

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

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

SUMMARY

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

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

bacteriochorophyll; a corphin; a chlorophyllin; a bacteriochlorin; or an isobacteriochlorin moiety complexed to an iron ion). Such highly conjugated heterocycylic moieties

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

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

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

Food products for use in the present disclosure include those that have an iron-complex (e.g., a heme cofactor such as a heme-containing protein), and one or more flavor precursor molecules included therein. The iron-complex such as a heme cofactor (e.g., a heme-containing protein) and the one or more flavor precursor molecules can be homogenously or heterogeneously included in the food products. A heme protein can be isolated and purified prior to inclusion in the food product. Non-limiting examples of consumable food products which can include an iron complex such as a heme-cofactor (e.g., a heme-containing protein) and one or more flavor precursor molecules include animal-based or non-animal (e.g., plant-based), or combinations of

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

Consumable food products for use in the present disclosure can be flavor additive compositions, e.g., for addition to another consumable food product before, during, or after its cooking process. A flavor additive composition can include an iron complex such as a heme-cofactor (e.g., a heme-containing protein), and one or more flavor precursors. A flavor additive composition can include a heme protein, e.g., an isolated and purified heme protein; such a flavor additive composition can be used to modulate the flavor and/or aroma profile of a consumable food product that comprises one or more flavor precursor molecules or compositions. A flavor additive composition can include one or more flavor precursor molecules or compositions; such a flavor additive composition can be used to modulate the flavor and/or aroma profile of a consumable food product that comprises the heme protein, e.g., an isolated and purified heme protein.

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

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

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

In one aspect, this document features a food product that includes an iron complex such as a heme moiety, or a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochlorophyll, a corphin, a chlorophyllin, a bacteriochlorin, or an isobacteriochlorin moiety complexed to an iron ion and one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1 ,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide -bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, sunflower oil, canola oil, olive oil, a free fatty acid, cysteine,

methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone. The heme moiety can be a heme-containing protein, a heme moiety bound to a non-peptidic polymer, or a heme moiety bound to a solid support. The heme-containing protein can be a plant, mammalian, a yeast or filamentous fungi, or bacterial heme-containing protein. The food product can include two to one hundred, two to fifty flavor precursors, two to forty flavor precursors, two to thirty-five flavor precursors, two to ten flavor precursors, or two to six flavor precursors. In some embodiments, the one or more flavor precursor molecules are selected from the group consisting of glucose, ribose, cysteine, a cysteine derivative, thiamine, alanine, methionine, lysine, a lysine derivative, glutamic acid, a glutamic acid derivative, IMP, GMP, lactic acid, maltodextrin, creatine, alanine, arginine, asparagine, aspartate, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, linoleic acid, and mixtures thereof. The heme-containing protein can be a non-symbiotic hemoglobin or a leghemoglobin (e.g., a plant leghemoglobin such as one from soybean, alfalfa, lupin, pea, cow pea, or lupin). The heme-containing protein can include an amino acid sequence having at least 80% sequence identity to a polypeptide set forth in SEQ ID NOs: 1-26. The heme-containing protein can be isolated and purified. The food product further can include a food-grade oil, a seasoning agent, a flavoring agent, a protein, a protein concentrate, an emulsifier, a gelling agent, or a fiber. The food product can be a meat substitute, a soup base, stew base, snack food, bouillon powder, bouillon cube, a flavor packet, or a frozen food product. Any of the food products can be free of animal products. The food product can be sealed within a packet or shaker.

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

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

In another aspect, this document features a method for producing a flavor compound. The method includes combining an iron complex, such as a heme-containing protein, and one or more flavor precursor molecules to form a mixture, the one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, methionine, cysteine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture to form one or more flavor compounds set forth in Tables 3, 8, or 9. For example, the flavor precursors can include cysteine, a sugar, and one or more other precursors.

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

the flavoring composition together, a meat like flavor (e.g., beef-like, chicken like, pork-like, lamb- like, turkey-like, duck-like, deer-like, or bison-like) is imparted to the food product. In some embodiments, the iron complex is removed from the food product. The flavoring composition further can include a seasoning agent, a flavoring agent, a protein, a protein concentrate, or an emulsifier. The flavoring composition can be sealed within a packet or shaker.

In another aspect, this document features a method of making a food product.

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

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

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

DESCRIPTION OF THE DRAWINGS

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

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

DETAILED DESCRIPTION

This document is based on methods and materials for modulating the taste and/or aroma profile of food products. As described herein, compositions containing one or more flavor precursors and one or more highly conjugated heterocyclic rings complexed to an iron (referred to herein as an iron complex) can be used to modulate the taste and/or aroma profile of food products. Such iron complexes include heme

moieties or other highly conjugated heterocylic rings complexed to an iron ion (referred to as an iron complex). "Heme" refers to a prosthetic group bound to iron (Fe <2+>or Fe <3+>) in the center of a porphyrin ring. Thus, an iron complex can be a heme moiety, or a porphyrin, porphyrinogen, corrin, corrinoid, chlorin, bacteriochorophyll, corphin, chlorophyllin, bacteriochlorin, or isobacteriochlorin moiety complexed to iron ion. The heme moiety that can be used to modulate the taste and/or aroma profile of food products can be a heme cofactor such as a heme-containing protein; a heme moiety bound to a non-peptidic polymer or other macromolecule such as a liposome, a polyethylene glycol, a

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

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

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

TABLE 1

Flavor Precursor Molecules

Sugars, sugar alcohols, sugar acids, and sugar derivatives: glucose, fructose, ribose, sucrose, arabinose, glucose-6-phosphate,fructose-6-phosphate, fructose 1 ,6-diphosphate, inositol, maltose, molasses, maltodextrin, glycogen, galactose, lactose, ribitol, gluconic acid and glucuronic acid, amylose, amylopectin, or xylose

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

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

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

guanosine, guanoside monophosphate (GMP), adenosine, adenosine

monophosophate (AMP)

Vitamins: thiamine, vitamin C, Vitamin D, Vitamin B6, or Vitamin E

Misc: phospholipid, lecithin, pyrazine, creatine, pyrophosphate

Acids: acetic acid, alpha hydroxy acids such as lactic acid or gly colic acid, tricarboxylic acids such as citric acid, dicarboxylic acids such as succinic acid or tartaric acid

Peptides and protein hydrolysates: glutathione, vegetable protein hydrolysates, soy protein hydrolysates, yeast protein hydrolysates, algal protein hydrolysatess, meat protein hydrolysates

Extracts: a malt extract, a yeast extract, and a peptone

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

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

Non- limiting examples of heme-containing polypeptides can include an androglobin, a cytoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a myoglobin, an erythrocruorin, a beta hemoglobin, an alpha hemoglobin, a protoglobin, a cyanoglobin,

a cytoglobin, a histoglobin, a neuroglobins, a chlorocruorin, a truncated hemoglobin (e.g., HbN or HbO), a truncated 2/2 globin, a hemoglobin 3 (e.g., Glb3), a cytochrome, or a peroxidase.

Heme-containing proteins that can be used in the compositions and food products described herein can be from mammals (e.g., farms animals such as cows, goats, sheep, pigs, ox, or rabbits), birds, plants, algae, fungi (e.g., yeast or filamentous fungi), ciliates, or bacteria. For example, a heme-containing protein can be from a mammal such as a farm animal (e.g., a cow, goat, sheep, pig, ox, or rabbit) or a bird such as a turkey or chicken. Heme-containing proteins can be from a plant such as Nicotiana tabacum or Nicotiana sylvestris (tobacco); Zea mays (corn), Arabidopsis thaliana, a legume such as Glycine max (soybean), Cicer arietinum (garbanzo or chick pea), Pisum sativum (pea) varieties such as garden peas or sugar snap peas, Phaseolus vulgaris varieties of common beans such as green beans, black beans, navy beans, northern beans, or pinto beans, Vigna unguiculata varieties (cow peas), Vigna radiata (Mung beans), Lupinus albus (lupin), or Medicago sativa (alfalfa); Brassica napus (canola); Triticum sps. (wheat, including wheat berries, and spelt); Gossypium hirsutum (cotton); Oryza sativa (rice); Zizania sps. (wild rice); Helianthus annuus (sunflower); Beta vulgaris (sugarbeet);

Pennisetum glaucum (pearl millet); Chenopodium sp. (quinoa); Sesamum sp. (sesame); Linum usitatissimum (flax); or Hordeum vulgare (barley). Heme-containing proteins can be isolated from fungi such as Saccharomyces cerevisiae, Pichia pastoris, Magnaporthe oryzae, Fusarium graminearum, Aspergillus oryzae, Trichoderma reesei, Myceliopthera thermophile, Kluyvera lactis, or Fusarium oxysporum. Heme-containing proteins can be isolated from bacteria such as Escherichia coli, Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Synechocistis sp., Aquifex aeolicus, Methylacidiphilum infernorum, or thermophilic bacteria such as Thermophilus. The sequences and structure of numerous heme-containing proteins are known. See for example, Reedy, et ah, Nucleic Acids Research, 2008, Vol. 36, Database issue D307-D313 and the Heme Protein Database available on the world wide web at http ://hemeprotein.info/heme .php . For example, a non-symbiotic hemoglobin can be from a plant selected from the group consisting of soybean, sprouted soybean, alfalfa, golden flax, black bean, black eyed pea, northern, garbanzo, moong bean, cowpeas, pinto beans, pod peas, quinoa, sesame, sunflower, wheat berries, spelt, barley, wild rice, or rice.

Any of the heme-containing proteins described herein that can be used for producing food products can have at least 70% (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%), 98%o, 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: I), 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: I 1, group I truncated), a hemoglobin from

Aspergillus niger (SEQ ID NO: 12), or a mammalian myoglobin protein such as the Bos taurus (SEQ ID NO: 18) myoglobin, Sus scrofa (SEQ ID NO: 19) myoglobin, Equus caballus (SEQ ID NO:20) myoglobin, a heme -protein from Nicotiana benthamiana (SEQ ID NO:21), Bacillus subtilis (SEQ ID NO:22), Corynebacterium glutamicum (SEQ ID

NO:23), Synechocystis PCC6803 (SEQ ID NO:24), Synechococcus sp. PCC 7335 (SEQ ID NO:25), or Nostoc commune (SEQ ID NO:26).

The percent identity between two amino acid sequences can be determined as follows. First, the amino acid sequences are aligned using the BLAST 2 Sequences (B12seq) program from the stand-alone version of BLASTZ containing BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from Fish & Richardson's web site (e.g., www.fr.com/blast/) or the U.S. government's National Center for

Biotechnology Information web site (www.ncbi.nlm.nih.gov). Instructions explaining how to use the B12seq program can be found in the readme file accompanying BLASTZ. B12seq performs a comparison between two amino acid sequences using the BLASTP algorithm. To compare two amino acid sequences, the options of B12seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seql .txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two amino acid sequences: C:\B12seq -i c:\seql .txt -j c:\seq2.txt -p blastp -o c:\output.txt. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology, then the designated output file will not present aligned sequences.

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

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

It will be appreciated that a number of nucleic acids can encode a polypeptide having a particular amino acid sequence. The degeneracy of the genetic code is well known to the art; i.e., for many amino acids, there is more than one nucleotide triplet that serves as the codon for the amino acid. For example, codons in the coding sequence for a

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

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

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

Arabidopsis, or mammalian cells). In some cases, standard polypeptide synthesis techniques (e.g., liquid-phase polypeptide synthesis techniques or solid-phase

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

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

In some embodiments, the isolated and purified protein is substantially in its native fold and water soluble. In some embodiments, the isolated and purified protein is more than

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

Modulating Flavor and/or Aroma Profiles

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

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

TABLE 2

Image available on "Original document"
yeasty goaty

vegetable broth

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

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

TABLE 3

Compounds Produced

Image available on "Original document"
benzaldehyde 2-hexyl-thiophene l-penten-3-one

(E)-2-Nonenal 4-cyclopentene- 1 ,3-dione dimethyl sulfide pyrazine 1-nonanol thiirane

1-pentanol (E)-2-decenal (E)-2-octen-l-ol trans-2-(2-pentenyl)furan 4-ethyl-benzaldehyde 2,4-dimethyl- 1 -heptene l,3-bis(l,l-

1-hexanol l,7-octadien-3-ol dimethylethyl)-benzene

1-heptanol octanoic acid heptane

dimethyl trisulfide 2-ethyl-5 -methyl-pyrazine 4,7-dimethyl-undecane

3 -ethy 1-2 , 5 -dimethy 1-

2-nonanone pyrazine acetophenone

2-pentanone 1 ,3,5-cycloheptatriene tridecane

thiophosphoramide, s-

2-heptanone 2-ethyl- 1 -hexanol methyl ester

2,3-butanedione 4-methyl-octanoic acid 2-methyl-thiazole

3 -(1 -methy lethoxy)- heptanal m-aminophenylacetylene propanenitrile,

2,4-bis(l,l- nonanal benzene dimethylethyl)-phenol

3-ethyl-2,2-dimethyl-

2-octanone thiophene pentane

2-butanone 2-methyl-furan 3-ethyl-pentane octanal pyridine 2,3 ,4-trimethyl-pentane

1-octanol furan 2 ,4 , 6 -trimethy l-o ctane

3 -ethylcyclopentanone butanal 2,6-dimethyl-nonane

8 -methyl- 1 -undecene 2-ethyl-furan 2-hexyl-furan

4-methyl-5-

3-octen-2-one carbon disulfide thiazoleethanol

2,4-Heptadienal, (E,E)- Furan, 2-hexyl- 2 4-penten-2-one

(Z)-2-heptenal 3 -methy 1-butanal 4-methylthiazole

6-methyl-2-heptanone 2-methyl-butanal 2-methyl-3 -pentanone

(Z)-4-heptenal methacrolein 2,3-pentanedione

(E,Z)-2,6-nonadienal octane (E)-2-tridecen- 1 -ol

2-

3 -methyl-2-butenal ethanol thiophenemethanamine

2-pentyl-furan 2-methyl-propanal (Z)-2-nonenal, thiazole acetone methyl thiolacetate

(E,E)-2,4-decadienal propanal methyl ethanoate hexanoic acid methyl-thiirane isothiazole

1 -ethyl-5 -methylcyclopentene acetaldehyde 3 ,3 -dimethyl-hex ane (E,E) -2,4-nonadienal 2-propenal 4-methyl-heptane

(Z)-2-decenal 2-propyl-furan 2,4-dimethyl-heptane dihydro-5-propyl-2(3H)- dihydro-5-pentyl-2(3h)-furanone furanone 2,3 ,4-trimethyl-heptane trans-3 -nonen-2-one dihydro-3 -(2H)-thiophenone 2-methyl-heptane

(E,E)-3,5-octadien-2-one 2,2,6-trimethyl-decane 2-methyl-3 -furanthiol

4-amino- 1,2,5-

3,3'-dithiobis[2-methyl- oxadiazole-3-

(Z)-2-octen-l-ol furan carbonitrile

1 ,2-benzisothiazol-

5-ethyldihydro-2(3h)-furanone 1-heptene 3(2H)-one

2-butenal 1,3-octadiene 2-acetyl-propen-2-ol, l-penten-3-ol 1-nonene l-decen-3-one

1 -(ethylthio)-2-(methylthio)-buta- 1,3- diene

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

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein is heated in the presence of cysteine and glucose or other combinations of flavor precursors to provide a different profile of volatile odorants than when any subset of the three components are used individually.

Volatile flavor components that are increased under these conditions include but are not limited to furan, acetone, thiazole, furfural, benzaldehyde, 2-pyridinecarboxaldehyde, 5- methyl-2-thiophenecarboxaldehyde, 3 -methyl-2-thiophenecarboxaldehyde, 3 - thiophenemethanol and decanol. See, e.g., Tables 8 and 9. Under these conditions, cysteine and glucose alone or in the presence of iron salts such as ferrous glucanate

produced a sulfurous, odor, but addition of heme-containing proteins reduced the

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

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

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

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

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

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

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

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

pentanone, 3 -methyl butanal, 2-methyl butanal, 2-heptenal, 1-octene, nonanal, 2-propenal, 2-decenal, 2-nonanone, 2-octanone, 2-tridecen-l-ol, 2-octanone, 2-octenal, 4- methyl-2-heptanone, octanal, 2-undecenal, butyrolactone, l-octen-3-one, 3-methylheptyl acetate, and 2-pentyl-thiophene. These differences in flavor compounds can change the overall taste profile.

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

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

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

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

Food Products

Food products containing one or more flavor precursors and one or more heme-containing proteins can be used as a base for formulating a variety of additional food products, including meat substitutes, soup bases, stew bases, snack foods, bouillon powders, bouillon cubes, flavor packets, or frozen food products. Meat substitutes can be formulated, for example, as hot dogs, burgers, ground meat, sausages, steaks, filets, roasts, breasts, thighs, wings, meatballs, meatloaf, bacon, strips, fingers, nuggets, cutlets, or cubes. In addition, food products described herein can be used to modulate the taste and/or aroma profile of other food products (e.g., meat replicas,

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

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

Food products described herein can include additional ingredients including food-grade oils such as canola, corn, sunflower, soybean, olive or coconut oil, seasoning agents such as edible salts (e.g., sodium or potassium chloride) or herbs (e.g., rosemary, thyme, basil, sage, or mint), flavoring agents, proteins (e.g., soy protein isolate, wheat glutin, pea vicilin, and/or pea legumin), protein concentrates (e.g., soy protein

concentrate), emulsifiers (e.g., lecithin), gelling agents (e.g., k-carrageenan or gelatin), fibers (e.g., bamboo filer or inulin), or minerals (e.g., iodine, zinc, and/or calcium).

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

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

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

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

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

In some embodiments, a food product described herein can be compared to another food product (e.g., meat or meat substitute) based upon olfactometer readings. In various embodiments, the olfactometer can be used to assess odor concentration and

odor thresholds, odor suprathresholds with comparison to a reference gas, hedonic scale scores to determine the degree of appreciation, or relative intensity of odors.

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

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

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

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

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

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

EXAMPLES

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

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

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

Panelists were encouraged to rinse their mouths with water between samples, and to fill out a survey to record their evaluation of each sample.

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

TABLE 4

Replica Burger Ingredients

Repl ica biiruer " .) precooked w w

Pea vicilin 3.86

Soy protein concentrate (SPC) 2.52

Bamboo fiber 0.34
Image available on "Original document"

TABLE 5

Sensory evaluation of replica burger with Heme

Image available on "Original document"
Example 2: Replica burgers with a flavor precursor mixture taste beefy and bloody

Replica burgers containing a flavor precursor mixture of glucose, cysteine, thiamine, and glutamic acid and 1% LegH pre-cooked w/w (see Table 4) were prepared as described in Example 1 , and evaluated by a trained sensory panel after the burgers were cooked for 5 minutes at 150 C. Control burgers included LegH and all other ingredients except for the flavor precursor mixture. Panelists were asked to evaluate the samples overall improvement in taste and descriptively analyze each sample using a 5-point scale from l=dislike extremely, to 5=like extremely. Panelists were encouraged to rinse their mouths with water between samples, and to fill out a survey to record their evaluation of each sample. The replicate burgers which included LegH and the flavor precursor mixture were described as having bouillon, gravy, meaty, bloody, savory, and beefy notes on taste, and were preferred to the same replica burger with LegH but no added flavor precursor mixture. See, Table 6

TABLE 6

Improvement of overall taste with precursors added to LegH burgers
Image available on "Original document"

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

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

Flavors generated by addition of precursors to LegH (1 %)
Image available on "Original document"

Image available on "Original document"

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

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

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

Notably, the control mixtures of cysteine with ribose or glucose heated in the absence of the LegH heme-protein did not generate the same set of flavor compounds. The flavor reaction mixtures containing LegH also were evaluated by a blinded trained sensory panel, which described the samples with ribose as having beefy, savory, brothy, and gravy-like notes, and the samples with glucose as savory, bloody, metallic, raw meat, and bouillon-like. TABLE 8

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

Image available on "Original document"
trichloromethane X

2-methyl-furan X X furan X X pyrazine X X thiophene X X

1 ,3-dimethyl-benzene X X octane X octanal X X thiazole X X

2-pentanone X furfural X X

2-nonanone X X

(Z)-2-heptenal X X

(E)-2-heptenal X X

1-octene X X formic acid, heptyl ester X X

2-pentyl-thiophene X l-octen-3-one X X

3-pentyl-furan X X

2-propenal X

(E)-2-tridecen- 1 -ol X benzene X

(E)-4-octene X l-penten-3-one X

4-penten-2-one X X

2-methyl-thiazole X methyl-pyrazine X trans-2-(2-pentenyl)furan X

3-ethylcyclopentanone X pyrrole X X

2-thiophenecarboxaldehyde X

3 -thiophenecarboxaldehy de X

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

The addition of thiamine in a flavor reaction mixtures with a heme protein and other flavor precursors affected the formation of 5-Thiazoleethanol, 4-methyl-furan, 3,3'-

dithiobis[2-methyl-thiazole, and 4-methylthiazole. These compounds are known to be present in meat and have beefy, meaty taste notes.

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

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

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

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

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

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

The addition of lactic acid in mixes with heme protein, ribose, and cysteine controlled

the formation of the flavor compounds nonanal, thiazole, 2-acetylthiazole, and 8-methyl-l-undecene. These compounds are known to be present in meat.

Reactions containing heme protein at 1%, cysteine (10 mM), and ribose (20 mM), and lactic acid (lmM), pH 6.0, were prepared and heated to 150C for 3 minutes.

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

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

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

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

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

Example 9 - Flavor compound production by different Heme-proteins

The addition of different types of heme-proteins (LegH, Barley, B. myoglobin, or A. aeolicus) in flavor reaction mixtures containing one or more flavor precursor compounds results in many of the same key meat flavors, including but not limited to 2-pentyl-furan, 2,3-Butanedione, Thiophene, 2-methyl-thiazole, Pyrazine, Furan, Pyrrole,

2-methyl-furan and distinct differences in the flavor compounds, including but not limited to 2-pentyl-thiophene, Nonanal, 2-Nonanone, and l-Octen-3-one. These differences in flavor compounds can change the overall taste profile. The different types of heme-protein were LegH, Barley, B. myoglobin, ox A. aeolicus used at 1% w/w in a reaction mixed with cysteine (10 mM) and ribose (10 mM) at pH 6. The pre-reaction mixture was heated to 150 C for 3 minutes; this reaction created flavor compounds known to be present in meat; see Table 9. The characteristic flavor and fragrance components are mostly produced during the cooking process where the flavor precursor molecules react with the heme-protein. Samples were evaluated by GCMS to identify the flavor compounds generated after heating and also evaluated for their sensory profiles.

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

TABLE 9

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

Name LegH Barley B. myoglobin A. aeolicus

Furan X X X X

Thiazole X X X X

benzaldehyde X X X X

2-acetylthiazole X X X X 2-methyl-propanal X X X X furfural X X X X

2,3-butanedione X X X X

2-pentyl-furan X X X X

2-pentanone X X

pyrazine X X X X dimethyl trisulfide X X X X

3 -methy 1-butanal X X X

2-methyl-thiazole X X X X pentanal X X X X

1 ,3,5-cycloheptatriene X X X X methacrolein X X X X heptanal X X X X

2-methyl-butanal X X X isothiazole X X X X thiophene X X X X propanal X X X X

2-heptenal X X X methyl-pyrazine X X X X

1-octene X X X butanal X X X X

2-acetyl-propen-2-ol X X X X pyrrole X X X X

2-methyl-furan X X X X nonanal X X X

2-propenal X X X

2-decenal X X X

2-nonanone X X

2-octanone X X X

2-tridecen-l-ol, X X

2-octanone X

2-octenal X X

4-methyl-2-heptanone X X octanal X X

2-undecenal X butyrolactone X l-octen-3-one X

3-methylheptyl acetate X x

Example 10 - Generation of Meat flavors from different lipids

Several different samples including oils (canola oil or coconut oil), free fatty acids (FFA) (linoleic acid (C18:2), oleic acid (C18: l), stearic acid (C18:0), or myristic acid (Cl 4:0)) and phospholipids (PL) (beef heart polar lipids extract, Biolipon95 (from

Perimond), or NatCholinePC40 (from Perimond)) were tested for their ability to produce beefy flavor in the absence and in the presents of other precursors. Oils, FFAs, and PLs were added to 50 mM potassium phosphate buffer (PPB) pH 6.0 or a MaiUard reaction mix (MRM) containing 50 mM potassium phosphate pH 6.0, 5 mM Cysteine, 10 mM Glucose, 0.1 mM Thiamine, and 0.1% (w/v) LegHemoglobin. Lipids in combination with MRM were designed to capture the cross reactions of lipid degradation and MaiUard reaction productions while lipids in phosphate buffer functioned as a lipid control. The oils were added at 3% of the total 1 mL volume of solution while FFAs and PLs were added at 1% of the total 1 mL volumes. All samples were cooked at 150°C for 3 mins, cooled to 50°C and then analyzed using GCMS (SPME fiber sampling of headspace). After all samples were analyzed by GCMS the caps were removed and samples were smelled by a trained flavor scientist and aromas recorded.

Table 10

Legend showing components of each sample

Sample Name Solution Additives

MRM None MaiUard Reaction Mix None

MRM Linoelic Acid MaiUard Reaction Mix 1% linoleic acid

MRM Oleic Acid MailJard Reaction Mix 1% oleic acid

MRM C14 MailJard Reaction Mix 1% C14:0 free fatty acid

MRM C18 MailJard Reaction Mix 1% Cl 8:0 free fatty acid

MRM Canola MailJard Reaction Mix 3% Canola Oil

MRM Coconut MailJard Reaction Mix 3% Coconut Oil

MRM BeefHeart MailJard Reaction Mix 1% Beef Heart Polar Lipids Extract

MRM_Biolipon95 MailJard Reaction Mix 1% Biolipon95 (emulsifier)
MRM_NatCholinePC40 Maillard Reaction Mix 1% NatCholinePC40 (emulsifier)

KPhos6 Linoelic Acid PPB, pH 6 1% linoelic acid

KPhos6_01eic Acid PPB, pH 6 1% oleic acid

KPhos6_C14 PPB, pH 6 1% C14:0 free fatty acid

KPhos6_C18 PPB, pH 6 1% Cl 8:0 free fatty acid

KPhos6 Canola PPB pH 6 3% Canola Oil

KPhos6_Coconut PPB, pH 6 3% Coconut Oil

KPhos6_BeefHeart PPB, pH 6 1% Beef Heart Polar Lipids Extract

KPhos6_Biolipon95 PPB, pH 6 1% Biolipon95 (emulsifier)

KPhos6_NatCholinePC40 PPB, pH 6 1% NatCholinePC40 (emulsifier)

Table 11 contains the aroma descriptions and Table 12 contains the GCMS data from the most interesting samples analyzed. Many of the lipids introduced a "fatty" aroma to MRM that was otherwise absent. The combinations of Linoleic Acid or

NatCholinePC40 in MRM produced the greatest abundance of fatty compounds suggesting that these lipids may improve the flavor perception of beef tallow. Linoleic Acid and NatCholinePC40 also showed high abundance of earthy-mushroom aromas. The addition of lipids to MRM significantly increased the abundance of "nutty & roasted" aromas. Less desirable "green" aroma compounds were most prominent in samples with unsaturated free fatty acids (linoleic acid or oleic acid) or phospholipids. In general, the addition of lipids significantly increased the number of target beef

compounds made.

TABLE 11

Aroma descriptions of each sample after it was cooked.

Image available on "Original document"
KPhos6_NatCholinePC4

0 light fatty, fresh

MRM_NatCholinePC40 fatty, beef tallow, brothy

K-Phos6_C14 light/faint plastic/waxy

MRM_C14 brothy, beefy, minty, fresh

K-Phos6_C18 light/faint plastic/waxy

MRM_C18 beefy with cucumber &/or pepper aroma

K-Phos6_Canola fresh, cucumber

MRM_Canola fatty, brothy, oil, roasted nuts

K-Phos6_Coconut nothing

MRM_Coconut brothy. beefy, slight fatty, crackers

K-Phos6_Oleic Acid fresh, cucumber, camphorous/minty-like

MRM_Oleic Acid herbal, plas IMRMt on yic, slight cheesy, brothy

K-Phos6_I_inoelic Acid light plastic

MRM_Linoelic Acid fatty, light waxy, brothy. herbal

Table 12 fMRMBH teeea r

List of aromatic compounds found in Beef by GCMS and a chart showing which were detected in each lipid plus MRM sam hliC MRMN taonp_ le.

C 40P ei MRMLno <ec>

i da c

Compounds in Beef

(s)-isopropyl lactate N N N N l-ethyl-5- methylcyclopentene Y Y Y Y

1-heptanol N Y N N l-hepten-3-ol N Y Y Y

1-heptene N Y Y Y

2-methyl- 1 -heptene N N N N

1-hexanol N Y Y Y

2-ethyl- 1 -hexanol N N N N

1-nonanol N N |||||||:|||||| N

1-nonene N Y nilli;iiillii;i;illi N

1-octanol N Y Y N l-octen-3-ol N Y Y Y l-octen-3-one ŷ Y Y Y

1-octene N N N N

1-pentanol N Y Y Y l-penten-3-ol N Y Y N

1-propanol N N N N

8-methyl- 1 -undecene N Y Y Y

1,3-hexadiene N N N Y

3-ethyl-2-methyl-l,3- hexadiene N Y Y Y

1,3-octadiene Y N N Y

1 ,3,5-cycloheptatriene N N N N

2.3- dihydro-5,6-dimethyl-

1.4- dioxin N N N N l,7-octadien-3-ol N Y N N lh-pyrrole-2- carboxaldehyde N N N N

2-methyl- 1 H-pyrrole N N N N

2-acetyl-2-thiazoline 1111 N N N

2-acetylthiazole Y Y Y Y

2-butanone N Y Y Y

2-butenal N Y Y Y

2-ethyl-2-butenal N N N Y

3 -methyl-2-butenal N N Y Y

3 -methyl-2-cyclohexen- 1 - one N N N N

2-decanone Y Y Y N

(E)-2-decenal N N N N

(Z)-2-decenal Y Y Y Y

2-furanmethanol N N N N

2-heptanone Y Y Y Y

6-methyl-2-heptanone N N Y N

(E)-2-heptenal N Y Y Y

(Z)-2-heptenal N N N Y

(E)-2-hexenal N Y Y Y

2-ethyl-2-hexenal N N N N

2-methyl-2-heptene llll N N N

2-n-heptylfuran llll N N N

2-n-octylfuran Y Y Y N 2-nonanone N Y N

(E)-2-nonenal Y Y Y Y

(Z)-2-nonenal N N N Y

2-octanone v Y Y Y

(Z)-2-octen-l-ol Y Y Y Y

(E)-2-octenal N Y Y Y

2-pentanone N Y Y N

1 -propoxy-2-propanol N N N N

1 -(acetyloxy)-2-propanone Y N N N

1 -hydroxy-2-propanone Y N N N

2-propenal N N N Y

2-thiophenecarboxaldehyde Y Y Y Y

2-undecenal N Y Y Y

2,3-butanedione N N N Y

2,3-pentanedione N N N N

(E,E)-2,4-decadienal N Y Y Y

2,4-decadienal N N N Y

(E,E)-2,4-heptadienal N Y Y Y

(E,E)-2,4-nonadienal N Y Y Y

2,6-dimethylpyrazine N N N N

(E,Z)-2,6-nonadienal N N Y N

5-ethyldihydro-2(3H)- furanone N Y Y Y

5-methyl-2(3H)-furanone N N N N dihydro-5-pentyl-2(3H)- furanone N N Y Y dihydro-5-propyl-2(3H)- furanone N N N N

2(5H)-furanone N N N N tetrahydro-6-methyl-2H- pyran-2-one N N N N

3 -ethylcyclopentanone N Y Y Y

3-hexanone N N N N

3-methyl-2- thiophenecarboxaldehyde N N N N

3-oetanone Y Y N Y

3-octen-2-one N Y Y

3 -thiophenecarboxaldehyde N Y Y Y
Image available on "Original document"
ethanol N N N Y l-(l(H)-pyrrol-2-yl)- ethanone N N N N

1 -(2-furanyl)-ethanone N N N N ethosuximide 1111 N N N formic acid, heptyl ester Y Y N N furan Y N N Y

2-ethyl-furan iiiii N N N

2-hexyl-furan 1111 N N Y

2-methyl-furan N N N Y

2-pentyl-furan N Y Y Y

2-propyl-furan N N Y Y

3-methyl-furan Y N N N

3-pentyl-furan Y Y Y Y furfural N Y Y Y heptanal N Y Y Y heptanoic acid N N N Y

2-methyl-hex-2-yn-4-one N N N N hexanoic acid N N N Y hydrogen sulfide N N N N m-aminophenylacetylene N N N N maleic anhydride N N N N methacrolein N N N N methanethiol N N N N methyl ethanoate N N N N methyl isobutyl ketone 1111 N N N n-caproic acid vinyl ester N Y N nonanal N Y Y Y

3 -methy 1-nonane iiiii N N N nonanoic acid 1111 N N N octanal N Y Y Y octane N N N Y octanoic acid N N N Y oxalic acid, isobutyl pentyl

ester Y N N N p-cresol N N N N pentanal N N N Y pentanoic acid Y N N Y
Image available on "Original document"
Image available on "Original document"

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

KPhos6_BeefHeart, MRM BeefHeart, MRM_BioLipon95, MRM_NatCholinePC40, Kphos6_Canola, MRM Canola, KPhos6_01eic Acid, KPhos6_Linoleic acid and

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

MRM_BioLipon95, MRM BeefHeart, and MRM Linoleic acid samples. For (E,E)-2,4-nonadienal, the strongest signal intensity was in the MRM Canola and MRM Linoleic acid samples. (E,E)-2,4-nonadienal also was detected in the Kphos6_Canola,

MRM_NatCholinePC40, MRM_BioLipon95, MRM BeefHeart, and KPhos6_Linoleic acid samples. For 2,4-decadienal, the strongest signal intensity was in the

MRM Linoleic acid sample. 2,4-decadienal also was detected in KPhos6_Linoleic acid, MRM Canola, and KPhos6_01eic Acid samples.

In samples having earthy or mushroom aromas, 3-octen-2-one, l-octen-3-one, 3-octanone, and/or l-octen-3-ol were detected in the KPhos6_BeefHeart, MRM BeefHeart, Kphos_BioLipon95, MRM_BioLipon95, Kphos_NatCholinePC40,

MRM_NatCholinePC40, MRM Canola, KPhos6_01eic Acid, MRM Oleic Acid, KPhos6_Linoleic acid, and MRM Linoleic acid samples. For l-octen-3-ol, the strongest signal intensity was in the MRM Linoleic acid sample, followed by

MRM _NatCholinePC40, KPhos6_Linoleic acid, MRM BeefHeart, KPhos6_BeefHeart, MRM Canola, MRM_BioLipon95, KPhos6_01eic Acid, and MRM Oleic Acid samples. 3 -octanone was detected in the MRM Oleic Acid, KPhos6_Linoleic acid, and

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

MRM_NatCholinePC40, KPhos6_BeefHeart, KPhos6_01eic Acid, MRM Oleic Acid, MRM BeefHeart, MRM_BioLipon95, MRM Canola, Kphos_BioLipon95, and Kphos_NatCholinePC40. Pyrazine was detected in the MRM Coconut, MRM C18, MRM C14, and MRM_BioLipon95 samples.

In samples having a nutty and roasted aroma, thiazole and 2-acetylthiazole were the

most abundant compounds detected, along with pyrazine, methyl pyrazine, trimethyl pyrazine, and 3-ethyl-2,5-dimethylpyrazine. 2-acetylthiazole was detected in all samples with MRM and most abundant in samples with MRM Beefheat, MRM_biolipon95, MRM Canola, and MRM coconut. Thiazole was created in samples with MRMCoconut, MRM BeefHeat, MRM_Biolipon95, MRM C14, MRM C18, MRM Canola, MRM Oleic acid and MRM Linoleic acid and MRM_NatCholinePC40. Pyrazine was present in the largest amount in samples with MRM-Coconut, followed by samples MRM BeefHeat, MRM_Biolipon95, MRM C14, MRM C18, MRM Canola having roughly equal amount, MRM Oleic acid and MRM Linoleic acid sample had even less. Methyl-pyrazine was present in MRM_Biolipon95 and MRM Coconut. 3-ethyl-2,5- dimethyl-pyrazine and trimethyl- pyrazine, were present only without phospholipids in the MRM.

In samples having green, vegetable, or grass aromas, 1-heptanol, l-hepten-3-ol, 1-hexanol, (E)-2-heptenal, (Z)-2-heptenal, (E)-2-hexenal, 2-pentyl-furan, and/or heptanal were detected in the KPhos6_BeefHeart, MRM BeefHeart, Kphos_BioLipon95, MRM_BioLipon95, Kphos_NatCholinePC40, MRM_NatCholinePC40, Kphos_C14, MRM C14, Kphos_C18, MRM C18, MRM Canola, MRM Coconut, KPhos6_01eic Acid, MRM Oleic Acid, KPhos6_Linoleic acid, and MRM Linoleic acid samples. For 2- pentyl-furan, the strongest signal intensity was in the KPhos6_BeefHeart sample, followed by the KPhos6_Linoleic acid, MRM_BioLipon95, MRM Linoleic acid, MRM BeefHeart, MRM Oleic Acid, MRM_NatCholinePC40, MRM Canola,

KPhos6_01eic Acid, and Kphos_NatCholinePC40 samples. For (E)-2-heptenal, the strongest signal intensity was in the MRM BeefHeart, MRM Canola, MRM Oleic Acid, and KPhos6_Linoleic acid samples, followed by the KPhos6_01eic Acid,

MRM_BioLipon95, KPhos6_BeefHeart, MRM Linoleic acid, MRM_NatCholinePC40, Kphos_BioLipon95, and Kphos_NatCholinePC40 samples. For (Z)-2-heptenal, the strongest signal intensity was in the MRM Linoleic acid sample. MRM Linoleic acid also was detected in the KPhos6_Linoleic acid sample. For heptanal, the strongest signal intensity was in the MRM Oleic Acid sample, followed by the KPhos6_01eic Acid, MRM C14, MRM C18, MRM Canola, MRM BeefHeart, MRM_NatCholinePC40, MRM Linoleic acid, and KPhos6_BeefHeart samples. For , (E)-2-hexenal, the strongest signal intensity was in the MRM Linoleic acid sample, followed by the

MRM_NatCholinePC40, KPhos6_Linoleic acid, and MRM Oleic Acid samples.

Example 11 - Creation of beefy flavors using complex precursor mixtures

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

The meat replica sample containing only LegH, was compared to the meat replica sample containing the magic mix and LegH by a sensory panel and GCMS analysis. Five tasters rated the flavored meat replicas for beefmess, bitterness, and levels of savory flavors, and off flavors. Each property was rated on a 7 point scale in which 7 was the highest amount of the specified property (e.g., a standard 80:20 ground beef

would be rated 7 on the beefy scale). The Magic Mix flavor was rated one point higher in beefy character than the LegH only sample (FIG. 1).

To determine which chemical products were produced upon heating, a solution of Magic Mix was prepared with 1% w/v LegH at pH 6.0. The samples were cooked with shaking at 150°C for three minutes, then Solid Phase Micro Extraction (SPME) was performed for twelve minutes at 50°C to extract the volatile compounds above the headspace of the reaction. A search algorithm was used to analyze the retention time and mass fingerprint information of the volatile compounds and assign chemical names to peaks. Table 14 shows the compounds identified in both the Magic Mix + LegH (MM, average of two samples) and in the LegH alone in buffer (LegH, average of five samples) samples. The compounds in Table 14 are listed in order of the retention time (R.T., in seconds), and are designated as having a zero peak area (0), or a small (S), medium (M), or large (L) average peak area. Hundreds of compounds were identified between the samples, many of which are characteristic of beefy aroma, including but not limited to l,3-bis(l,l-dimethylethyl)-benzene, 2-methyl 3-furanthiol, and Bis (2-methyl-4,5- dihydro-3-furyl) disulfide, which increased in the samples containing the Magic Mix and LegH.

Table 13

Chemical entities added to the Magic Mix

Image available on "Original document"
Valine 0.9

glucose 5.6

Ribose 6.7

Maltodextrin 5.0

Thiamine 0.5

GMP 0.24

IMP 0.6

Lactic acid 1.0

creatine 1.0

NaCl 10

KC1 10

Kphos pH 6.0 10

TABLE 14

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

LegH alone (average of five samples)

MM

with LegH

R.T.(s) Name LegH alone

248 acetaldehyde L S

256.3 carbon disulfide L S

264.3 dimethyl sulfide S 0

265 oxalic acid, isobutyl pentyl ester M 0

268.1 2,3 ,4-trimethyl-pentane M 0

269.2 methanethiol S 0

283.4 propanal M 0

285.4 octane M 0

287.1 furan M 0

295.3 2-methyl-propanal L s

297.6 acetone L s

319.3 2-propenal M s

338.1 2-methyl-furan M s

342.1 butanal L s 344.2 2,4-dimethyl- 1 -heptene M 0

346.3 methacrolein M 0

357.4 methyl-thiirane L 0

360.2 3-methyl-furan S 0

363.7 butanone L s

368.9 2,3 -dihydro-5 -methyl-furan M s

376.4 2-methyl-butanal L M

381.1 3 -methy 1-butanal L M

390.6 isopropyl alcohol 0 s

399.6 ethanol L M

406.2 2-propenoic acid, methyl ester M 0

408.2 benzene S 0

414.4 methyl vinyl ketone M 0

416.4 2,2,4,6, 6-pentamethyl-heptane M 0

422.6 2-ethyl-furan S 0

438.4 2-ethylacrolein M 0

449.9 2-pentanone S 0

453.2 pentanal/2,3-butanedione L 0

453.8 2,3-butanedione L M

472.8 4,7-dimethyl-undecane M s

485.9 2-methyl-pentanal M 0

492.6 2-methyl- 1 -penten- 1 -one S 0

496.6 (E)-3-penten-2-one M 0

508.6 l-penten-3-one M 0

510.6 trichloromethane M M

520.4 p-dithiane-2,5-diol M 0

525.5 3 -methyl-pentanal M 0

535.1 (E)-5-decene M 0

536.5 toluene M s

537.9 2-butenal M s

543.8 4-penten-2-one M 0

550.8 methyl thiolacetate M 0

683.7 p-xylene S 0

727.4 dimethyl selenone M 0

738.3 methyl isopropyl disulphide M 0

755 2-heptanone M 0

758.7 heptanal L 0

781.9 1 ,3 -diisopropoxy-1 ,3 -dimethyl- 1 ,3- S M disilacyclobutane

789.4 3 -methy 1-2 -butenal M 0

793.4 4-methyl-2-heptanone M 0

810.4 pyrazine M 0

818.8 isothiazole S 0

827.1 acetyl valeryl M 0

831.8 2-pentyl-furan L 0

851 2-methyl-thiazole S 0

853.3 isothiocyanato-methane S 0

870.9 thiazole L 0

879.2 styrene M 0

890.7 1 -(methylthio)-propane M 0

895.6 methyl-pyrazine M 0

910.5 thiocyanic acid, methyl ester S 0

918.6 4-methylthiazole M 0

921.4 2-octanone M 0

923.9 2-methyl-cyclopentanone M 0

927.9 octanal L s

934.3 tridecane M 0

948.8 trans-2-(2-pentenyl)furan S 0

961.9 1 -hydroxy-2-propanone M 0

974.5 (E)-2-heptenal M 0

987.4 5 -methyl- 1 -undecene M 0

993.8 2-hexyl-furan M 0

1007.8 7-methyl-(E)-5 -undecene M 0

1024.1 2-methyl-5 -(methylthio)-furan, S 0

1058.6 2-butyl-l-decene M 0

1079.3 dimethyl trisulfide L s

1085.3 2-nonanone M 0

1093.2 nonanal L M

1142.3 1 ,3 -bis(1 , 1 -dimethylethyl)-benzene M 0

1149.6 (E)-2-octenal M 0

1164.5 1-heptanol M 0

1193.5 methional L 0

1198.8 acetic acid M s

1207.2 furfural M 0

1242.1 2-decanone M 0

1250.8 decanal M 0 1265.2 l-decen-3-one M 0

1283.3 pyrrole M 0

1292.6 5 -ethenyl-4-methyl-thiazole M 0

1294.3 benzaldehyde L M

1303.7 2-n-octylfuran M 0

1305.6 (E)-2-nonenal M 0

1341.4 1-octanol M 0

1361.1 2-methyl- 1 (H)-pyrrole S 0

1391.7 2-undecanone M 0

1401.2 (E)-2-octen-l-ol M 0

1448 butyrolactone S s

1456.3 (E)-2-decenal M 0

1462.4 phenylacetaldehyde L s

1466.3 2-acetylthiazole L 0

1471.3 acetophenone M s

1475.4 1-nonanol M 0

1487 methyl (methylthio)methyl disulfide M 0

1497.1 5-(2-chloroethyl)-4-methylthiazole L 0

1497.5 1 -(ethylthio)-2-(methylthio)-buta- 1 ,3-diene L s

1512 3 -thiophenecarboxaldehyde M 0

1518.8 2-nonen-4-one M 0

1531.7 2-thiophenecarboxaldehyde S 0

1543.9 dodecanal M 0

1551.6 4-ethyl-2-methyl-pyrrole S 0

1558.2 3 -(methy lthio)-propanenitrile S 0

1561.2 3-decen-2-one M 0

1613.1 bis(2-methyl-4,5-dihydro-3-furyl) disulfide M 0

1615.6 1,10-undecadiene M 0

1619.5 2-undecenal S 0

1668.9 2-phenylpropenal M 0

1692.3 (Z)-3-decen-l-ol, acetate M 0

1733.1 3-phenyl-furan S 0

4-nitrophenyl 2-thiophenecarboxylic acid

1739.7 ester S 0

1741.2 5 -formyl-4-methylthiazole M 0 pentanoic acid, 2,2,4-trimethyl-3-hydroxy-,

1749.7 isobutyl ester M 0

1765.5 benzyl alcohol S 0 pentanoic acid, 2,2,4-trimethyl-3-hydroxy-,

1774.2 isobutyl ester S 0

1796.9 dodecanal M 0

1806.1 (1 -ethyl- 1 -propenyl)-benzene S 0

1825.6 1-undecanol M s

1827.9 2-methyl-3 -furanthiol M 0

1828.3 2-methyl-3-(methylthio) furan M 0

1836.1 4-chloro-2,6-bis(1 , 1 -dimethylethyl)-phenol S 0

1844.1 phenol S s

1845.3 [(methylsulfonyl)methyl]-benzene s 0

1850.3 (e)-2-tridecen- 1 -ol M 0

1 -heptyl- 1 ,2,3 ,4-tetrahydro-4-methyl-

1859.9 naphthalene s 0

1863.2 2,4-decadienal s 0

1905.1 3 ,3 '-dithiobis [2-methyl]-furan M 0

1906.9 3,5-di-tert-butylbenzoic acid s 0

1909.6 4-ethoxy-benzoic acid, ethyl ester s 0

1921.5 3-(phenylmethyl)-2,5-piperazinedione s 0

1944.5 9-octadecenal M 0

1959.7 3 ,5 -bis(1 , 1 -dimethylethyl)-phenol M s

1968.4 4-methyl-5 -thiazoleethanol M s

2007.8 1 , Γ-(1 ,2-cyclobutanediyl)bis-cis-benzene S 0

2019.8 benzoic acid s s

2026.4 4-quinolinecarboxaldehyde s 0

2027.8 m-aminophenylacetylene M 0

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

Fresh green spinach (10 lb) was added to 500 mL water and finely ground in a

Vitamix blender to yield 2 L of green suspension. Acetone (8L) was added with mixing and the material was allowed to extract for 1 hour. The material was filtered through Whatman filter paper and the acetone was removed on a rotary evaporator (Buchi). To the residual green suspension (500 mL) was added 2 mL of 10 M HC1, causing the

suspension to turn brown. To this was added lg of FeCl 2.4H 20 in 10 mL H 20. The solution was shaken then left at 4°C for 16 hours. This suspension was extracted with diethyl ether (3x50mL) to give a bright green organic phase, the combined organics were washed with saturated sodium chloride solution, dried over sodium sulfate, filtered and evaporated to leave a black paste (l .lg). The pellet was dissolved in chloroform for fractionation.

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

TABLE 15

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

Image available on "Original document"

Preparation of Flavor Reaction Containing Ferrous Chlorin or Leghemoglobin

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

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

TABLE 16

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

1-heptanol acetone

1-hexanol acetonitrile

1-octanol benzaldehyde

l-octen-3-ol butanal

l-octen-3-one 2-methyl-butanal

1-pentanol dimethyl trisulfide

2-acetylthiazole ethyl acetate

2-butenal furan

3 -methyl-2-butenal, 2-ethyl-furan

(Z)-2-decenal 2-hexyl furan

6-methyl-2-heptanone 2-pentyl-furan

(E)-2-heptenal furfural

(E)-2-hexenal heptanal

2-methyl-3 -furanthiol aminophenylacetylene

(E)-2-nonenal methacrolein

(E)-2-octenal methional

2-pentanone octanal

1 -hydroxy-2-propanone octane

2-thiophenecarboxaldehyde oxalic acid, diallyl ester

2-undecenal 2,3-butanedione 3 -methyl-3 -buten-2-one 2-methyl-propanal

3 -thiophenecarboxaldehyde pyrazine

(E)-4-octene, 2,3- dimethy 1-pyr azine methyl-pyrazine 2,5-dimethyl-pyrazine thiazole

Example 13 - Flavor creation by immobilized Hemin

Preparation of hemin linked CM Sepharose.

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

inky black in color.

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

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

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

TABLE 17

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

2-methyl-5-(methylthio)-thiophene Low

dihydro-5-propyl-2(3H)-furanone Low

octane Low

pyrrole Low Low

methanethiol Low Low

2-thiophenecarboxaldehyde Low Low

methyl-pyrazine Low Low

1 -hydroxy-2-propanone Low Low

propanal Low Low thiophene Low medium pyridine Low Low

2-methyl-furan Low medium oxalic acid, butyl propyl ester Low Low pyrazine medium Low oxalic acid, diallyl ester medium medium

2-butenal medium large furfural medium medium nonanal medium medium

2-ethyl-furan medium Low ethanol medium very large

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3 ,3 '-dithiobis [2 -methyl] -furan medium medium m-aminophenylacetylene medium medium

2,5-dihydro-3,4-dimethyl-furan medium medium

2-acetylthiazole medium medium cyclohexane medium

ethyl tert-butyl ether medium

carbon disulfide medium medium thiazole medium medium acetonitrile medium large

2-pentyl-furan medium Low

3 -thiophenecarboxaldehyde medium medium

2-methyl-butanal medium medium thiazole medium large

2-methyl-3 -furanthiol larege large

2-propenal large large

3 -methy 1-2 -butenal large medium

2-methyl-3-(methylthio) furan large large ethyl acetate large medium methacrolein large medium methyl-thiirane large large methional large large methyl alcohol large medium

2-butanone large Low

2,3-butanedione large medium acetone large large furan large medium benzaldehyde large medium

methyl thiolacetate large medium

acetaldehyde very large very large

2-methyl-propanal very large very large

dimethyl trisulfide very large very large

3 -methy 1-butanal very large very large

propyl-cyclopropane medium

(E)-2-octenal medium

2-n-propylaziridine medium

thiirane medium

ethyl formate medium

methyl vinyl ketone medium

2-propenoic acid, ethyl ester medium

1-nonanol large

1-octene large

1-heptanol large

1-dodecene large

phorone very large

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

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

Flavor molecules created by the combination of LegH and precursor mix.
Image available on "Original document"
2-nonanone medium

propanal medium

pyrazine medium

(Z)-2-heptenal medium

2-methyl- 1 -heptene medium

2-ethyl-furan medium

octanal medium

(E)-4-octene low

(E)-2-octenal low

2-methyl-thiazole low

2-propenal low

l-octen-3-one low

1-octene low

2-octanone low

dimethyl sulfide low

3-pentyl-furan low

2-n-octylfuran low

2-pentyl-thiophene low

OTHER EMBODIMENTS

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

特　許　庁

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特　許　公　報

特許出願公告
昭42－22194
公告　昭42.10.31
（全3頁）

フレーバ（香味）添加剤の製造法

特　願　昭　40－6567
出　願　日　昭　40．2．8
優先権主張　1964．2．8（スイス国）
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1965．1．15（スイス国）
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発明の詳細な説明

本発明は肉類似の味を有し、且つ食品のフレーバ（香味）を増進するために用いることの出来るフレーバ添加物（flavouring preparation）の製造に関するものである。アミノ酸又は蛋白質を含有する物質が単糖類或いは多糖類の存在下で加熱されるときはメイラード（maillard）反応が起ることが知られている。この反応の特徴はアロマ（aroma）の生成及び反応剤の褐変を伴うことにある。反応の結果アロマが生ずるためカラメル或いはパン皮類似の味を有するフレーバ添加剤を製造するのに此の反応を利用することができるのである。

一般に肉エキス製造中の特徴的なロースト様（roasted）フレーバの生成は少くとも1部は此の反応に依ると考えられている。然しながら、反応は極めて複雑であつて、その機構はまだ充分には理解されていない。従つて選択した既知の原料をもつて反応を行つた場合でも、その結果を規定することはできない。

最近の研究の結果、ロースト肉の特徴的フレーバの生成には硫黄の存在が必須であることが判明した。更に、肉はかなりの量のグルタチオンを含有していること、そしてこの物質がロースト肉の特徴的味の発生に寄与しているらしいということが知られている。他方、生筋肉の液汁には認め得る程度の遊離アミノ酸及びペントーズやヘキソーズの燐酸エステルが含有されていることが見出された。従つて、肉のアロマは少くとも1部はアミノ酸及び糖とグルタチオンとの反応によつて生成するものと考えられる。

メイラード反応を含む方法がロースト肉類似のアロマを有するフレーバ添加剤を調整するために記載されている。ロースト肉類似の味を有する製品が、例えばシステン或いはシステインと、キシローズの如き単糖類及びその他の1種又は1種以上のアミノ酸を原料として製造できることが記載されている。各成分の反応は既知の方法に従い、水の存在下で下記に要約する所の定の特定条件の下で行なわれる。

水の使用量は単糖類の量の5－1500倍であり、反応は還流下で行なわれる。システン或いはシステイン対単糖類の比は0．4：1乃至2：1である。

然しながら、此の方法によつて得られた製品は比較的弱く、且つ最分不安定である。更に、これらの方法は非常に稀い溶液の真空蒸発或いは混合物の凍結乾燥を含んでおり、揮発性アロマのかなりの損失が起る。其の上、大量の水の存在はかなりの装置を必要とする。従つて、斯る方法は寧ろコスト高となる傾向がある。

本発明の目的は強いアロマを有し、且つ、又非常に安定なフレーバ添加剤を製造する簡単、且つ経済的方法を供与することにある。

即ち、本発明によれば、システイン及び（又は）グルタチオンと、単糖類の1つと及びその他の少なくとも1種のアミノ酸とを反応させることよりなり、而してシステイン及び（又は）グルタチオンと単糖類との重量比が0．05：1乃至0．4：1であり、反応を高温で水溶液又は水性懸濁液中で行なうことを特徴とする肉エキス様の風味を有するフレーバ添加剤の製造方法が提供される。反応混合物は重量で30％以下の水を含有するのが好ましく、アミノ酸とシステイン及び（又は）グルタチオンとの重量比が50：1乃至600：

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１にあるのが好都合である。望むならばグルタチオン及び／或いはシステインは天然の蛋白性物質の加水分解、特に酵母、血液或いは肝臓の酵素的加水分解物の形として用いることも出来る。

アミノ酸源として特に適当なものは、動物或いは植物蛋白の酸、アルカリ又は酵素的加水分解物例えば肉、血液、或いは小麦グルテンの加水分解物である。或いは、それらの代りに純アミノ酸の人工混合物を用いることも出来る。

此の様な加水分解物は、好ましくは使用前に脱色及び脱臭（例えば活性炭と共に高温で加熱することによつて）しなければならない。此の処理後得られた稀い加水分解物は、例えば全固形物８０－９０％を含有するペーストまで濃縮するのがよい。

塩酸の作用によつて得られた加水分解物は若干量の食塩を含んでいる、がしかしこのものは反応には影響しない。アミノ酸源として用いられる加水分解物は取扱いを容易にするために粘稠なペーストの形となして約７０℃に加熱される。

本発明に従う方法は、例えば下記の如くして実施される。

ペントーズ（例えばキシローズ或いはリボーズ）及び／或いはヘキソーズと－ＳＨ基を含有する天然物（システイン、グルタチオン）或いは酵母、血液又は肝臓の酵素水解物の混合物を蛋白水解物（アミノ酸８０－７０％を含有し、上記の如くして、脱色脱臭した）によつて供給されるアミノ酸或いはアミノ酸類に加える。含硫化合物を供給する加水分解物は濃縮して、少くとも約８０％の固形物を有するペーストとするのが好ましく、且つ０．１－２．０％のグルタチオン及び／或いはシステインを含有しなければならない。或いは又、加水分解物を貯蔵する必要がある場合には濃縮後乾燥することが出来る。そして使用時に適当量の水と混ぜ合わせて所望する固形物濃度とすることが出来る。望むならばリボーズ及び含硫化合物は酵母水解物によつて供給出来る。

反応自体は、好ましくは攪拌をしつつ、温度８０－１１０℃、好ましくは９０℃で行なわれる。加熱の影響下で、液は先づ第一に泡立つ、そしてついで褐色に変る。反応剤及び最終製品の所望する味によつて反応時間は１０分間から６時間まで変り得る。反応が完結したら、混合物を苛性ソーダで中和する。

仕上げペーストは直接貯蔵容器中に満すことが出来る。ペーストはそこで固化する。好ましくは製品は充分に充てんした密閉容器中に１０－１５℃以下の温度で貯蔵しなければならない。此の方法で、製品は長期の保存後でも変質しないのである。

本発明の好ましい具体例に従つて、反応混合物中に乳酸塩（例えば乳酸カルシュウム又はカリウム）を混入することによつて製品のフレーバを更に増進することが出来る。此の添加は、好ましくは反応の前又は終で行なわれる。例えばアミノ酸を含有する加水分解物に、この水解物を濃縮する前に相当する塩基及び乳酸を添加することによつて行なわれる。最終製品中の乳酸塩濃度は約２５％まで、好ましくは１０－１５重量％範囲とすることが出来る。

又イノシン酸、又は５′－グアニル酸、或いはこれらの酸の塩を添加することによつて更に製品の品質を改良することも出来る。

本発明法に従つて製造されるフレーバ添加剤は水に溶かし、此の形態で食品用の調味料として用いることが出来る。

下記の実施例は本発明の実施法を説明するものであるが、しかし本発明はその記載条件に規定されるものではない。尚、パーセントは重量パーセンを示めす。

実施例　１

小麦グルテン９０ｋｇを塩酸と共に還流下或いは加圧下で加熱することによつて加水分解する。水解物は苛性ソーダ又は炭酸塩でｐＨ５－６、５まで中和する。不溶物を濾過を行つて除き、濾液は食塩の沈澱が現われるまで蒸発する。数週間放置した後、溶液を再び濾過し、９０％乳酸７ｋｇと活性炭（カルボラフインＣ或いはノーリット）３ｋｇを加える。混合物を７５℃に加熱し、２時間攪き混ぜる。次に苛性カリ４ｋｇを水に溶かして加え、溶液を濾過して活性炭を除き、濾液のｐＨを５－６、５の間の値に調節する。

黄色溶液を蒸発して８５－８８％固形物を含有するペーストとする。斯して得られたペースト１１５ｋｇにキシローズ１５００ｇとシステイン３００ｇを混ぜ合わせて、ステンレススチール釜に入れ、９５℃で３０－６０分間加熱する。これによつて、肉エキス類似の非常に香のよいペーストが得られる。

加熱停止時に３０％苛性ソーダ５．０ｇ及びグアニル酸ソーダ、或いはイノシン酸ソーダ１２０

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g、或いは両者の混合物を加え、肉エキス代用品として用いることが出来る製品を得ることも出来る。ペーストを水に溶かせば、顕著な肉様味を有する非常に良好な食品調味料が得られる。

実施例　2

新鮮肉或いは抽出肉（例えば肉エキス製造のスクラップ）を塩酸で加水分解し、実施例1記載の如く処理する。屠殺場の肉スクラップも同様に用いることが出来る。他方、魚ミールはトリエチルアミンを完全に除去した場合にのみ適当である。

動物蛋白水解物は、つづいて実施例1記載の如くして反応させて呈味剤を得る。製品の味を強めるためにシステイン－キシローズ混合物の量を水解物の約5％まで増すことが出来る。然しながらシステイン／キシローズ比は約0．2：1に保たなければならない。

実施例　3

2．5kgの市販の乾燥酵母（トルラユチリス）を温水20l中に懸濁する。温度を95℃に10分間保ち、ついで80℃に冷す。

パパインI：350、10gを添加し、pHを7．0に調節する。6時間反応後、混合物を95℃で10分間加熱し、そしてpHを6．0に調節する。次に懸濁液を濾過し、残渣を温水で洗う。合した濾液を減圧下で蒸発すれば83％の固形物及び約0．7％のグルタチオン及び／或はシステインを含有する黄色ペースト1．8kgが得られる。

新鮮肉（筋肉、胃、肝臓、腎臓）を細く砕き、濃塩酸1．25kgを用い135℃で4時間加水分解する。次に水解物を炭酸ソーダ700gで中和し、そして濾過する。乳酸160g及び活性炭60gを加え、混合物を70℃で90分間加熱する。次に30％苛性カリ300gを加え、生成物を濾過する。黄色の濾液が得られる。此れを減圧下で蒸発すれば86％固形物及びアミノ酸約60％を含有する黄色のペースト2kgが得られる。

上記の如くして製造した酵母水解物1．8kgと肉水解物2．0kgを蔗糖450g及びキシローズ90gと良く混ぜ合わせる。得られたペーストを攪き混ぜながら90℃で約1時間加熱する。生成物を70℃に冷却し、30％苛性ソーダ120mlを加える。最後にイノシン酸ソーダ45gをペーストと充分に混ぜ合わせる。肉エキスと非常に似た香のよい製品4．3kgが得られる。

本発明は特許請求の範囲記載のフレーバ添加剤の製造法に関するものであるが、更に下記の態様をも包含するものである。

(1)　システイン及び（又は）グルタチオンの給源として天然の蛋白質の加水分解物、好ましくは酵母、肝臓又は血液の酵素的水解物を使用する特許請求の範囲に記載の方法。

(2)　反応溶液或いは懸濁液が30％重量以下の水を含量している特許請求の範囲及び前記(1)項のいずれかに記載の方法。

(3)　アミノ酸に対するシステイン及び（又は）グルタチオンの重量比が50：1乃至500：1である特許請求の範囲及び前記(1)～(2)項のいずれかに記載の方法。

(4)　アミノ酸の給源として濃縮、脱色及び脱臭した動物又は植物性蛋白質の加水分解、好ましくは血液、肉又は小麦グルテンの酸加水分解物を使用する特許請求の範囲及び前記(1)～(3)項のいずれかに記載の方法。

(5)　単糖類が少なくとも1種のペントーズ又はヘキソーズである特許請求の範囲及び前記(1)～(4)項のいずれかに記載の方法。

(6)　ペントーズがリボーズ又はキシローズである前記(5)項に記載の方法。

(7)　反応を乳酸塩の存在下に実施する特許請求の範囲及び前記(1)～(6)項のいずれかに記載の方法。

(8)　乳酸とカルシウム又はカリウムの塩基とをアミノ酸又はアミノ酸給源に添加し、乳酸塩の全量が反応混合物の重量の約25％以下である前記(7)項に記載の方法。

(9)　イノシン酸、5′－グアニル酸又はこれらの酸の塩の1つ或いはこれらの混合物をフレーバ添加剤に加える特許請求の範囲及び前記(1)～(8)項のいずれかに記載の方法。

特許請求の範囲

1　システイン及び（又は）グルタチオンと、単糖類の1つと及びその他の少なくとも1種のアミノ酸とを反応させることよりなり且つシステイン及び（又は）グルタチオンの単糖類に対する重量比が0．05：1乃至0．4：1であり、上記反応を高温で水溶液或いは懸濁液中で行なうことを特徴とする肉エキス類似の風味を有するフレーバ添加剤の製造法。

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(54)牛肉のフレーバーのする物質の製造方法

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発明の詳細な説明

本発明は新規且つ有用な調味料組成物に係り、更に詳しくは糖類とアミノ酸、植物蛋白加水分解物及び5—リボヌクレオチドの反応生成物からなり食品に牛肉様フレーバーを与えるのに有用な混合物に関するものである。

スープ、グレービーの如き多くの市販食品中の牛肉様フレーバーは該食品中に牛肉抽出物と称せられる物を添加することにより得られている。牛肉抽出物は時間のかゝる高価な方法で作られるが該方法は一般に屠殺牛の肉組織を酸で加水分解することからなるものである。工業的に一般に用いられている方法では肉を加水分解し、次で混合物を中和し、液状部が大部分蒸発してしまう迄濃縮し褐色の実質的完全に加水分解された残留物、所謂牛肉抽出物を得る。この方法は比較的大きな装置を必要とし大量の牛肉組織を最終物たる比較的少量の濃縮抽出物にする為多くの熱を必要とする為高価である。

牛肉抽出物は、肉が比較的安く又加工費も少い

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地域から米国に輸入されている商品である。多くの輸入商品と同様に、牛肉抽出物の価格は年間の季節により又食卓用の肉の要求量によつても変動する傾向がある。

又、抽出物の質は、業者がその製造に当り質の悪い切り肉を主として使用する為変動する傾向がある。特に良質の肉の価が高い場合にはこういつた結果になる。こういつた諸問題がある為食品加工業者は牛肉抽出物にたよらない方法を求めていたのである。本発明の調味料組成物を使用することによつて牛肉のフレーバーを付した食品製造に牛肉抽出物にたよらなくても良いことになつた。即ち牛肉抽出物の代りに価格の安定している又将来は益々に安価になつて行くことの期待される市場で入手可能な化学的並びに植物性物質から作られた組成物を使用することにより上記目的は達成せられる。この新規組成物の特に重要な利点は牛肉抽出物と価格を比較すれば容易に理解される、即ち食品製造に牛肉抽出物を使う場合は本発明の調味料組成物を用いる場合に比し約3倍も高価になる。

従つて本発明の主要な目的の一つは食品中の牛肉抽出物の全量又は一部をそれで以て置換することの出来る牛肉のフレーバーのする組成物を提供するにある。

本発明の別の目的は肉を使用せずに牛肉のフレーバーのする食品を実質的安価に得る為の組成物を提供するにある。

本発明の更に別の目的は均質で、年中容易に入手出来、安価な調味料組成物を提供するにある。

本発明の上記並びに他の諸目的は本発明の組成物を使用することにより容易に達成せられる。

本発明に係る組成物は：

(a) ヘキソーズ及びペントーズ単糖類からなる群から選ばれる化合物を水の存在下にシステイン及びシスチンと加熱することにより作られる牛肉のフレーバーのする物質

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(b) 前記牛肉のフレーバーのする物質がシステンを用いて作られた場合には該物質1重量部当り約3～約15重量部、又システインを用いて作られた場合には該物質1重量部当り約3乃至5重量部以下の植物蛋白加水分解物

(c) 前記牛肉のフレーバーのする物質1部当り約0.02～約1.5重量部に当る、イノシン酸、グアニル酸、それらの生理学的に許容しうる塩、それらの混合物からなる群から選ばれる5′－リボヌクレオチド

から成るものである。

特に良好なバランスの良くとれた牛肉フレーバーを与える本発明の一具体例に於ては上述の組成物に、前記牛肉のフレーバーのする物質1部当り約2～約15重量部の、グルタミン酸及びその生理学的に許容しうる塩からなる群から選ばれる化合物を含ましめる。

又、本発明の別の具体例では前記ヘキソーズがデキストローズであり前記ペントーズがアラビノーズである。この組成物はフレーバー及び経済性のバランスが非常によくとれている。

更に又別の具体例では牛肉のフレーバーのする物質1重量部当り約0.05～0.5重量部のアラニン又はグリシンを含む前記の如きフレーバーの弱い組成物が提供せられる。

又、特に弱いフレーバーで高濃度で用いてもにがみのない組成物として前記牛肉のフレーバーのする物質1重量部当り約1～4重量部のシュークローズの含有される上述の如き組成物が提供せられる。

味のパネル試験であらゆる点で牛肉抽出物と全く同等であることが見出された本発明の具体例では

(a) デキストローズ及びアラビノーズを水の存在でシステイン又はシステンと共に加熱して作られる牛肉のフレーバーのする物質

(b) システンを用いた場合は前記牛肉のフレーバーのする物質

1重量部当り約3～約15重量部、システインが用いられた場合には牛肉のフレーバーのする物質1重量部当り約3～約5重量部に当る植物蛋白加水分解物

(c) 前記牛肉のフレーバーのする物質1重量部当り約2～約15重量部のモノソジウム　グルタメート

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(d) 前記牛肉のフレーバーのする物質1重量部当り約1～約4重量部のシュークローズ

(e) 前記牛肉のフレーバーのする物質1重量部当り各々約0.05乃至約0.5重量部のアラニン及びグリシン、及び

(f) グアニル酸1重量部に対し約1重量部のイノシン酸からなる混合物を前記牛肉のフレーバーのする物質1重量部当り約0.02～約1.5重量部存在せしめる。

なる組成を有する。

上記の組成物の各成分は商品として容易に入手できるか、または合成で得ることができる。

前記の(a)における「牛肉のフレーバーのする物質」はMorton 他の米国特許第2934437号およびその中の例に示されている。

牛肉のフレーバーのする物質を作るのに用いられる単糖類はペントーズ、又はヘキソーズ又はそれらの混合物である。ペントーズの代表的なものはリボーズ、アラビノーズ、キシローズ等であり、ヘキソーズの代表的なものはグルコース、デキストローズ、フルクトーズ等である。本願明細書に於て使用せる「植物蛋白加水分解物」なる語は穀類、特に小麦中に存在する植物蛋白の混合物、グルテンとして知られているものの如き植物性蛋白を酸、酵素又は他の方法で加水分解して得られる蛋白の人工的消化物を意味する。自由に入手しうるこの蛋白加水分解物は原料蛋白の構成分を代表する各種アミノ酸からなつている。本願明細書に於て使用せる「生理学的に許容しうる塩」なる語は5′－リボヌクレオチド類、その中でイノシン－5′－フオスフオリックアシド即ちイノシン酸と呼ばれているものは特に重要、及びグルタミン酸の非毒性アルカリ金属類、アルカリ土類金属塩又は有機塩基塩を意味する。入手の容易なこと及び毒性の少いことでナトリウム塩及びカルシウム塩は特に有用である。

又、その他で重要なものはカリウム塩、アンモニウム塩、グリシンとの塩及び他の非毒性有機塩基との塩である。

イノシン酸と共に或はその代りに他の5′－リボヌクレオチドも本発明の実施に用いうることが見出された。この点に関し、通常使用されるイノシン酸源即ちイーストリボヌクレイックアシドから

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誘導される或種の5′－リボヌクレオチド即ちサイチジン－5′－フオスフオリックアシド、ユリジン－5′－フオスフオリックアシド及びグアノジン－5′－フオスフオリック　アシド（このものは通常グアニル酸と呼ばれている）について特に普及されている。

牛肉抽出物の代用として適している組成物に対しては牛肉のフレーバーのする物質、植物蛋白加水分解物及び5′－リボヌクレオチドの三成分の全てが存在する必要がある。例えばスープ　ベースに牛肉抽出物の代りに新規組成物を用いて味のパネル　テストを行つた結果完全に満足すべき結果を得たが植物蛋白加水分解物の入らぬものや5′－ヌクレオチドの入らぬものを用いた場合にがみがあり全然代用し得ないことが判つた。

本発明の実施に対しては植物蛋白加水分解物対牛肉のフレーバーのする物質の比を本願明細書に記載し且つ特許請求の範囲の項に記載してある値に保つことが重要である。もし牛肉のフレーバーのする物質1重量部当り約15重量部以上の加水分解物を使用すると、牛肉の味はもはや大してしなくなる。他方、もし、牛肉のフレーバーのする物質1重量部当り約3重量部以下の加水分解物を用いると明らかににがみが認められるようになる。

又、本発明の実施には、牛肉のフレーバーのする物質に対する5′－リボヌクレオチドの比を本願明細書記載の値に保つことが極めて望ましい。もし牛肉のフレーバーのする物質1重量部当り、1.5重量部以上のヌクレオチドが存在すると本願発明の経済的利点が失われるし又、その量が0.02重量部以下だと牛肉のフレーバーが少くなる傾向が認められる。バランスのよくとれた適度のフレーバーと経済性という観点から特に有用な各成分の重量比は牛肉のフレーバーのする物質：植物蛋白加水分解物：5′－リボヌクレオチドー1：5：0.30であることが判つた。

後段に於て例示されている如く、本発明に係る組成物は実質的に乾燥せる固体、ペースト、又は溶液の形で得られ且つ使用せられる。本発明に係る調味料組成物を牛肉抽出物の代りに用いる場合には最も望ましいフレーバー又はエッセンスにする為には最終製品の各々の好み及び性質に応じて使用濃度を幾分調節する必要がある。

こういつた調節は当業技術者により容易になしうるところであるし、又本発明の合成混合物のフレーバーの強さを牛肉抽出物の強さと同じにすることにより需要家が調節する必要性を大巾に少くすることが出来る。かゝる組成物は適当な濃度を選択することにより容易に得られ、又後段に実施例として具体的に示されている。

ロースト　ミートのフレーバーとにおいをもつた牛肉のフレーバーのするグレービーを作るのに特に適した本発明の一具体例に於ては、上述せる如き組成物に、前記牛肉のフレーバーのする物質1重量部当り約0.5～約15部の食用脂肪を添加する。

最も強いロースト　ミート　フレーバーを得る為には、具体例に示されている如く、牛肉のフレーバーのする物質と他の有効成分とを約70℃～約100℃に10分～約4時間加熱することが望ましい。当業技術者には明らかな如く、温度を高くすればする程、適当なフレーバーを得る為に必要な加熱時間は短かくなる。優れた牛肉のフレーバーのする食品は又、本発明に係る牛肉抽出物代用物と最終食品とを加熱することにより得られる。

食用脂肪に関しては、動物性及び植物性脂肪何れをも用いうる。例えば豚の脂肪、牛肉の脂肪、マーガリン、バター、ベニバナ油、等を用いることが出来る。ロースト　ミートのにおい及び味になる反応は明瞭には理解されていないが、脂肪と共に混合物をこういつた条件下に加熱すると、脂肪がない場合に比し極めて望ましい生成物が得られる。

食用脂肪の量は変更可能である。例えば牛肉のフレーバーのする物質に基いてその1重量部当り約0.5～約15重量部使用することが出来る。特に望ましい結果が得られるのは牛肉のフレーバーのする物質1重量部当り食用脂肪が8重量部の場合である。

各成分を混合する為の便利な方法は下記の如くである。

(a)　ヘキソーズ及びペントーズ単糖類からなる群から選ばれた化合物とシステイン及びシステインからなる群から選ばれたアミノ酸との混合物を水の存在で牛肉のフレーバーのする混合物を得られる迄加熱する。

(b)　前記牛肉のフレーバーのする混合物1重量部当り、システインを用いた場合には約3～約15

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重量部の植物蛋白加水分解物を、又、システインを用いた場合には約3乃至5重量部以下の植物蛋白加水分解物を加え更に約0.02～約1.5重量部の5′－リボヌクレオチドを加え少くとも70℃に約2時間加熱する。

牛肉のフレーバーのする物質はシステインと約4～4½重量部の単糖類と約5重量部の水を混合し、90～100℃に2時間加熱し、55℃に冷却し、50%の苛性ソーダ水溶液でPHを約6.9に調節することにより作るのが便利である。これを次にその約½重量の水で処理し、植物蛋白加水分解物と5′－ヌクレオチドを加え次に混合物を70℃に約2時間加熱し、冷却、ホモゲナイズして需要家に特に便利に使用せられる形の重い蜂蜜様ペーストにする。以下実施例により本発明の詳細を説明するが、これらは本発明を何ら限定する意図のものではなく各種の変更が可能である。

実施例　1

デキストローズ　12.5g、アラビノーズ9.75g及びシステイン塩酸塩6.5gの混合物に水25mlを加え、混合物を100℃に2時間加熱し、55℃に冷却し、50%苛性ソーダ水溶液を加えてPHを3.2から6.9になし、水35ml、植物蛋白加水分解物275g及びイノシン酸とグアニル酸の1：1重量比混合物18gを加え、次に70℃に2時間加熱し、冷却し、滑らかな蜂蜜様のものが得られる迄撹拌することにより牛肉のフレーバーのする組成物を作つた。

上記方法に於て、イノシン酸とグアニル酸の混合物の代りに、各々ジナトリウム　イノシネート、ジカリウム　イノシネート、カルシウム　イノシネート、ジーアンモニウム　イノシネート及びジナトリウム　イノシネートとジナトリウム　グアニレートの1：1重量比混合物を用いて牛肉のフレーバーのする組成物を作つた。又、上記方法でモノナトリウム　グルタメートの代りにグルタミン酸、モノカリウム　グルタメート、モノカルシウム　ジグルタミン酸及びモノアンモニウム　グルタメートを用いて各々牛肉のフレーバーのする組成物を作つた。

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実施例　2

デキストローズ　12.05g、アラビノーズ9.75g、モノナトリウム　グルタメート9.75g、システイン6.5g、アラニン4.65g及びグリシン3.3gを水300mlに溶解した。混合物を激しく撹拌しつつ100℃に2時間加熱し、次に10%のNaOH　水溶液でPH 6.7に調節した。混合物を75～80℃で濃縮し蜂蜜様暗褐色の牛肉のフレーバーのする物質を得た。

この牛肉のフレーバーのする物質と、植物蛋白加水分解物261.3g、シュークローズ59.75g、モノナトリウム　グルタメート97.80g及びイノシン酸とグアニル酸の1：1重量比混合物17.15gを水500mlにとかし、激しく撹拌しつつ100℃に6時間加熱し、冷却して牛肉抽出物の代用として適した組成物を得た。

上記方法を、アミノ酸、糖、植物蛋白加水分解物及び5′－リボヌクレオチドの濃度を各々変えて繰返し実施した。何れの場合にも満足すべき牛肉のフレーバーのする組成物が得られたが、それらの各組成を示せば第1表の如くである。

第　1　表

アラビノーゼーデキストローゼーシステイン、＊ 重量部	植物蛋白加水分解物、 重量部	5′－リボヌクレオチド 重量部	アラニン 重量部	グリシン 重量部	シュークローズ 重量部	MSG＊＊ 重量部
1	3	0.30	0.18	0.09	1.2	3.0
1	5	0.30	0.10	0.05	1.2	7.65
1	5	0.30	0.10	0.05	1.2	4.0
1	3	0.2	0.10	0.05	1.2	4.0
1	3	0.2	0.05	0.05	1.2	2.0
1	3	0.02	0.05	0.05	1.0	2.0

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＊　牛肉のフレーバーのする物質混合重量
＊＊　一モノナトリウム　グルタメート

実施例　3

実施例2の方法により、又最終加熱工程の前に水500mlに牛肉脂肪200gを加えて、ロースト　ミートのにおいと味のするグレービーを作るのに適した牛肉のフレーバーのする組成物を作つた。

100℃に6時間加熱した後、165gの脂肪をすくい取り、水118gを含みグレービーを作るのに用いうる生成物689gを残存せしめた。実施例2の表1に記載の調味組成物とアラビノーゼ－デキストローゼ－システイン混合物1重量部当り0.5部重量部の牛肉脂肪を用いて上述の方法を繰返しロースト　ミートのにおいとフレーバーのするグレービー　ベースを得た。

実施例　4

システイン塩酸塩13.30g、デキストローゼ33.24g、アラビノーゼ26.09g、モノナトリウムグルタメート26.09g、グリシン塩酸塩6.65g及びアラニン13.30gを水66.48gに溶解して、混合物を90℃に加熱し、同温度に激しく撹拌しつつ2時間保つた。混合物を20℃に冷却し、水99.71gを加えた。50%苛性ソーダ水溶液を12.8ml加えて混合物のPHを6.8にした。得られた牛肉のフレーバーのする物質を激しく撹拌しつつ、これに166.19gのシュークローズ、531.80gのモノナトリウムグルタメート、99.71gの牛肉脂肪、及び731.23gの植物蛋白加水分解物を加えた。激しく撹拌しつつ混合物を70℃に加熱し、この温度に4時間保つた。混合物を50～55℃に冷却し、ジナトリウム　イノシネートとジナトリウム　グアニレートの50：50重量混合物39.89gを混合し15分間混合を続け食品調味用組成物を得た。

実施例　5

ジナトリウム　イノシネートとジナトリウム　グアニレートの50：50混合物の代りにジナトリウムグアニレート39.89gを用いて実施例4の方法を繰返し実施した。食品の調味用として適した組成物を得た。

実施例　6

システインの代りに13.30gのシスチンを用いて実施例4の方法を繰返し実施し、食品の調味用として適した組成物を得た。

参考例　1

(1)　米国特許第2934437号の例Ⅰの方法に正確に従つて褐色の溶液を製造した。

(2)　上記の方法で用いたD－リボースの代りにL－アラビノースを用いて上記の特許の例Ⅰの方法に従い第2の褐色の溶液を製造した。ここでL－アラビノースを用いた理由はL－アラビノースが本発明において好適なペントースであるからである。

(3)　上記の米国特許の例Ⅰに従い、D－リボースの代りにL－アラビノースを用い、そして混合物を90－95℃で2時間加熱して第3の褐色の溶液を製造した。

試験用の最適濃度を決定する目的で4名の味覚パネル試験官に上記(1)，(2)および(3)の溶液をそのままで、また水の1，2，3，4および6部で稀釈した後に試験させた。

この結果、溶液1部を水3部で稀釈したものが最も良好であることが見出された。これより稀釈度の低いものは苦味がありかつ非常に不快であつた。一方稀釈度の大きいものは本発明のビーフのフレーバーを有する組成物と比較するには水気の多い味であつた。

次にイノシン酸ジナトリウムとグアニル酸ジナトリウムの混合物の代りに5′－イノシン酸ナトリウムを用いて本発明の例1の方法に従つて製造した市販の生成物〔Pfizer Meat Flavor，Type MF－2（ロット番号68962）〕の5gを水100mlに加えて実験上試験に最適であることが示された5%の濃度の溶液を得た。

結　果

上記の試料で試験した結果、味覚のパネル試験官は全員が下記の通りの結論を出した。

(a)　前記(1)，(2)および(3)の溶液の1：3稀釈物は全て味が悪く、幾分不快なフレーバーを有し、明らかにビーフではないがある種の肉に僅かに似たフレーバーを有した。

(b)　本発明のビーフのフレーバーを有する組成物の5%溶液はビーフ・ブロスに非常に似た味を

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有し、快くかつ食欲を誘発するものであつた。

この試料は先行技術の3種よりも非常に優れた味のものであつた。

参考例　2

例2による組成物に関して、下記のごとく調整した3種類の試料についてパネル分析を実施した。

6970－3A

デキストロース	12.05g
L(+)アラビノース	9.75g
グルタミン酸モノナトリウム	9.75g
L－システイン	6.50g
β－アラニン	4.65g
グリシン	3.30g

上記の成分を撹拌しかつ僅かに温めながら（約35℃）水300mlに溶かした。僅かに混濁したほとんど無色の溶液が得られた：容量、328ml。この溶液を等量の2部に分けた。1部6970－3Aのラベルを付け、冷蔵庫に貯蔵した。（例2の最初の成分を水300mlに溶かしたもの）

6970－4B

上記で得られた第2の部分を、撹拌器、温度計および還流凝縮器を備えた300ml容の3頸のフラスコに移した。これを激しく撹拌しながら115－120℃に維持した油浴を用いて100℃で2時間加熱し；室温まで冷却し、次いで10%NaOH 水溶液の15.3mlでpH 3.5からpH 6.7に調節した。

次に蒸留用に凝縮器を付け、圧力を調節して75－80℃の内部温度を維持しながら反応混合物をハチミツの稠度になるまで濃縮した。この濃縮物27.1gをビンに移し、6970－4Bのラベルを付け、冷蔵庫で貯蔵した。

6970－7C

例2に示した通りのビーフエキス代替物の完全な製品を5のスケールで製造した。

デキストロース	4.02g
L(+)アラビノース	3.25g
グルタミン酸ナトリウム	3.25g
L－システイン	2.17g

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β－アラニン	1.55g
グリシン	1.10g
水	100　ml

上記の成分を、撹拌器、温度計および還流凝縮器を備えた250mlの3頸フラスコに入れた。反応体を100℃に加熱し、激しく撹拌しながらこの温度で2時間維持した。混合物を次に室温まで冷却し、10%NaOH 水溶液の10.0mlでpHを3.5から6.7に調節した。この溶液を次いで75－80℃、圧力<180mmで水を蒸発させることによりハチミツの稠度まで濃縮して濃縮物の18.5gを得た。

上記の濃縮物に下記の成分を加えた：

マギーの4BEペースト（ネツスル社製、加水分解した植物性蛋白質）	87.1　g
蔗　糖	19.9　g
グルタミン酸モノナトリウム	32.6　g
イノシン酸ジナトリウムとグアニル酸ジナトリウム1：1混合物	5.72g
水	167.0　ml

得られた混合物を500ml容の3頸フラスコに移し、激しく撹拌しながら100℃で6時間加熱した。生成物を次いで冷却し、冷蔵庫で貯蔵した。最終重量は313.8gで、これに6970－7Cのラベルを付けた。

これらのパネル試験は予備実験で上記の試料の各々の最適濃度を決定した後にこれらをアロマとフレーバーについて比較して行つた。試料Aは20%の濃度（20mlを水で100mlに稀釈）で使用し、試料Bは3%（3g／100ml）、試料Cは6%（6g／100ml）で使用し、全ての試料はパネル評価中約50℃に維持した。パネル試験官にこれらの3種類の試料と質問事項を提供してアロマとフレーバーの両方の試験を要求し、次いで好ましい順序を示すように要求し、さらに試験官には各々の試料についての簡単な印象を記載することを要求し、その結果として10名の試験官の全員が下記の如く同じ順序で試料を等級付けした。

選択順位第1位 ……C
〃　第2位 ……B

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選択順位第3位 …… A　　※一フフレーバーであると判定し、そしてAおよび
試験官の殆んどが試料Cを良好または満足なビ※　Bについてはしばしば「不快」であると報告した。

本願発明の例2により製造したビーフエキス代替物の試料の感覚評価

パネル試験官名	選択順位 1位 2位 3位	所見 アロマ	所見 フレーバー
K.T	C B A	C－コラール（Corral）ビーフに類似。	満足なビーフフレバー。
		B－僅かに肉様。	不快、水っぽい。
		A－不快、イオウ臭い。	酸味あり、不快。
N.B.K	C B A	C－肉様。	良好なビーフフレバー。
		B－イオウ臭い。	味気ない（flat）、イオウ臭い。
		C－イオウ臭い。	塩味強く、イオウ臭い。
R.S	C B A	C－ビーフ。	かなり良好なブイヨン。
		B－ブイヨン。	味気ない。
		A－魚臭い。	不快。
W.W.	C B A	C－やや良好なローストミート。	非常に豊かなビーフ・ブロス。
		B－イオウ臭く肉様でない。	酸味あり、肉様でない。
		A－臭気なし。	酸味あり、肉の品質なし。
A.T.	C B A	C－優れている。ビーフ様。	顕著に塩味あり、ビーフ様。
		B－イオウ臭い、化学剤様である。	軽度のイオウ臭、甘味－塩味あり。
		A－Bに類似するがさらにイオウ臭い。	酸味あり、塩味あり、イオウ臭あり。
B.T.	C B A	C－ブイヨン。	塩味のある、甘味のあるブイヨンレバー。
		B－弱い。	不快、塩味のある、イオウ臭い。
		A－弱い。	酸味あり、非常に不快。
R.D.	C B A	C－ビーフブイヨン。	ビーフ・ブイヨン、酸味あり。
		B－ポップコーン様。	酸味あり。
		A－イオウ臭い、〃濡れた犬〃の臭い。	非常に酸味あり、不快。

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D.H.	C B A	C―ビーフ様。	塩味のあるビーフブロス。
		B―〃 薄れた犬〃の臭い。	かなり良好なビーフブロス。
		A―古い肉。	薬品臭い、腐つた肉。
Ph.T.	C B A	C―焼肉。	非常に快い肉ブロス。
		B―快い肉ブロス。	酸味あり、軽度の肉ブロス。
		A―薄れた毛髪様と肉ブロス。	僅かに肉様のクエン酸溶液に似ている。
		C―強い典型的なビーフブロス。	HVP の味、幾分鋭い。
		B―弱いがしかし快い。	新鮮でない味、口当りがよい。
		A―非常に弱い。	酸味あり、非常に不快。

A＝水に入れた6970－3Aの20％（V／V）溶液（20㎖を100㎖に稀釈）
B＝水に入れた6970－4Bの3％（W／V）溶液（3.0g を100㎖に溶解）
C＝水に入れた6970－7Cの6％（W／V）溶液（6.0g を100㎖に溶解）
全ての試料は試験期間中50℃の水浴に入れて温めた。

追加の関係

本願発明は特許第602791号（特公昭44－31751号）発明の追加発明であつて、上記原発明に於てはヘキソーゼまたはペントーゼモノサツカライドをシステインと水の存在で混合しビーフの風味を有する混合物が得られるまで加熱し、混合物1重量部に対し植物性蛋白質加水分解物約0.5～1.5部及び5′－リボヌクレオチド約0.5～1.5部を加え、少くとも70℃に約2時間加熱していたが、本願発明はシステインのみならずシスチンも有効であることの発見に基き使用可能アミノ酸の点で原発明の範囲を拡大せるものである。

㊀特許請求の範囲

1　ヘキソーズ又はペントーズ単糖類とシステン又はシスチンの混合物を水の存在で牛肉のフレーバーのする混合物が得られる迄加熱し、前記牛肉のフレーバーのする混合物1重量部当り、シスチンを用いた場合には約3乃至約15重量部の、又システインを用いた場合には約3乃至約5重量部以下の植物蛋白加水分解物と約1.5重量部の5′－リボヌクレオチドとを加え、混合物を少くとも70℃に約2時間加熱することからなる牛肉のフレーバーのする物質の製造方法。

㊁引用文献

米国特許　2934437

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Case No.: IPR2023-00208
U.S. Patent No. 9,943,096

United States Patent Office 3,761,287
Patented Sept. 25, 1973

3,761,287

PROCESS FOR THE MANUFACTURE OF MEAT FLAVORS

Kurt Jaeggi, Dubendorf, Switzerland, assignor to Givaudan Corporation, Clifton, N.J.

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ABSTRACT OF THE DISCLOSURE

A process for the manufacture of flavors and aromas for food by heating a mixture containing a pentose, a polyalcohol, proline, methionine, cysteine and possibly other amino acids.

RELATED APPLICATION

This application claims priority from applicant's copending Swiss application S.N. 19,071/70, filed Dec. 23, 1970.

FIELD OF THE INVENTION

Synthetic meat aromas.

SUMMARY OF THE INVENTION

The present invention relates to synthetic meat aromas. More particularly, the invention is concerned with a process for the manufacture of synthetic meat aromas, with foodstuffs, delicacies and feedstuffs containing said aromas and with a method for the aromatization of foodstuffs, delicacies and feedstuffs by means of said aromas.

According to the process provided by the present invention, synthetic meat aromas are manufactured by heating a charge comprising a pentose, a polyalcohol, and a mixture of amino acids, said mixture containing at least some proline, cysteine, and methionine.

The amino-acids aforesaid can be employed as such or in the form of derivatives or salts thereof which form the free amino-acids under the conditions of the process.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The amino acids charged may comprise not only proline, cysteine and methionine, but may also comprise other amino acids; for example, smaller amounts of the amino acids known to occur in meat, such as, for example, alanine, arginine, cysteine, glycine, histidine, isoleucine, leucine, lysine, serine, threonine, valine, anserine, carnosine or taurine. There may also be utilized the acid salts of these amino acids. Any physiologically acceptable amino acid salts may be utilized, however, the hydrochlorides we deemed especially suitable.

The amount of amino acids present in the mixture expediently amounts to 2 to 40%, preferably 5 to 30 wt. percent. If less than 2 wt. percent of amino acids are present, no powerful aroma is obtained and, indeed, it can even be markedly weak.

If more than 40 wt. percent are present, undesired side-reactions can occur.

According to a preferred embodiment of the present process, proline is employed as the main component of the three amino acids aforesaid. The use of a mixture in which over 50 wt. percent of the amino acid ingredients consists of proline is preferred. On the other hand, however, this amount should not exceed 85 to 90%, since otherwise the aroma which is obtained is reminiscent of bread.

Ribose is preferably employed as the pentose, although other pentoses such as, for example, arabinose or xylose can also be employed. Mixtures of such pentoses can also be employed. The amount of pentose present expediently amounts to 0.4 to 18 wt. percent and preferably 1 to 10 wt. percent.

As polyalcohols there can be employed, for example, trihydric alcohols such as glycerine, or glycols such as, for example, propylene glycol. The polyalcohol can be present in the mixture in an amount of 9 to 90 wt. percent, preferably 40 to 90 wt. percent.

Suitable proportions of the main components of the mixture used in accordance with the present invention are given in the following Table I:

TABLE I

	Percent by weight
Proline	1.0–40
Cysteine	0.1–30
Methionine	0.1–16
Pentose	0.4–18
Polyalcohol	9.0–90

As a rule, the desired meat aroma is formed when the mixture used as the starting material is heated to temperatures of 90°–170° C., in particular to about 110°–130° C. The heating time is normally inversely proportional to the heating temperature; it expediently amounts to about 15 minutes to 2 hours.

Besides the aforementioned main components, there can also be present in the mixture other ingredients such as aroma enhancers (e.g. 0.1–200% monosodium glutamate, 0.1–100% disodium inosine-5'-monophosphate (IMP) and disodium guanosine-5-phosphate (GMP)), fats (e.g. groundnut oil) or special aroma substances (e.g. butter or synthetic butter composition).

The products obtained after the heating are highly concentrated synthetic meat aromas which may be diluted or distributed in edible materials in a manner known per se. They can be converted according to methods which are known per se into the usual forms of presentation such as solutions, pastes or powders. The product can be spray-dried, vacuum-dried or lyophilised. As carrier materials there can be used, for example, gum arabic, salts or brewers yeast or similar adsorbancies. Indoles, maltol, dienals, spice oleoresins or smoke aromas can also be added as flavour-improvers.

The thus formulated products can serve for the aromatization of many kinds of foodstuffs, delicacies and feedstuffs; for example, meat-like products manufactured from natural or synthetic proteins as well as chips etc.

The following Table II gives a basis for suitable concentrations of the synthetic meat aromas in various forms of presentation.

TABLE II

Form of presentation	General, percent	Preferred, percent
Solutions	1–100	10–60
Pastes	1–100	10–60
Spray-dried powder	3–80	15–35
Lyophilised vacuum-dried powder	2–30	5–18

The following examples illustrate the present invention:

Example 1

An intimate mixture containing:

Proline	g.	1.5
Cysteine	g.	1.25
Methionine	g.	0.3
Ribose	g.	0.8
Butter aroma	g.	0.3
Glycerine	ml.	20

is heated with constant stirring to 120° C. held at this temperature for 1 hour. The resulting product has a powerful meat aroma. By dilution with hot water (2 g. to 1 litre), there is obtained a clear broth with a marked meat aroma.

Example 2

An intimate mixture containing:

Proline	g.	16
Cysteine	g.	2.5
Methionine	g.	0.6
Ribose	g.	2.5
Glycerine	ml.	40
Butter	g.	0.2
Groundnut oil	ml.	20
IMP	g.	20

is cautiously heated with stirring to 120° C. and with constant further stirring held at this temperature for 1 hour. Addition of 0.2% of this product to a sauce imparts to the latter a marked aroma of boiled meat.

Example 3

An intimate mixture containing:

Proline	g.	6.0
Cysteine	g.	5.0
Methionine	g.	1.2
Ribose	g.	3.2
Glycerine	ml.	5
Butter	g.	0.6
Guanylic acid	g.	20
GMP	ml.	15

is cautiously heated with stirring to 120° C. and then held at this temperature for 1 hour. The product has a flavour of boiled meat with a fatty note. By mixing this product with 0.1–5%, preferably 6%, of an artificial smoke aroma, there results a product with marked ham note which can be used for aromatizing broths, meat substitutes from protein-containing materials, crackers or potato crisps.

Example 4

An intimate mixture of:

Proline	g.	30
Cysteine	g.	7
Methionine	g.	2
Ribose	g.	7
Glycerine	g.	100
Water	ml.	50

is cautiously heated with stirring to 120° C. and held at this temperature for 1 hour. The product has marked roast beef flavour. It can, for example, be used in amounts of 0.2–4% for imparting the typical roast beef flavour to purely synthetic fibrous meat substitute.

Example 5

An intimate mixture of:

Proline	g.	7
Cysteine	g.	4.0
Methionine	g.	0.4
Xylose	g.	3.3
Glycerine	ml.	50
Butter aroma	g.	0.25
GMP	g.	10
Groundnut oil	ml.	50
Water	ml.	15

is cautiously heated with stirring to 120° C. and held at this temperature for 1 hour. The product can be added to a waffle filling to which it imparts a delicious flavour of roast liver in amounts of about 1.5%.

Example 6

An intimate mixture of:

Proline	g.	28.0
Cysteine	g.	6.6
Methionine	g.	1.6
Ribose	g.	6.8
Glycerine	ml.	57.0
Water	ml.	50.0

is cautiously heated with stirring to 120° C. and held at this temperature for 1 hour. The product has a marked roast beef flavour and can be used for the manufacture of a "dip" for potato crisps.

Example 7

A potato crisp dip is manufactured according to the following process:

	G.
Product of Example 6	5.8
Edible curd	200
Whipped cream	100
Salt	2
Paprika	0.1
Pepper	0.1
Chives (fresh)	1.5

The edible curd is firstly stirred to a fine mass and the whipped cream is cautiously admixed therewith. The spices are added to the resulting homogeneous mass.

I claim:

1. A process for the manufacture of synthetic meat

1. A process for the manufacture of synthetic meat

tures within the range 90–170° C. for about 15 minutes to 2 hours, a mixture comprising a pentose, a polyalcohol and a mixture of amino acids or the physiologically acceptable salts thereof, said mixture of amino acids comprising at least proline, methionine and cysteine, said mixture of amino acids comprising between 2 and 40 wt. percent of the total weight of the reactants.

2. A process according to claim 1, wherein the said amino acids comprise 5 to 30 wt. percent of the reactants.

3. A process according to claim 1, wherein proline, cysteine and methionine are the only amino acids charged.

4. A process according to claim 1, wherein 50 to 90 wt. percent of the amino acid mixture consists of proline.

5. A process according to claim 1, wherein the reactants comprise 1 to 40% proline, 0.1 to 30% cysteine, 0.1 to 16% methionine, 0.4 to 18% of a pentose and 9 to 90% of a polyalcohol.

6. A process according to claim 1, wherein the reactants also comprise water.

7. A process according to claim 1, wherein the reactants are heated to a temperature of between 110° C. and 130° C.

8. A process according to claim 1, wherein said pentose comprises 0.4 to 18 wt. percent of the reactants.

9. A process according to claim 8, wherein said pentose comprises 1 to 10 wt. percent of the reactants.

10. A process according to claim 9, wherein the pentose is ribose.

11. A process according to claim 1, wherein 9 to 90 wt. percent of the reactants comprise a polyalcohol.

12. A process according to claim 11, wherein 40 to 90 wt. percent of the reactants comprise a polyalcohol.

13. A process according to claim 12, wherein the polyalcohol is glycerine.

14. A synthetic meat flavour composition, manufactured according to the process of claim 1.

15. A foodstuff which comprises an olfactible amount of a synthetic meat flavour composition of claim 14.

16. A method for the aromatization of foodstuffs which comprises adding an olfactible amount of a synthetic meat flavour composition of claim 14 to foodstuffs.

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食品、嗜好品の風味付けの方法

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方式審査

明細書

1. 発明の名称
食品、嗜好品の風味付け方法

2. 特許請求の範囲
プロリン、システイン、メチオニン、ペントースおよびポリアルコールを含む混合物を加熱することにより製造した合成の肉アロマを食品又は嗜好品に加えることを特徴とする、食品、嗜好品の風味付け方法。

3. 発明の詳細な説明
本発明は合成の肉アロマより食品又は嗜好品に風味をつける方法に関する。

本発明方法で使用する合成アロマはプロリン、システイン、メチオニン、ペントースおよびポリアルコールを含有する混合物を加熱してつくる。

前記アミノ酸はそのまゝ用いてもよく、又はその工程の条件で遊離アミノ酸を形成するその誘導体もしくは塩の形で用いてもよい。たとえばシステイン塩酸塩をシステインの代りに用いてもよい。プロリン、システインおよびメチオニンの他にこの混合物はその他のアミノ酸、たとえば肉の中に存在することが知られている少量のアミノ酸、たとえばアラニン、アルギニン、シスチン、グリシン、ヒスチジン、イソロイシン、ロイシン、リジン、セリン、スレオニン、バリン、アンセリン、カルノシンもしくはオウリンを含有してもよい。

この混合物中のアミノ酸の量は3ないし40重量%、望ましくは5ないし30重量%であることが便宜である。3重量%以下のアミノ酸では強力な風味が得られず、事実それは非常に高い。40重量%以上では望ましくない副反応が起り得る。

本発明方法の所望の態様によれば、前記三つのアミノ酸の主成分としてプロリンを用いる。そのアミノ酸成分の50重量%以上がプロリンから成る混合物を用いることが望ましい。しかし、一方この量は85ないし90%を越えてはいけない。何となれば、さもないと得られる風味がパンを思い出させることになる。

ペントースとしては、たとえば、アラビノースもしくはキシロースを用いてもよいが、リボース

を用いることができる。これらのペントースの混合物を用いてもよい。ペントースのなかでもリボースおよびキシロース、もしくはリボースおよびアラビノースであることが好ましい。

ポリアルコールとしては、たとえば、三価アルコール、たとえばグリセリン、双目グリコール、たとえばポリエチレングリコールを用いる。その混合物中のポリアルコールの割合は1%ないし9%、望ましくは4%ないし8%である。

本発明によって得られる乳合物の主成分の混合割合を次表に示す。

表1

	重量%
グリセリン	1.0 - 5.5
レシチン	0.1 - 3.0
メチオニン	0.1 - 1.6
ペントース	0.4 - 1.8
ポリアルコール	3.0 - 9.0

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たとえば、アラビアゴム、もしくはビーズ油のごとき油脂もしくはそのエステル類を用いることができる。イソトール、ペントース、ソルビール、多価アルコールおよびこれらの混合物は乳化剤および安定剤として、とくに好ましい。

ポリアルコールおよび乳合物は多くの種類のペプチド、たとえばアミノ酸もしくは合成のキレート化合物と組合せることができ、たとえばチアゾールおよびそのエステルと組合せる。

ロードアミンおよびその誘導体のようなアミノ酸もしくは合成キレート化合物を用いる。

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原料として、お茶のようなカフェイン含有植物を用いる場合、カフェインを除去するのが好ましい。カフェインの除去は熱水抽出前に茶葉に対して行なうのが好ましい。

抽出は、茶葉の重量に対して、たとえば、0.5 - 200重量%の水、好ましくは1 - 100重量%、とくに好ましくは2 - 50重量%の水を用いて行なう。抽出温度は、たとえば、室温（15℃）および沸点（100℃）、好ましくは、高温度（80）以上の温度で行なう。抽出時間は、たとえば、1分ないし2時間、好ましくは3分ないし1時間である。

抽出は、茶葉を水に浸漬するか、または茶葉に水を通すことによって行なう。抽出液は、茶葉から分離する。

抽出液に用いる乳合物は前記したような乳化剤のグループから、それぞれ単独で、または組合せて用いる。これらの乳合物は、好ましくは、たとえば、乳糖、ペントースおよびポリアルコールを含有する。

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例 1

グリセリン	4.5 g
レシチン	1.2 g
メチオニン	0.3 g
リボース	0.8 g
ペントース混合物	0.2 g
ポリオキシエチレン	30 ml

上記組成の乳化剤を含有する乳合物を紅茶抽出液に添加し、その後冷却して、乳化剤を含有する紅茶飲料を得る。

例 2

グリセリン	4 g
レシチン	3.5 g
メチオニン	0.6 g
リボース	2.5 g
ポリオキシエチレン	50 ml
ペクチン	0.2 g
蒸留水	30 ml

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と、新規なヘムの特性をもする生成が得られ、これはプロス、タンパク含有材料のための代用品、クランカーもしくはポテトクリップドル材をつけるのに用いることができる。

例　4

グロリン	3.0g
シスチン	7g
ノナオニン	2g
リボース	9g
グリセリン	100g
水	50ml

上記成分を含有するよく溶ぜんのしそれぞれながら往意深くノ2.0℃に加熱し、その後に1時間保つ。この生成物は新規なヘーストビーフの風味を有する。そのとき、純すいなそばの乳代用品にこの新規なヘーストビーフの香りをえるにはそれを0.2～5%を用いればよい。

例　5

グロリン	9g
システィン	4.0g

ながら往意深くノ2.0℃に加熱し、その後に1時間保つ。この生成物は新規なヘーストビーフの風味を有し、これはポテトクリスプ・フィンガー「ディップ（dip）」の製造に用いることができる。

例　7

ポテトクリスプディップを次のように作る。

本品の生成物	5.8g
食用カード	200g
あわ立てクリーム	100g
塩	2g
パプリーカ	0.1g
こしょう	0.1g
チャイブ（chives）（新鮮なもの）	1.5g

まず食用カードをかき混ぜてクリーム状とし、これにあわ立てクリームを注意深く混ぜよ。つぎに他の一般的な風味香料を加える。

代理人　[illegible]

ミルク	200g

上記成分をよく混ぜた組合物をかきまぜながらおよそノ2.0℃に加熱し、さらにまず1時間なおこの温度に1時間保つ。この生成物0.2%をソースに加えると、そのソースは新鮮なおいしさの風味を示す。

例　3

グロリン	4.0g
システィン	5.0g
ノナオニン	1.2g
リボース	3.2g
グリセリン	1ml
バター	0.4g
ジャガイモ澱	20g
水	15ml

上記成分を含有するよく混ぜた組合物を攪拌しながらおおよそノ2.0℃に加熱し、次にこの温度に1時間保つ。この生成物は肉類の特有をおしたポテトの風味を有する。この生成物を0.1～5%、もしくはそれ以上の人造ベーコン調味料と混ぜる

ノナオニン	0.4g
イノシトール	3.5g
グリセリン	50ml
バター調味料	0.25g
水	10g
牛乳脂	50ml
塩	1.5ml

上記成分を含有するよく混ぜた組合物をかきまぜながらおよそノ2.0℃に加熱し、この温度に1時間保つ。この生成物をマッシュの詰め物に添加すると、それはおよそ1.5%の量でそれにおいしいローストビーフの風味をおえる。

例　4

グロリン	28.0g
システィン	4.2g
ノナオニン	1.8g
リボース	4.8g
グリセリン	37.0ml
水	30.0ml

上記成分を含有するよく混ぜた組合物を加熱し

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6. 添付書類の目録

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

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

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

CROSS-REFERENCE TO RELATED APPLICATIONS

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

TECHNICAL FIELD

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

BACKGROUND

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

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

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

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

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

SUMMARY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DESCRIPTION OF THE DRAWINGS

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

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

DETAILED DESCRIPTION

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

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

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

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

TABLE 1

Flavor Precursor Molecules

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Modulating Flavor and/or Aroma Profiles

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

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

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

TABLE 2

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

yeasty	goaty
vegetable broth	

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

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

TABLE 3

Compounds Produced

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Food Products

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

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

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

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

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

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

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

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

Assessment of Food Products

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

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

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

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

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

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

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

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

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

EXAMPLES

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

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

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

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

TABLE 4

Replica Burger Ingredients

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

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

TABLE 5

Sensory evaluation of replica burger with Heme

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

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

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

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

TABLE 6

Improvement of overall taste with precursors added to LegH burgers

	with precursors	without precursors
Average	3.5	1.8
STDV	0.6	0.5

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

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

TABLE 7

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

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

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

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

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

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

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

TABLE 8

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

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

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

pyrrole	X	X
2-thiophenecarboxaldehyde		X
3-thiophenecarboxaldehyde		X

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Example 9 - Flavor compound production by different Heme-proteins

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

TABLE 9

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

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

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

2-pentyl-thiophene				x

Example 10 - Generation of Meat flavors from different lipids

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

Table 10

Legend showing components of each sample

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

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

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

TABLE 11

Aroma descriptions of each sample after it was cooked.

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

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

Table 12

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Example 11 - Creation of beefy flavors using complex precursor mixtures

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

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

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

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

Table 13

Chemical entities added to the Magic Mix

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

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

TABLE 14

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

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

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

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

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

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

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

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

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

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

TABLE 15

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

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

Preparation of Flavor Reaction Containing Ferrous Chlorin or Leghemoglobin

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

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

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

TABLE 16

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

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

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

Example 13 - Flavor creation by immobilized Hemin

Preparation of hemin linked CM Sepharose.

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

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

Flavor reaction

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

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

TABLE 17

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

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

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

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

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

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

Table 18

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

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

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

OTHER EMBODIMENTS

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

WHAT IS CLAIMED IS:

1. A food product comprising:

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

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

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

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

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

5. A food product comprising:

a) a heme-containing protein; and

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

FIGURE 1

SEQ ID NO:1 *Vigna radiata*

MTTTLERGFTEEQEALVVKSWNVMKKNSGELGLKFFLKIFEIAPSAQKLFSFLRDSTVP
LEQNPKLKPHAVSVFVMTCDSAVQLRKAGKVTVRESNLKKLGATHFRTGVANEHFEVTK
FALLETIKEAVPEMWSPAMKNAWGEAYDQLVDAIKYEMKPPSS

SEQ ID NO:2 *Methylacidiphilum infernorum*

MIDQKEKELIKESWKRIEPNKNEIGLLFYANLFKEEPTVSVLFQNPISSQSRKLMQVLG
ILVQGIDNLEGLIPTLQDLGRRHKQYGVVDSHYPLVGDCLLKSIQEYLGQGFTEEAKAA
WTKVYGIAAQVMTAE

SEQ ID NO:3 *Aquifex aeolicus*

MLSEETIRVIKSTVPLLKEHGTEITARMYELLFSKYPKTKELFAGASEEQPKKLANAII
AYATYIDRLEELDNAISTIARSHVRRNVKPEHYPLVKECLLQAIEEVLNPGEEVLKAWE
EAYDFLAKTLITLEKKLYSQP

SEQ ID NO:4 *Glycine max*

MGAFTEKQEALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLSNGVDPSNPKL
TGHAEKLFGLVRDSAGQLKANGTVVADAALGSIHAQKAITDPQFVVVKEALLKTIKEAV
GDKWSDELSSAWEVAYDELAAAIKKAF

SEQ ID NO:5 *Hordeum vulgare*

MSAAEGAVVFSEEKEALVLKSWAIMKKDSANLGLRFFLKIFEIAPSARQMFPFLRDSDV
PLETNPKLKTHAVSVFVMTCEAAAQLRKAGKITVRETTLKRLGGTHLKYGVADGHFEVT
RFALLETIKEALPADMWGPEMRNAWGEAYDQLVAAIKQEMKPAE

Magnaporthe oryzae, (SEQ ID NO:6)

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

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

Fusarium oxysporum (SEQ ID NO:7)

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

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

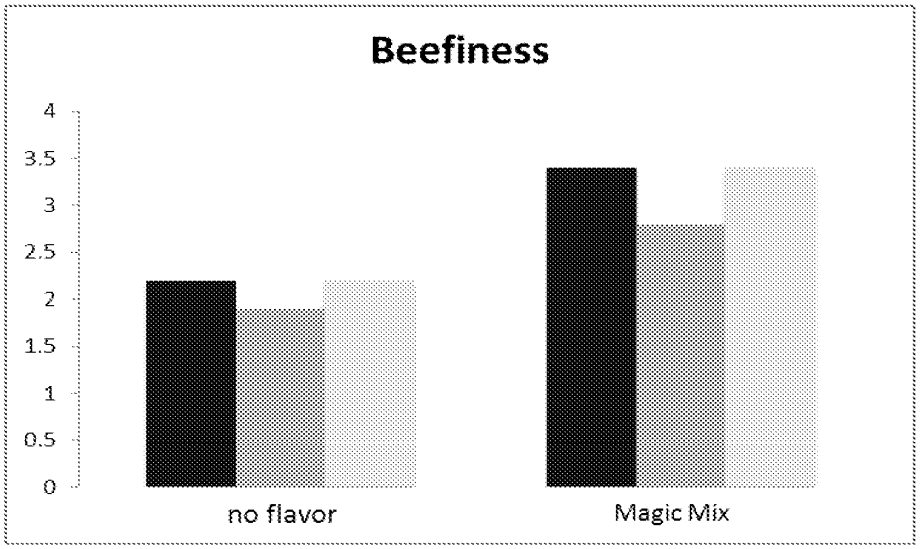
MDVALLEKSFEQISPRAIEFSASFYQNLFHHHPELKPLFAETSQTIQEKKLIFSLAAI
IENLRNPDILQPALKSLGARHAEVGTIKSHYPLVGQALIETFAEYLAADWTEQLA
TAWVEAYDVIASTMIEGADNPAAYLEPELTFYEWLDLYGEESPKVRNAIATLTH
FHYGEDPQDVQRDSRG

SEQ ID NO: 26 Nostoc commune

MSTLYDNIGGQPAIEQVVDELHKRIATDSLLAPVFAGTDMVKQRNHLVAFLAQIF
EGPKQYGGRPMDKTHAGLNLQQPHFDAIAKHLGERMAVRGVSAENTKAALDR
VTNMKGAILNK

SEQ ID NO: 27 Bacillus megaterium
MREKIHSPYELLGGEHTISKLVDAFYTRVGQHPELAPIFPDNLTETARKQKQFLTQ
YLGGPSLYTEEHGHPMLRARHLPFEITPSRAKAWLTCMHEAMDEINLEGPERDE
LYHRLILTAQHMINSPEQTDEKGFSH

FIGURE 2



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Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) Date of publication of the international search report:
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WO 2014/110532 A3

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

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/11347

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A23D 7/005, 9/013; A23L 1/30 (2014.01)
USPC - 426/92, 641, 647
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A23D 7/00, 59/013, 9/00, 9/06, 7/02; A23L 1/30, 1/226, 1/226, 1/217, 1/235 (2014.01)
USPC: 426/613, 506, 535, 808, 537, 92, 540, 96, 644, 643, 641, 647, 250, 646

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google/Google Scholar; PubMed; ProQuest; flavor, taste, precursor, iron, 'heterocycline ring,' heme, phenylacetaldehyde, food, consumable, beef, chicken, pork, lamb, turkey

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2009/0274817 A1 (YAMAGUCHI, S et al.); November 05, 2009: paragraphs [0024], [0029], [0035], [0040], [0045], [0053], [0078]; Table 9	1-10, 35-37, 39 ----- 28-34
Y	US 3829582 A (GUADAGNI, DG et al.); August 13, 1974; abstract; column 1, lines 30-35; Claims 1-3	28-34
Y	US 5443852 A (SHAHIDI, F et al.); August 22, 1995; column 8, lines 10-25; column 8, lines 50-60; column 42, lines 5-15	29-33

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 June 2014 (09.06.2014)	03 JUL 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/11347

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/11347

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

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

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

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

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

The inventions listed as Groups I+-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I+ include a method for imparting a meat-like flavor to a food product comprising contacting said food product with a flavoring composition, wherein after heating said food product and said flavoring composition together, a meat-like flavor is imparted to said food product, which are not present in Group II, the special technical features of Group II including a heme-moiety bound to a solid support.

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

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/11347

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

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

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

Electronic Patent Application Fee Transmittal

Application Number:	15398479
Filing Date:	04-Jan-2017
Title of Invention:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES
First Named Inventor/Applicant Name:	Rachel Fraser
Filer:	M. Angela Parsons/Shannon Stadnick
Attorney Docket Number:	38767-0015002

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt

EFS ID:	30986630
Application Number:	15398479
International Application Number:	
Confirmation Number:	2358
Title of Invention:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES
First Named Inventor/Applicant Name:	Rachel Fraser
Customer Number:	26191
Filer:	M. Angela Parsons/Shannon Stadnick
Filer Authorized By:	M. Angela Parsons
Attorney Docket Number:	38767-0015002
Receipt Date:	17-NOV-2017
Filing Date:	04-JAN-2017
Time Stamp:	15:48:30
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	Transmittal.pdf	70249 426bf16b3c25e1e8069a0b1c587502bb3cac167a	no	1

Warnings:

Information:					
This is not an USPTO supplied IDS fillable form					
2	Information Disclosure Statement (IDS) Form (SB08)	1449.pdf	120895 4b1e70c300b29c518f65923c67a8bb65adfed962	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Foreign Reference	EP0136428.pdf	942655 4c410ad7dad174bece366cc0a4e92798789900f4	no	29
Warnings:					
Information:					
4	Foreign Reference	EP2943072.pdf	5424594 fc51a34a632991d05a8edf293229fcfd5de41017	no	46
Warnings:					
Information:					
5	Foreign Reference	JPS4222194.pdf	342899 165094e6eb3e6cda84973220faca383b5ccdad25	no	3
Warnings:					
Information:					
6	Foreign Reference	JPS4939824.pdf	680694 eaeae23e605de2578962aab9382518e5e3601cd6	no	8
Warnings:					
Information:					
7	Foreign Reference	JPS5163971.pdf	442625 e703bedc23d60499a9ac628547b86bfd801a46fd	no	7
Warnings:					
Information:					
8	Foreign Reference	WO2014110532.pdf	4412492 d43052b1572fe996f2490e2d418ded1a8a333ee9	no	88
Warnings:					

Information:					
9	Non Patent Literature	ESSR_EP15774164_2017_10_27.pdf	380986 9060cbe60b5d6b1e092b8eb63d36ab514c4d6dae	no	11
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Information:					
10	Fee Worksheet (SB06)	fee-info.pdf	30832 7262df74799ba6ee9d6a875c9cd5a30efb1a4c9e	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	12848921	

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Electronic Patent Application Fee Transmittal

Application Number:	15398479
Filing Date:	04-Jan-2017
Title of Invention:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES
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Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
Submission- Information Disclosure Stmt	1806	1	180	180
		Total in USD ($)		**180**

Electronic Acknowledgement Receipt

EFS ID:	30993414
Application Number:	15398479
International Application Number:	
Confirmation Number:	2358
Title of Invention:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES
First Named Inventor/Applicant Name:	Rachel Fraser
Customer Number:	26191
Filer:	M. Angela Parsons/Shannon Stadnick
Filer Authorized By:	M. Angela Parsons
Attorney Docket Number:	38767-0015002
Receipt Date:	20-NOV-2017
Filing Date:	04-JAN-2017
Time Stamp:	09:37:20
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$180
RAM confirmation Number	112017INTEFSW00011855061050
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Fee Worksheet (SB06)	fee-info.pdf	30833 0436349630195f1134194a2ec7c83eae669f189c	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	30833		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
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New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Receipt date: 11/17/2017

Attorney Docket No.: 38767-0015002 / Meat3CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Rachel Fraser	Art Unit	:	1791
Serial No.	:	15/398,479	Examiner	:	Elizabeth A. Gwartney
Filed	:	January 4, 2017	Conf. No.	:	2358
Title	:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES			

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Please consider the references listed on the enclosed PTO-1449 Form. Foreign patent documents and non-patent literature are enclosed; U.S. patents will be provided upon request. Copies of a Communication from a foreign Patent Offices in a counterpart application also is enclosed.

This statement is being filed after a first Office Action on the merits, but before receipt of a Final Office Action or a Notice of Allowance. A submission fee in the amount of $180 as required under 37 CFR § 1.17(p) is being paid herewith. Please apply any other charges, or credits, to Deposit Account No. 06-1050, referencing the above-indicated Attorney Docket Number.

Please contact the undersigned if there are any questions regarding this statement.

Respectfully submitted,

Date: /November 17, 2017/

/M. Angela Parsons/
M. Angela Parsons, Ph.D.
Reg. No. 44,282

Customer Number 26191
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Facsimile: (877) 769-7945

61594507.doc

Receipt date: 11/17/2017

Sheet 1 of 1

Substitute Disclosure Form / U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No.	Application No.
Information Disclosure Statement by Applicant (Use several sheets if necessary) (37 CFR §1.98(b))	38767-0015002	15/398,479
	Applicant: Impossible Foods Inc.	
	Filing Date: January 4, 2017	Group Art Unit: 1791

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
/E.G./	1.	2,934,437	04/26/1960	Morton et al.			
/E.G./	2.	3,394,017	07/23/1968	Giacino et al.			
/E.G./	3.	5,039,543	08/13/1991	Lee et al.			

Foreign Patent Documents or Published Foreign Patent Applications

Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Class	Subclass	Translation Yes	Translation No
/E.G./	4.	EP 0136428	04/10/1985	EPO				
/E.G./	5.	EP 2943072	11/18/2015	EPO				
/E.G./	6.	JP S42-22194	10/31/1942	JAPAN				
/E.G./	7.	JP S49-39824	10/29/1974	JAPAN				
/E.G./	8.	JP S51-63971	10/24/1975	JAPAN			Engl. Tranls.	
/E.G./	9.	WO 2014/110532	07/17/2014	WIPO				

Other Documents (include Author, Title, Date, and Place of Publication)

Examiner Initial	Desig. ID	Document
/E.G./	10.	Supplementary European Search Report in European Application No. 15774164, dated October 27, 2017, 11 pages

Examiner Signature	Date Considered
/ELIZABETH GWARTNEY/	11/18/2017

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form

Receipt date: 09/12/2017

Sheet 1 of 1

Substitute Disclosure Form / U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No.	Application No.
Information Disclosure Statement by Applicant (Use several sheets if necessary) (37 CFR §1.98(b))	38767-0015002	15/398,479
	Applicant: Impossible Foods Inc.	
	Filing Date: January 4, 2017	Group Art Unit: 1791

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
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Foreign Patent Documents or Published Foreign Patent Applications

Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Class	Subclass	Translation Yes	Translation No
	1.	CN 101156632	4/9/2008	CHINA			English Abstract	
/E.G./	2.	CN 101489422	7/22/2009	CHINA			English Abstract	
/E.G./	3.	CN 101861895	10/20/2010	CHINA			English Abstract	
/E.G./	4.	CN 101897418	12/1/2010	CHINA			English Abstract	
/E.G./	5.	CN 102440302	5/9/2012	CHINA			English Abstract	
/E.G./	6.	CN 102578544	7/18/2012	CHINA			English Abstract	
/E.G./	7.	CN 102835460	12/26/2012	CHINA			English Abstract	
/E.G./	8.	JP S53115846	10/9/1978	JAPAN			Machin. Translat.	
/E.G./	9.	JP H08140627	6/4/1996	JAPAN				
/E.G./	10.	JP 2012016336	1/26/2012	JAPAN				

Other Documents (include Author, Title, Date, and Place of Publication)

Examiner Initial	Desig. ID	Document
/E.G./	11.	Naike, "Food Flavor Chemistry," 1st Edition China Light Industry Press, pages 236-243 (1996) (English Translation)
/E.G./	12.	Office Action in Chinese Application No. 201480014349.9, dated July 24, 2017, 52 pages (English Translation)
/E.G./	13.	Office Action in Chinese Application No. 201280041713.1, dated July 13, 2017, 28 pages (English Translation)
/E.G./	14.	Office Action in Chinese Application No. 201480013778.4, dated August 08, 2017, 24 pages (English Translation)
/E.G./	15.	Xiaoling, "Practical Technology and Quality Management of Deep Processing of Meat Products," China Textile & Apparel Press, pages 9 and 10 (English Translation), 10 pages

Examiner Signature	Date Considered
/ELIZABETH GWARTNEY/	11/18/2017

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form

Notice of References Cited	Application/Control No. 15/398,479	Applicant(s)/Patent Under Reexamination Fraser et al.	
	Examiner ELIZABETH A GWARTNEY	Art Unit 1791	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
	A					
	B					
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Proulx et al., "Iron bioavailability of hemoglobin from soy root nodules suing a Caco-2 cell culture model" – J. Agric. Food Chem., 22 February 2006, 54(4), pp. 1518-1522.
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

BIB DATA SHEET

CONFIRMATION NO. 2358

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
15/398,479	01/04/2017	426	1791	38767-0015002

APPLICANTS

Impossible Foods Inc., Redwood City, CA;

INVENTORS

Rachel Fraser, San Francisco, CA;
Patrick O'Reilly Brown, Stanford, CA;
Jessica Karr, San Francisco, CA;
Celeste Holz-Schietinger, East Palo Alto, CA;
Elysia Cohn, Sunnyvale, CA;

**** CONTINUING DATA **************************

This application is a CON of 14/797,006 07/10/2015 PAT 9700067
which is a CON of PCT/US2014/011347 01/13/2014
which is a CON of 13/941,211 07/12/2013
and claims benefit of 61/908,634 11/25/2013
and claims benefit of 61/751,816 01/11/2013

**** FOREIGN APPLICATIONS **************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

01/13/2017

Foreign Priority claimed	☐ Yes ☑ No	☐ Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
35 USC 119(a-d) conditions met	☐ Yes ☑ No		CA	5	30	2
Verified and Acknowledged	/ELIZABETH A GWARTNEY/ Examiner's Signature	Initials				

ADDRESS

FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022
UNITED STATES

TITLE

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

FILING FEE RECEIVED	FEES: Authority has been given in Paper	☐ All Fees
2400	No._____ to charge/credit DEPOSIT ACCOUNT	☐ 1.16 Fees (Filing)
	No._____ for following:	☐ 1.17 Fees (Processing Ext. of time)
		☐ 1.18 Fees (Issue)
		☐ Other _____
		☐ Credit

BIB (Rev. 05/07).

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/398,479	Fraser et al.
	Examiner	**Art Unit**
	ELIZABETH A GWARTNEY	1791

CPC						
Symbol					**Type**	**Version**
A23L	/	27	/	26	F	2016-08-01
A23L	/	13	/	424	I	2016-08-01
A23L	/	13	/	426	I	2016-08-01
A23L	/	27	/	00	I	2016-08-01
A23L	/	27	/	20	I	2016-08-01
A23L	/	33	/	185	I	2016-08-01

CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
	/		/					

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	24	
/ELIZABETH GWARTNEY/ Primary Examiner, Art Unit 1791	20 November 2017	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

U.S. Patent and Trademark Office — Part of Paper No.: 20171117

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/398,479	Fraser et al.
	Examiner	**Art Unit**
	ELIZABETH A GWARTNEY	1791

INTERNATIONAL CLASSIFICATION				
CLAIMED				
A23L	/	27	/	26

NON-CLAIMED				
	/		/	

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 24	
/ELIZABETH GWARTNEY/ Primary Examiner, Art Unit 1791 (Primary Examiner)	20 November 2017 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure 1

U.S. Patent and Trademark Office — Part of Paper No.: 20171117

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/398,479	Fraser et al.
	Examiner	**Art Unit**
	ELIZABETH A GWARTNEY	1791

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
-	1	-	10	-	19	-	28	6	37	13	46	20	55		
-	2	-	11	-	20	-	29	7	38	-	47	21	56		
-	3	-	12	-	21	-	30	8	39	-	48	-	57		
-	4	-	13	-	22	1	31	9	40	14	49	22	58		
-	5	-	14	-	23	-	32	10	41	15	50	23	59		
-	6	-	15	-	24	2	33	-	42	16	51	-	60		
-	7	-	16	-	25	3	34	-	43	17	52	24	61		
-	8	-	17	-	26	4	35	11	44	18	53				
-	9	-	18	-	27	5	36	12	45	19	54				

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 24	
/ELIZABETH GWARTNEY/ Primary Examiner, Art Unit 1791 (Primary Examiner)	20 November 2017 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure 1

U.S. Patent and Trademark Office

Part of Paper No.: 20171117

Applicant-Initiated Interview Summary	**Application No.** 15/398,479	**Applicant(s)** Fraser et al.	
	Examiner ELIZABETH A GWARTNEY	**Art Unit** 1791	**AIA Status** No

All participants (applicant, applicants representative, PTO personnel):

(1) ELIZABETH A. GWARTNEY. (3) Teresa Lavoie.

(2) Ian Lodovice. (4) _____.

Date of Interview: 11 September 2017.

Type: ☑ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☑ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☑112 ☐102 ☐103 ☑Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: 31 and 41..

Identification of prior art discussed: NA.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

In the interview Mr. Lodovice and Ms. Lavoie discussed possible claim amendments to overcome the prior art rejections of record and to further clarify the significant aspects of the invention. No specific agreements were reached.
.

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/ELIZABETH GWARTNEY/ Primary Examiner, Art Unit 1791	

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiners responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicants correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,-
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicants record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiners version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, Interview Record OK on the paper recording the substance of the interview along with the date and the examiners initials.

Examiner-Initiated Interview Summary	**Application No.**	**Applicant(s)**	
	15/398,479	Fraser et al.	
	Examiner	**Art Unit**	**AIA Status**
	ELIZABETH A GWARTNEY	1791	No

All participants (applicant, applicant's representative, PTO personnel):

(1) ELIZABETH A. GWARTNEY. (3) _____.

(2) Ian Lodovice. (4) _____.

Date of Interview: 16 November 2017.

Type: ☑ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☑ No.
If Yes, brief description: _____.

Issues Discussed ☐ 101 ☐ 112 ☐ 102 ☐ 103 ☑ Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: 31 and 41.

Identification of prior art discussed: NA.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

See Continuation Sheet.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/ELIZABETH GWARTNEY/
Primary Examiner, Art Unit 1791

Continuation of Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: In the interview, Mr. Ian Lodovice approved the Examiner's Amendment to claims 41 and 42 set forth in the attached Notice of Allowability.

In a follow-up interview 20 November 2017, Mr Lodovice approved the Examiner's Amendment of claims 47, 48, 57, 58, 59 and 60 in the attached Notice of Allowability.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

26191 7590 12/06/2017
FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

EXAMINER	
GWARTNEY, ELIZABETH A	
ART UNIT	PAPER NUMBER
1791	

DATE MAILED: 12/06/2017

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/398,479	01/04/2017	Rachel Fraser	38767-0015002	2358

TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	03/06/2018

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

26191 7590 12/06/2017
FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/398,479	01/04/2017	Rachel Fraser	38767-0015002	2358

TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$960	$0	$0	$960	03/06/2018

EXAMINER	ART UNIT	CLASS-SUBCLASS
GWARTNEY, ELIZABETH A	1791	426-533000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

❑ Issue Fee

❑ Publication Fee (No small entity discount permitted)

❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

❑ A check is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

❑ Applicant asserting small entity status. See 37 CFR 1.27

❑ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/398,479	01/04/2017	Rachel Fraser	38767-0015002	2358

26191 7590 12/06/2017

FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

EXAMINER	
GWARTNEY, ELIZABETH A	

ART UNIT	PAPER NUMBER
1791	

DATE MAILED: 12/06/2017

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance. Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 15/398,479	Applicant(s) Fraser et al.	
	Examiner ELIZABETH A GWARTNEY	Art Unit 1791	AIA Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to Amendment filed 25 September 2017 .
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____ .

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are See Continuation Sheet . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file areply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☑ Notice of References Cited (PTO-892)
2. ☑ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 20170714; 20170912; 20171117
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____
4. ☑ Interview Summary (PTO-413), Paper No./Mail Date. _____ .
5. ☑ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____ .

/ELIZABETH GWARTNEY/
Primary Examiner, Art Unit 1791

Continuation Sheet (PTOL-37) **Application No. 15/398,479**

Continuation of 3. The allowed claim(s) is/are: 31,33-41,44-46,49-56,58-59 and 61

NOTICE OF ALLOWABILITY

1. **Claims 31, 33-41, 44-46, 49-56, 58, 59 and 61 are allowed.**

2. The previous rejections under 35 U.S.C. 112, first and second paragraph and 35 U.S.C. 102 have been withdrawn in light of Applicants' amendment and remarks filed 25 September 2017.

3. The previous objections to the Specification and Drawings have been withdrawn in light of Applicants amendment to the Specification and Replacement sheet for FIG. 2 filed 25 September 2017.

EXAMINER'S AMENDMENT

4. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in an interview with Ian Lodovice on November 16, 2017 and November 21, 2017.

Claim 41:

A food flavor additive composition prepared by a process of:

a) combining an isolated heme-containing protein; a compound ~~a sugar, sugar alcohol, sugar acid, or a sugar derivative~~ selected from glucose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose and glactose, and mixtures of two or more thereof; and a compound selected from cysteine, cysteine, ~~selenocysteine,~~ thiamine, methionine, and mixtures of two or more thereof, to form a heme flavor reaction mixture, and

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

wherein the food flavoring additive composition contains no animal products ~~no animal tissue derived components~~; and

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

Claim 42: (CANCEL)

Claim 47: (CANCEL)

Claim 48: (CANCEL)

Claim 57: (CANCEL)

Claim 58:

The food flavor additive composition of claim 41, further comprising one or more of an extract or hydrolysate selected from a vegetable protein hydrolysate, a soy protein hydrolysate, a yeast extract, a yeast protein hydrolysate, and an algal protein hydrolysate, and ~~or an albumin~~ or a globulin.

Claim 59:

A process for imparting a meaty flavor and/or meaty aroma to a consumable food product, comprising:

adding to the consumable food product a food flavor additive composition of claim 31[.]; and

heating the consumable food product after the adding step.

Claim 60: (CANCEL)

Reasons for Allowance

5. **The following is an examiner's statement of reasons for allowance:**

While the various pieces of applied prior art disclose compositions comprising animal derived heme-containing protein, sugars and amino acids (see Non-Final Office Action mailed 14 April 2017), the references do not disclose food flavor additive compositions that contain ***no*** animal products as presently claimed.

Amended **claims 31 and 41** require that the flavor additive composition contains ***no*** animal products. The present Specification discloses that any of the inventive food products, including food flavor additives, can be ***free*** of animal products, i.e. contain no animal products. The present Specification also discloses that the food products can be non-animal based. A food flavor additive containing no animal products means that no component or ingredient of the animal product has been sourced from or obtained from an animal source.

Note, while Proulx et al. ("Iron bioavailability of hemoglobin from soy root nodules suing a Caco-2 cell culture model" – *J. Agric. Food Chem.*, 22 February 2006, 54(4), pp. 1518-1522) that hemoglobin from soy root nodules (i.e. non-animal heme-containing protein) is a good source of bioavailable iron and can be used as a food fortificant, there is no motivation to include the fortificant, in combination with the claimed sugars and amino acids to produce meat associated flavors.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ELIZABETH A GWARTNEY whose telephone number is (571)270-3874. The examiner can normally be reached on M-F: 8 a.m. - 4 p.m..

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Donald (Larry) Tarazano can be reached on 571-272-1515. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

ELIZABETH A. GWARTNEY
Primary Examiner
Art Unit 1791

/ELIZABETH GWARTNEY/
Primary Examiner, Art Unit 1791

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/398,479	Fraser et al.
	Examiner	Art Unit
	ELIZABETH A GWARTNEY	1791

CPC - Searched*		
Symbol	Date	Examiner
A23L1/221, 231, 31427 (text limited)	4/10/2017	EG

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
PALM inventor search completed	4/7/2017	EG
Review of search history for 14/797,006	4/7/2017	EG
Google/Google Scholar - composition of blood from cow/cattle, composition of cow muscle, hemoglobin content of cow blood and muscle	4/7/2017	EG
A23L1/221, 231, 31427 (text limited)	4/10/2017	EG
EAST text search - interference	11/19/2017	EG

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
A23L	27/26, 13/424, 13/426	11/19/2017	EG

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	3013	A23L1/231	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/12/14 16:15
S2	3	A23L1/231 AND heme	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/12/14 16:15
S3	1132	A23L1/31427	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/12/14 16:16
S4	20791	A23L1/221	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/12/14 16:16
S5	95	(heme ADJ containing ADJ protein)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/12/14 16:17
S6	1	"14797006"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/12/14 18:04
S7	6	leghemoglobin WITH food	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/12/14 18:59
S8	67	leghemoglobin WITH soybean	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/12/14 19:00
S9	232	leghemoglobin	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/12/14 19:01
S10	155	(soybean WITH monophosphate)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/12/14 19:41
S11	42	(soybean with (meat ADJ substitute))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/12/14 19:43
S12	1632	frozen WITH soybean	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/12/15 08:25
S13	2	frozen WITH soybean WITH (meat WITH substitute)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/12/15 08:26
S14	107	frozen WITH soybean WITH meal	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/12/15 08:26
S15	95	(heme ADJ containing ADJ protein)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2015/12/15 08:36
S16	1	"14797006"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	OFF	2016/05/31 12:28

EAST Search History

			IBM_TDB			
S17	3	A23L1/231 AND heme	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2016/05/31 12:29
S18	913	heme ADJ protein	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2016/05/31 12:30
S19	83	(heme ADJ protein) AND meat	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2016/05/31 12:30
S20	2	"20030152500"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2016/05/31 15:10
S21	44	"9808603"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2016/05/31 16:00
S22	22	"2008069650"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2016/05/31 20:12
S23	8	"2003059778"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2017/03/10 13:20
S24	4	"20120093994"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2017/03/14 15:26
S25	5	A23J1/14 AND heme	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2017/03/15 12:04
S34	3	A23L1/231 AND hemoglobin	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2017/04/10 10:52
S35	1	A23L1/31427 AND heme	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2017/04/10 10:52
S36	8	A23L1/221 AND heme	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2017/04/10 10:53
S40	62	"2934437"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2017/11/18 11:59
S41	40	"3394017"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2017/11/18 11:59
S42	22	"5039543"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	OFF	2017/11/18 12:00

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	6	A23L27/26	US-PGPUB; USPAT	OR	OFF	2017/11/19 09:40
L2	5	A23L13/424	US-PGPUB; USPAT	OR	OFF	2017/11/19 09:41
L3	13	A23L13/426	US-PGPUB; USPAT	OR	OFF	2017/11/19 09:42

EAST Search History

L4	49	("heme-containing").clm.	US-PGPUB; USPAT	OR	OFF	2017/11/19 09:44
S26	0	A23J1/14 AND heme	USPAT	OR	OFF	2017/03/15 12:04
S27	0	A23J1/14 AND leghemoglobin	USPAT	OR	OFF	2017/03/15 12:04
S28	77	A23J1/14	USPAT	OR	OFF	2017/03/15 12:05
S29	260	A23J1/14	US-PGPUB; USPAT	OR	OFF	2017/03/15 12:05
S30	198	leghemoglobin	US-PGPUB; USPAT	OR	OFF	2017/03/15 12:05
S31	260	A23J1/14	US-PGPUB; USPAT	OR	OFF	2017/03/15 12:40
S32	109	A23J3/14	US-PGPUB; USPAT	OR	OFF	2017/03/15 12:40
S33	344	(heme WITH protein).clm.	US-PGPUB; USPAT	OR	OFF	2017/03/15 12:41
S37	8	A23L13/426	US-PGPUB; USPAT	OR	OFF	2017/08/16 15:58
S38	2	A23L27/26 AND heme	US-PGPUB; USPAT	OR	OFF	2017/08/16 15:58
S39	4	A23L13/424	US-PGPUB; USPAT	OR	OFF	2017/08/16 15:59

11/19/2017 9:45:41 AM
C:\Users\egwartney\Documents\EAST\Workspaces\15398479.wsp

Receipt date: 07/14/2017

Substitute Disclosure Form (37 CFR §1.98(b))	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No.	Application No.
Information Disclosure Statement by Applicant (Use several sheets if necessary)		38767-0015002	15/398,479
		Applicant: Impossible Foods Inc.	
		Filing Date: January 4, 2017	Group Art Unit

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
/E.G./	1.	2002/0034570	3/21/2002	Krammer et al.			
/E.G./	2.	2003/0212281	11/13/2003	Sinha et al.			
/E.G./	3.	2006/0204644	9/14/2006	Cavallini et al.			
/E.G./	4.	2008/0254199	10/16/2008	Orcutt et al.			
/E.G./	5.	2010/0196575	8/5/2010	Sanchez			
/E.G./	6.	2010/0310738	12/9/2010	Ludwig			
/E.G./	7.	2015/0296834	10/22/2015	Geistlinger et al.			
/E.G./	8.	2015/0296835	10/22/2015	Anderson et al.			
/E.G./	9.	5,597,594	1/28/1997	Matsuura et al.			

Foreign Patent Documents or Published Foreign Patent Applications

Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Class	Subclass	Translation Yes	Translation No
/E.G./	10.	WO 2013/013292	1/31/2013	WIPO				
/E.G./	11.	WO 2014/110540	7/17/2014	WIPO				
/E.G./	12.	WO 2015/127388	8/27/2015	WIPO				

Other Documents (include Author, Title, Date, and Place of Publication)

Examiner Initial	Desig. ID	Document
/E.G./	13.	Australian Patent Examination Report No. 1 in Australian Application No. 2012281064, dated January 25, 2016, 5 pages
/E.G./	14.	Australian Patent Examination Report No. 1 in Australian Application No. 2012281069, dated September 25, 2015, 5 pages
/E.G./	15.	International Preliminary Report on Patentability in Internation Application No. PCT/US2014/011361, dated July 14, 2015, 13 pages
/E.G./	16.	International Preliminary Report on Patentability in International Application No. PCT/US 2012/046560, mailed 1/23/2014, 12 pages
/E.G./	17.	International Preliminary Report on Patentability in International Application No. PCT/US 2012/46552, mailed 1/23/2014, 9 pages
/E.G./	18.	International Preliminary Report on Patentability in International Application No. PCT/US14/11362, dated July 23, 2015, 10 pages

Examiner Signature	Date Considered
/ELIZABETH GWARTNEY/	11/18/2017

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form

Receipt date: 07/14/2017

Substitute Disclosure Form / U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 38767-0015002	Application No. 15/398,479
Information Disclosure Statement by Applicant (Use several sheets if necessary) (37 CFR §1.98(b))	Applicant: Impossible Foods Inc.	
	Filing Date: January 4, 2017	Group Art Unit:

Other Documents (include Author, Title, Date, and Place of Publication)		
Examiner Initial	Desig. ID	Document
/E.G./	19.	International Search Report and Written Opinion in International Application No. PCT/US 2012/046560, mailed 12/14/2012, 11 pages
/E.G./	20.	International Search Report and Written Opinion in International Application No. PCT/US 2012/46552, mailed 11/19/2012, 12 pages
/E.G./	21.	International Search Report and Written Opinion in International Application No. PCT/US14/11361, dated June 16, 2014, 26 pages
/E.G./	22.	International Search Report and Written Opinion in International Application No. PCT/US14/11362, dated June 13, 2014, 19 pages
/E.G./	23.	International Search Report and Written Opinion in International Application No. PCT/US2015/017147, dated May 01, 2015, 15 pages
/E.G./	24.	International Search Report and Written Opinion in International Application No. PCT/US2015/023679, dated August 28, 2015, 26 pages
/E.G./	25.	Invitation to Pay Fees in International Application No. PCT/US14/11361, dated April 10, 2014, 4 pages

Examiner Signature	Date Considered
/ELIZABETH GWARTNEY/	11/18/2017

EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Substitute Disclosure Form

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/398,479	01/04/2017	Rachel Fraser	38767-0015002	2358

26191 7590 01/02/2018
FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

EXAMINER	
GWARTNEY, ELIZABETH A	

ART UNIT	PAPER NUMBER
1791	

NOTIFICATION DATE	DELIVERY MODE
01/02/2018	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PATDOCTC@fr.com

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Application No. : 15398479
Applicant : Fraser
Filing Date : 01/04/2017
Date Mailed : 01/02/2018

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Notice of Allowance Mailed

This application has been accorded an Allowance Date and is being prepared for issuance. The application, however, is incomplete for the reasons below.

Applicant is given two (2) months from the mail date of this Notice within which to respond. This time period for reply is extendable under 37 CFR 1.136(a) for only TWO additional MONTHS.

The informalities requiring correction are indicated in the attachment(s). If the informality pertains to the abstract, specification (including claims) or drawings, the informality must be corrected with an amendment in compliance with 37 CFR 1.121 (or, if the application is a reissue application, 37 CFR 1.173). Such an amendment may be filed after payment of the issue fee if limited to correction of informalities noted herein. See Waiver of 37 CFR 1.312 for Documents Required by the Office of Patent Publication, 1280 Off. Gaz. Patent Office 918 (March 23, 2004). In addition, if the informality is not corrected until after payment of the issue fee, for purposes of 35 U.S.C. 154(b)(1)(iv), "all outstanding requirements" will be considered to have been satisfied when the informality has been corrected. A failure to respond within the above-identified time period will result in the application being ABANDONED.

See attachment(s).

A copy of this notice MUST be returned with the reply. Please address response to "Mail Stop Issue Fee, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450".

/Marsha Palmer/
Publication Branch
Office of Data Management
(571) 272-4200

Application No. 15398479

NON-COMPLIANT ILLUSTRATIONS IN SPECIFICATION OR CLAIMS

☐ **The illustration(s) in the location(s) referenced below do not come within the exceptions of 37 CFR 1.58(a). Please delete the illustration(s) from the specification or claims, provided the illustration(s) as part of the formal drawing(s) in accordance with 37 CFR 1.84, and amend the specification to provide a brief description of the drawings in accordance with 37 CFR 1.74. Please see the following item(s):**

- ☐ Specification document coded dated , page(s) .
- ☐ Claims document coded dated , claim(s) .
- ☐ Amendment document coded dated .
- ☐ Other: .

☒ **The chemical formula(s), mathematical formula(s), or table(s) in the location(s) referenced below contain shading (color/grayscale) and therefore do not comply with 37 CFR 1.52 and do not come within the exceptions of 37 CFR 1.58(a). Please delete chemical formula(s), mathematical formula(s), or table(s) with shading from the specification or claims, provide them as formal color drawing(s) in accordance with 37 CFR 1.84, including petition, fee, and amendment in accordance with 37 CFR 1.84(a)(2), and amend the specification to provide a brief description of the drawings in accordance with 37 CFR 1.74. Please see the following item(s):**

- ☒ Specification document coded SPEC dated 01/04/2017, page(s) 29,30,35,43-49,62-64.
- ☐ Claims document coded dated , claim(s) .
- ☐ Amendment document coded dated .
- ☐ Other: .

Attorney's Docket No.: 38767-0015002 / Meat3CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Rachel Fraser	Art Unit	:	1791
Serial No.	:	15/398,479	Examiner	:	Elizabeth A. Gwartney
Filed	:	January 4, 2017	Conf. No.	:	2358
Title	:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES			

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REPLY TO NOTICE TO FILE CORRECTED APPLICATION PAPERS MAILED JANUARY 2, 2018

Applicant, as a Large Entity, submits herewith a Substitute Specification in compliance with 37 CFR §1.52. As requested in the Notice to File Corrected Application Papers mailed January 2, 2018, shading in the Tables has been removed. No new matter has been added.

Applicant understands that this perfects the application and no additional papers or filing fees are required. If this is incorrect, please apply any charges, or credits, to Deposit Account No. 06-1050, referencing the above-indicated Attorney Docket Number.

Respectfully submitted,

Date: /January 18, 2018/

/M. Angela Parsons/
M. Angela Parsons, Ph.D.
Reg. No. 44,282

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

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Application No. 14/797,006 filed on July 10, 2105 which is a continuation of and claims priority to PCT/US2014/011347 which claims priority to U.S. Application Serial No. 13/941,211, filed July 12, 2013, U.S. Application Serial No. 61/908,634, filed November 25, 2013, and to U.S. Application Serial No. 61/751,816, filed January 11, 2013, and is related to the following co-pending patent applications: Application Serial No. PCT/US12/46560; Application Serial No PCT/US12/46552; Application Serial No. 61,876,676, filed September 11, 2013; and Application Serial No. 61/751,818, filed January 11, 2013, all of which are incorporated herein by reference.

TECHNICAL FIELD

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

BACKGROUND

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

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

In most cases, the plant or animal food source is fractionated into a variety of different portions, depending upon the purpose of the food. Often, certain portions of the

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

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

SUMMARY

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

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

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

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

amount of iron complex (e.g., a heme cofactor such as a heme-containing protein), the temperature of the reaction, and the amount of water activity in the product, among other factors.

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

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

Consumable food products for use in the present disclosure can be flavor additive compositions, e.g., for addition to another consumable food product before, during, or after its cooking process. A flavor additive composition can include an iron complex

such as a heme-cofactor (e.g., a heme-containing protein), and one or more flavor precursors.

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

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

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

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

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

The heme-containing protein can be isolated and purified. The food product further can include a food-grade oil, a seasoning agent, a flavoring agent, a protein, a protein concentrate, an emulsifier, a gelling agent, or a fiber. The food product can be a meat substitute, a soup base, stew base, snack food, bouillon powder, bouillon cube, a flavor packet, or a frozen food product. Any of the food products can be free of animal products. The food product can be sealed within a packet or shaker.

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

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

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

proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and heating the mixture to form one or more flavor compounds set forth in Tables 3, 8, or 9. For example, the flavor precursors can include cysteine, a sugar, and one or more other precursors.

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

In another aspect, this document features a method of making a food product. The method includes combining an isolated heme-containing protein and one or more flavor precursor molecules to form a mixture, the one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-

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

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

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

DESCRIPTION OF THE DRAWINGS

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

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

DETAILED DESCRIPTION

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

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

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

TABLE 1

Flavor Precursor Molecules

Sugars, sugar alcohols, sugar acids, and sugar derivatives: glucose, fructose, ribose, sucrose, arabinose, glucose-6-phosphate,fructose-6-phosphate, fructose 1,6-diphosphate, inositol, maltose, molasses, maltodextrin, glycogen, galactose, lactose, ribitol, gluconic acid and glucuronic acid, amylose, amylopectin, or xylose
Oils: coconut oil, mango oil, sunflower oil, cottonseed oil, safflower oil, rice bran oil, cocoa butter, palm fruit oil, palm oil, soybean oil, canola oil, corn oil, sesame oil, walnut oil, flaxseed, jojoba oil, castor, grapeseed oil, peanut oil, olive oil, algal oil, oil from bacteria or fungi
Free fatty acids: caprylic acid, capric acid, lauric acid, myristic acid, palmititic acid, palmitoleic acid, stearic, oleic acid, linoleic acid, alpha linolenic acid, gamma linolenic acid, arachidic acid, arachidonic acid, behenic acid, or erucic acid
Amino acids and derivatives thereof: cysteine, cystine, a cysteine sulfoxide, allicin, selenocysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, 5-hydroxytryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, or tyrosine
Nucleosides and Nucleotides: inosine , inosine monophosphate (IMP), guanosine, guanoside monophosphate (GMP), adenosine, adenosine monophosophate (AMP)
Vitamins: thiamine, vitamin C, Vitamin D, Vitamin B6, or Vitamin E
Misc: phospholipid, lecithin, pyrazine, creatine, pyrophosphate
Acids: acetic acid, alpha hydroxy acids such as lactic acid or glycolic acid, tricarboxylic acids such as citric acid, dicarboxylic acids such as succinic acid or tartaric acid
Peptides and protein hydrolysates: glutathione, vegetable protein hydrolysates, soy protein hydrolysates, yeast protein hydrolysates, algal protein hydrolysatess,

meat protein hydrolysates
Extracts: a malt extract, a yeast extract, and a peptone

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

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

stored, handled or prepared for consumption. The life-time of the unoxygenated Fe^{2+} state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption

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

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

Bacillus megaterium, Synechocistis sp., Aquifex aeolicus, Methylacidiphilum infernorum, or thermophilic bacteria such as *Thermophilus*. The sequences and structure of numerous heme-containing proteins are known. See for example, Reedy, *et al.*, *Nucleic Acids Research*, 2008, Vol. 36, Database issue D307–D313 and the Heme Protein Database available on the world wide web at http://hemeprotein.info/heme.php.

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

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

NO:23), Synechocystis PCC6803 (SEQ ID NO:24), Synechococcus sp. PCC 7335 (SEQ ID NO:25), or *Nostoc commune* (SEQ ID NO:26).

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

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

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

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

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

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

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

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

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

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

Modulating Flavor and/or Aroma Profiles

As described herein, different combinations of flavor precursors can be used with one or more iron complexes (e.g., a ferrous chlorin, a chlorin-iron complex, or a heme-cofactor such as a heme-containing protein or heme bound to a non-peptidic polymer such as polyethylene glycol or to a solid support) to produce different flavor and aroma profiles when the flavor precursors and iron complexes are heated together (e.g., during cooking). The resultant flavor and/or aroma profile can be modulated by the type and concentration of the flavor precursors, the pH of the reaction, the length of cooking, the

type and amount of iron complex (e.g., a heme-cofactor such as heme-containing protein, heme bound to non-peptidic polymer or macromolecule, or heme bound to a solid support), the temperature of the reaction, and the amount of water activity in the product, among other factors. In embodiments in which a heme moiety is bound to a solid support such as cellulose or a chromatography resin, graphite, charcoal, or diatomaceous earth, the solid support (e.g., beads) can be incubated with sugars and/or one or more other flavor precursors to generate flavors, and then the solid support with attached heme moiety can be re-used, i.e., incubated again with sugars and/or one or more other flavor precursors to generate flavors.

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

TABLE 2

Flavor Types	
beef	beef broth
beef dripping	cheesy
cold-cut deli meat	squash
bacon	sharp
meaty	fruity
brothy	floral
ramen	musty
egg	fried food
malty	caramel
bready	barbeque
sulfur	chocolate
fried chicken	sweet
browned	potato
pretzel	french toast

grassy	breadcrust
bloody	mushroom
broccoli	chicken
brothy	cumin
buttery	umami
metallic	raisin
yeasty	goaty
vegetable broth	

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

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

TABLE 3

Compounds Produced

phenylacetaldehyde	2-butenal, 2-ethyl-	1,3-hexadiene
1-octen-3-one	acetonitrile	4-decyne
2-n-heptylfuran		pentanal
2-thiophenecarboxaldehyde	(E)-2-Hexenal	1-propanol
3-thiophenecarboxaldehyde	4-ethyl-phenol,	heptanoic acid
1-octene	3-octanone	ethanethiol
butyrolactone	styrene	2-methyl-1-heptene
2-undecenal	furan, 3-pentyl-	(E)-4-octene
propyl-cyclopropane	formic acid, heptyl ester	2-methyl-2-heptene
methyl-pyrazine	(E)-2-Heptenal	pentanoic acid
1-hydroxy-propanone	6-methyl-5-hepten-2-one	nonanoic acid
acetic acid	n-caproic acid vinyl ester	1,3-dimethyl-benzene
furfural	2-ethyl-2-hexenal	
2-decanone	1-hepten-3-ol	toluene

pyrrole	1-ethyl-1-methyl-cyclopentane	1-butanol
1-octen-3-ol	3-ethyl-2-methyl-1,3-hexadiene	2,3,3-trimethyl-pentane
2-acetylthiazole	2-pentyl-thiophene	isopropyl alcohol
(E)-2-octenal	(Z)-2-nonenal	2,2,4,6,6-pentamethyl-heptane
decanal	2-n-octylfuran	phenol
benzaldehyde	2-hexyl-thiophene	1-penten-3-one
(E)-2-Nonenal	4-cyclopentene-1,3-dione	dimethyl sulfide
pyrazine	1-nonanol	thiirane
1-pentanol	(E)-2-decenal	(E)-2-octen-1-ol
trans-2-(2-pentenyl)furan	4-ethyl-benzaldehyde	2,4-dimethyl-1-heptene
1-hexanol	1,7-octadien-3-ol	1,3-bis(1,1-dimethylethyl)-benzene
1-heptanol	octanoic acid	heptane
dimethyl trisulfide	2-ethyl-5-methyl-pyrazine	4,7-dimethyl-undecane
2-nonanone	3-ethyl-2,5-dimethyl-pyrazine	acetophenone
2-pentanone	1,3,5-cycloheptatriene	tridecane
2-heptanone	2-ethyl-1-hexanol	thiophosphoramide, s-methyl ester
2,3-butanedione	4-methyl-octanoic acid	2-methyl-thiazole
heptanal	m-aminophenylacetylene	3-(1-methylethoxy)-propanenitrile,
nonanal	benzene	2,4-bis(1,1-dimethylethyl)-phenol
2-octanone	thiophene	3-ethyl-2,2-dimethyl-pentane
2-butanone	2-methyl-furan	3-ethyl-pentane
octanal	pyridine	2,3,4-trimethyl-pentane
1-octanol	furan	2,4,6-trimethyl-octane
3-ethylcyclopentanone	butanal	2,6-dimethyl-nonane
8-methyl-1-undecene	2-ethyl-furan	2-hexyl-furan
3-octen-2-one	carbon disulfide	4-methyl-5-thiazoleethanol
2,4-Heptadienal, (E,E)-	Furan, 2-hexyl-:2	4-penten-2-one
(Z)-2-heptenal	3-methyl-butanal	4-methylthiazole
6-methyl-2-heptanone	2-methyl-butanal	2-methyl-3-pentanone
(Z)-4-heptenal	methacrolein	2,3-pentanedione
(E,Z)-2,6-nonadienal	octane	(E)-2-tridecen-1-ol

3-methyl-2-butenal	ethanol	2-thiophenemethanamine
2-pentyl-furan	2-methyl-propanal	(Z)-2-nonenal,
thiazole	acetone	methyl thiolacetate
(E,E)-2,4-decadienal	propanal	methyl ethanoate
hexanoic acid	methyl-thiirane	isothiazole
1-ethyl-5-methylcyclopentene	acetaldehyde	3,3-dimethyl-hexane
(E,E)-2,4-nonadienal	2-propenal	4-methyl-heptane
(Z)-2-decenal	2-propyl-furan	2,4-dimethyl-heptane
dihydro-5-pentyl-2(3h)-furanone	dihydro-5-propyl-2(3H)-furanone	2,3,4-trimethyl-heptane
trans-3-nonen-2-one	dihydro-3-(2H)-thiophenone	2-methyl-heptane
(E,E)-3,5-octadien-2-one	2,2,6-trimethyl-decane	2-methyl-3-furanthiol
(Z)-2-octen-1-ol	3,3'-dithiobis[2-methyl-furan	4-amino-1,2,5-oxadiazole-3-carbonitrile
5-ethyldihydro-2(3h)-furanone	1-heptene	1,2-benzisothiazol-3(2H)-one
2-butenal	1,3-octadiene	2-acetyl-propen-2-ol,
1-penten-3-ol	1-nonene	1-decen-3-one
1-(ethylthio)-2-(methylthio)-buta-1,3-diene		

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

In some embodiments, an iron complex (e.g., a ferrous chlorin or a heme-cofactor such as a heme-containing protein) described herein is heated in the presence of cysteine and glucose or other combinations of flavor precursors to provide a different profile of volatile odorants than when any subset of the three components are used individually. Volatile flavor components that are increased under these conditions include but are not limited to furan, acetone, thiazole, furfural, benzaldehyde, 2-pyridinecarboxaldehyde, 5-

methyl-2-thiophenecarboxaldehyde, 3-methyl-2-thiophenecarboxaldehyde, 3-thiophenemethanol and decanol. See, e.g., Tables 8 and 9. Under these conditions, cysteine and glucose alone or in the presence of iron salts such as ferrous glucanate produced a sulfurous, odor, but addition of heme-containing proteins reduced the sulfurous odor and replaced it with flavors including but not limited to chicken broth, burnt mushroom, molasses, and bread.

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

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

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

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

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

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

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

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

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

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

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

Food Products

Food products containing one or more flavor precursors and one or more heme-containing proteins can be used as a base for formulating a variety of additional food

products, including meat substitutes, soup bases, stew bases, snack foods, bouillon powders, bouillon cubes, flavor packets, or frozen food products. Meat substitutes can be formulated, for example, as hot dogs, burgers, ground meat, sausages, steaks, filets, roasts, breasts, thighs, wings, meatballs, meatloaf, bacon, strips, fingers, nuggets, cutlets, or cubes.

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

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

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

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

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

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

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

Assessment of Food Products

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

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

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

In some embodiments, volatile chemicals identified using GCMS can be evaluated. For example, a human can rate the experience of smelling the chemical

responsible for a certain peak. This information could be used to further refine the profile of flavor and aroma compounds produced using a heme-containing protein and one or more flavor precursors.

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

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

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

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

EXAMPLES

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

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

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

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

TABLE 4

Replica Burger Ingredients

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

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

TABLE 5

Sensory evaluation of replica burger with Heme

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

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

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

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

TABLE 6

Improvement of overall taste with precursors added to LegH burgers

	with precursors	without precursors
Average	3.5	1.8
STDV	0.6	0.5

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

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

TABLE 7

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

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

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

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

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

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

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

TABLE 8

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

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

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

pyrrole	X	X
2-thiophenecarboxaldehyde		X
3-thiophenecarboxaldehyde		X

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Example 9 - Flavor compound production by different Heme-proteins

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

TABLE 9

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

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

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

2-pentyl-thiophene				x

Example 10 - Generation of Meat flavors from different lipids

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

Table 10

Legend showing components of each sample

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

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

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

TABLE 11

Aroma descriptions of each sample after it was cooked.

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

MRM_Biolipon95	fatty, brothy, hay, malty green
KPhos6_NatCholinePC 40	**light fatty, fresh**
MRM_NatCholinePC40	**fatty, beef tallow, brothy**
K-Phos6_C14	light/faint plastic/waxy
MRM_C14	brothy, beefy, minty, fresh
K-Phos6_C18	**light/faint plastic/waxy**
MRM_C18	**beefy with cucumber &/or pepper aroma**
K-Phos6_Canola	fresh, cucumber
MRM_Canola	fatty, brothy, oil, roasted nuts
K-Phos6_Coconut	**nothing**
MRM_Coconut	**brothy, beefy, slight fatty, crackers**
K-Phos6_Oleic Acid	fresh, cucumber, camphorous/minty-like
MRM_Oleic Acid	herbal, plastic, slight cheesy, brothy
K-Phos6_Linoelic Acid	**light plastic**
MRM_Linoelic Acid	**fatty, light waxy, brothy, herbal**

Table 12

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Example 11 - Creation of beefy flavors using complex precursor mixtures

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

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

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

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

Table 13

Chemical entities added to the Magic Mix

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

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

TABLE 14

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

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

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

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

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

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

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

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

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

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

TABLE 15

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

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

Preparation of Flavor Reaction Containing Ferrous Chlorin or Leghemoglobin

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

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

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

TABLE 16

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

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

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

Example 13 - Flavor creation by immobilized Hemin

Preparation of hemin linked CM Sepharose.

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

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

Flavor reaction

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

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

TABLE 17

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

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

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

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

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

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

Table 18

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

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

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

OTHER EMBODIMENTS

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

Electronic Acknowledgement Receipt

EFS ID:	31533626
Application Number:	15398479
International Application Number:	
Confirmation Number:	2358
Title of Invention:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES
First Named Inventor/Applicant Name:	Rachel Fraser
Customer Number:	26191
Filer:	M. Angela Parsons/Judith Witzel
Filer Authorized By:	M. Angela Parsons
Attorney Docket Number:	38767-0015002
Receipt Date:	18-JAN-2018
Filing Date:	04-JAN-2017
Time Stamp:	11:05:17
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Post Allowance Communication - Incoming	RespNFCAPs.pdf	51616 62b97577a7fa1c9a5f80ee31c6aa3a0dd92e4ba6	no	1

Warnings:

Information:					
2	Specification	Specification_clean.pdf	410435 0ccef2f531e9d4a18fb230e1ec1c5f4bc5f47f94	no	66
Warnings:					
Information:					
			Total Files Size (in bytes):	462051	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	15/398,479	Fraser et al.	
	Examiner	Art Unit	AIA Status
	ELIZABETH A GWARTNEY	1791	No

All participants (applicant, applicant's representative, PTO personnel):

(1) ELIZABETH A. GWARTNEY. (3) _____.

(2) Ian Lodovice. (4) _____.

Date of Interview: 09 January 2018.

Type: ☑ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☐ No.
If Yes, brief description: _____.

Issues Discussed ☐ 101 ☐ 112 ☐ 102 ☐ 103 ☑ Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: 44.

Identification of prior art discussed: NA.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

In the interview, Mr. Ian Lodovice approved the Examiner's Amendment set forth in the Supplemental Notice of Allowability..

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/ELIZABETH GWARTNEY/ Primary Examiner, Art Unit 1791	

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/398,479	Fraser et al.
	Examiner	**Art Unit**
	ELIZABETH A GWARTNEY	1791

CPC						
Symbol					**Type**	**Version**
A23L	/	27	/	26	F	2016-08-01
A23L	/	13	/	424	I	2016-08-01
A23L	/	13	/	426	I	2016-08-01
A23L	/	27	/	00	I	2016-08-01
A23L	/	27	/	20	I	2016-08-01
A23L	/	33	/	185	I	2016-08-01

CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
	/		/					

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 24	
/ELIZABETH GWARTNEY/ Primary Examiner, Art Unit 1791 (Primary Examiner)	(Date)	O.G. Print Claim(s) 1	O.G. Print Figure 1

U.S. Patent and Trademark Office — Part of Paper No.: 20180109

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/398,479	Fraser et al.
	Examiner	**Art Unit**
	ELIZABETH A GWARTNEY	1791

INTERNATIONAL CLASSIFICATION				
CLAIMED				
A23L	/	27	/	26

NON-CLAIMED				
	/		/	

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE (Assistant Examiner) (Date)	**Total Claims Allowed:** 24	
/ELIZABETH GWARTNEY/ Primary Examiner, Art Unit 1791 (Primary Examiner) (Date)	O.G. Print Claim(s) 1	O.G. Print Figure 1

U.S. Patent and Trademark Office — Part of Paper No.: 20180109

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/398,479	Fraser et al.
	Examiner	**Art Unit**
	ELIZABETH A GWARTNEY	1791

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
-	1	-	10	-	19	-	28	6	37	13	46	20	55		
-	2	-	11	-	20	-	29	7	38	-	47	21	56		
-	3	-	12	-	21	-	30	8	39	-	48	-	57		
-	4	-	13	-	22	1	31	9	40	14	49	22	58		
-	5	-	14	-	23	-	32	10	41	15	50	23	59		
-	6	-	15	-	24	2	33	-	42	16	51	-	60		
-	7	-	16	-	25	3	34	-	43	17	52	24	61		
-	8	-	17	-	26	4	35	11	44	18	53				
-	9	-	18	-	27	5	36	12	45	19	54				

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 24	
/ELIZABETH GWARTNEY/ Primary Examiner, Art Unit 1791 (Primary Examiner)	(Date)	O.G. Print Claim(s) 1	O.G. Print Figure 1

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Part of Paper No.: 20180109



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/398,479	01/04/2017	Rachel Fraser	38767-0015002	2358

26191 7590 01/24/2018
FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MINNESOTA 55440-1022

EXAMINER	
GWARTNEY, ELIZABETH A	

ART UNIT	PAPER NUMBER
1791	

NOTIFICATION DATE	DELIVERY MODE
01/24/2018	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PATDOCTC@fr.com

PTOL-90A (Rev. 04/07)

Supplemental Notice of Allowability	**Application No.** 15/398,479	**Applicant(s)** Fraser et al.	
	Examiner ELIZABETH A GWARTNEY	**Art Unit** 1791	**AIA Status** No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to Amendment filed 25 Septemeber 2017 .
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____ .

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are See Continuation Sheet . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file areply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____
4. ☑ Interview Summary (PTO-413), Paper No./Mail Date. 20180109 .
5. ☑ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____ .

/ELIZABETH GWARTNEY/
Primary Examiner, Art Unit 1791

Continuation Sheet (PTOL-37) **Application No. 15/398,479**

Continuation of 3. The allowed claim(s) is/are: 31,33-41,44-46,49-56,58-59 and 61

Supplemental Notice of Allowability

1. This is a Supplemental Notice of Allowability (*see* Examiner's Amendment set forth below).

2. **Claims 31, 33-41, 44-46, 49-56, 58, 59 and 61 are allowed.**

EXAMINER'S AMENDMENT

3. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in an interview with Mr. Ian Lodovice on 9 January 2018.

The application has been amended as follows:

Claim 44:

A food flavor additive composition of claim ~~42~~ 41, wherein the food flavoring wherein the food flavoring additive composition further comprises a compound selected from the group consisting of 2-methyl-furan, bis(2-methyl-3-furyl)disulfide, 2-pentyl-furan, 3,3'-dithiobis-2-methyl-furan, 2,5-dimethyl-pyrazine, 2-methyl-3-furanthiol, bis(2-methyl-4,5-dihydro-3-furyl) disulfide, dihydro-3-(2H)-thiophenone, 5-methyl-2-thiophenecarboxaldehyde, 3-methyl-2-thiophenecarboxaldehyde, 2-methyl-thiazole, dimethyl sulfide, decanal, 5-ethyldihydro-2(3H)-furanone, dihydro-5-pentyl-2(3H)-furanone, 2-octanone, 3,5-octadien-2-one, p-Cresol, and hexanoic acid.

Conclusion

4. Any inquiry concerning this communication or earlier communications from the examiner should be directed to ELIZABETH A GWARTNEY whose telephone number is (571)270-3874. The examiner can normally be reached on M-F: 8 a.m. - 4 p.m..

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Donald (Larry) Tarazano can be reached on 571-272-1515. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

ELIZABETH A. GWARTNEY
Primary Examiner
Art Unit 1791

/ELIZABETH GWARTNEY/
Primary Examiner, Art Unit 1791

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/398,479	01/04/2017	Rachel Fraser	38767-0015002	2358

26191 7590 01/25/2018
FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

EXAMINER	
GWARTNEY, ELIZABETH A	

ART UNIT	PAPER NUMBER
1791	

NOTIFICATION DATE	DELIVERY MODE
01/25/2018	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

PATDOCTC@fr.com

PTOL-90A (Rev. 04/07)

Response to Rule 312 Communication	Application No. 15/398,479	Applicant(s)
	Examiner	Art Unit

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address –

1. ☒ The amendment filed on *18 January 2018* under 37 CFR 1.312 has been considered, and has been:

a) ☒ entered.

b) ☐ entered as directed to matters of form not affecting the scope of the invention.

c) ☐ disapproved because the amendment was filed after the payment of the issue fee.
Any amendment filed after the date the issue fee is paid must be accompanied by a petition under 37 CFR 1.313(c)(1) and the required fee to withdraw the application from issue.

d) ☐ disapproved. See explanation below.

e) ☐ entered in part. See explanation below.

Charles Bowen

Publishing Division

Attorney's Docket No.: 38767-0015002 / Meat3CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Rachel Fraser	Art Unit	: 1791
Serial No.	:	15/398,479	Examiner	: Elizabeth A. Gwartney
Filed	:	January 4, 2017	Confirmation No.	: 2358
			Notice of Allowance Date: December 6, 2017	
Title	:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES		

MAIL STOP ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REPLY TO NOTICE OF ALLOWANCE

In response to the Notice of Allowance mailed December 6, 2017, enclosed is a completed Part B - Fee(s) Transmittal.

It is recognized that in accordance with MPEP § 1302.14, the Examiner's reasons for allowance need not set forth all of the details as to why the claims are allowed. In the above-referenced application, it is not conceded that the Examiner's stated reasons for allowance are the only reasons for which the claims are allowable. The Examiner's reasons for allowance indicate that particular claim elements are not disclosed or suggested by the prior art of record, yet the claims may be patentable for other reasons as well, including the inventive combination of all of the recited claim elements. It is not conceded that the specific limitations identified by the Examiner are necessary to distinguish the art of record or to satisfy the requirements of 35 U.S.C. § 112. Moreover, the Examiner does not assert, and it would not be conceded, that the Examiner's reasons have any bearing on the patentability of claims in any other applications directed to the disclosed subject matter.

In addition, each dependent claim stands on its own and may be allowable on its own merits. In particular, each dependent claim may be allowable on the basis of a combination of some of the features recited in the dependent claim and its base claim(s), which combination of features may not include all of the limitations identified in the Examiner's reasons for allowance.

The Issue Fee is being paid with this reply via the Electronic Filing System (EFS). Please apply those fees and any other charges, or credits, to Deposit Account No. 06-1050, referencing the above-indicated Attorney Docket Number.

Respectfully submitted,

Date: /March 6, 2018/

/M. Angela Parsons/

M. Angela Parsons, Ph.D.
Reg. No. 44,282

Customer Number 26191
Fish & Richardson P.C.
Telephone: (612) 335-5070
Facsimile: (877) 769-7945

61645216.doc

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

26191 7590 12/6/2017

FISH & RICHARDSON P.C.
P.O. BOX 1022
MINNEAPOLIS, MN 55440-1022

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/398,479	01/04/2017	Rachel Fraser	38767-0015002	2358

TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	LARGE	$1000	$0		$1000	03/06/2018

EXAMINER	ART UNIT	CLASS-SUBCLASS
GWARTNEY, ELIZABETH A.	1791	426-533000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

[ ] Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

[ ] "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Fish & Richardson P.C.
2
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: Impossible Foods Inc.

(B) RESIDENCE: (CITY and STATE OR COUNTRY): Redwood City, CA

Please check the appropriate assignee category or categories (will not be printed on the patent): [ ] Individual [X] Corporation or other private group entity [ ] Government

4a. The following fee(s) are submitted:

[X] Issue Fee

[ ] Publication Fee (No small entity discount permitted)

[ ] Advance Order - # of Copies _____

4b. Payment of Fee(s): (**Please first reapply any previously paid issue fee shown above**)

[ ] A check in the amount of the fee(s) is enclosed.

[ ] Payment by credit card. Form PTO-2038 is attached.

[X] The Director is hereby authorized to charge the required fee(s), or credit any overpayment, to Deposit Account Number 06-1050

5. **Change in Entity Status** (from status indicated above)

[ ] Applicant certifying micro entity status. See 37 CFR 1.29 — **NOTE**: Absent a valid certification of Micro Entity Status (see form PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

[ ] Applicant asserting small entity status. See 37 CFR 1.27. — **NOTE**: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

[ ] Applicant changing to regular undiscounted fee status. — **NOTE**: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

The Director of the USPTO is requested to apply the Issue Fee and Publication Fee (if any) or to re-apply any previously paid issue fee to the application identified above. NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature /M. Angela Parsons/ Date /March 6, 2018/

Typed or printed name M. Angela Parsons, Ph.D. Registration No. 44,282

61645219.doc

Electronic Patent Application Fee Transmittal

Application Number:	15398479
Filing Date:	04-Jan-2017
Title of Invention:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES
First Named Inventor/Applicant Name:	Rachel Fraser
Filer:	M. Angela Parsons/Judith Witzel
Attorney Docket Number:	38767-0015002

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
UTILITY APPL ISSUE FEE	1501	1	960	960

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**960**

Electronic Acknowledgement Receipt

EFS ID:	31971302
Application Number:	15398479
International Application Number:	
Confirmation Number:	2358
Title of Invention:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES
First Named Inventor/Applicant Name:	Rachel Fraser
Customer Number:	26191
Filer:	M. Angela Parsons/Rachel Guidarelli
Filer Authorized By:	M. Angela Parsons
Attorney Docket Number:	38767-0015002
Receipt Date:	06-MAR-2018
Filing Date:	04-JAN-2017
Time Stamp:	15:07:13
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$960
RAM confirmation Number	030718INTEFSW00001710061050
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Post Allowance Communication - Incoming	RespNOA.pdf	73204 78fc563da354b25c759753068ecc985a65a66abe	no	2
Warnings:					
Information:					
2	Issue Fee Payment (PTO-85B)	PartB.pdf	123907 c37fad41afe69f850f24cc443a7018e9c77826a8	no	1
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30823 8651912d4b2f884a8f69b6c365c845b0f80f43fb	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	227934		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Amendments to the Specification:

Change(s) applied to document, /B.D./ 12/20/2017

Please amend the paragraph on page 14, lines ~~5-29~~ lines 11-5, as follows:

Marked Up

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

Clean

Heme-containing proteins that can be used in the compositions and food products described herein can be from mammals (e.g., farms animals such as cows, goats, sheep, pigs, ox,

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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/398,479	04/17/2018	9943096	38767-0015002	2358

26191 7590 03/28/2018

FISH & RICHARDSON P.C. (TC)
PO BOX 1022
MINNEAPOLIS, MN 55440-1022

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Rachel Fraser, San Francisco, CA;
Impossible Foods Inc., Redwood City, CA;
Patrick O'Reilly Brown, Stanford, CA;
Jessica Karr, San Francisco, CA;
Celeste Holz-Schietinger, East Palo Alto, CA;
Elysia Cohn, Sunnyvale, CA;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

Attorney's Docket No.: 38767-0015002 / Meat3CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Rachel Fraser	Art Unit	: 1791
Patent No.	:	9,943,096	Examiner	: Elizabeth A. Gwartney
Issue Date	:	April 17, 2018	Conf. No.	: 2358
Serial No.	:	15/398,479		
Filed	:	January 4, 2017		

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

Attention Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION

Please issue a Certificate of Correction in accordance with the attached form.

One or more of the errors to be corrected were made by Applicant. The fee of $150 is being paid with this request. Please apply any other necessary charges or credits to Deposit Account No. 06-1050, referencing the above-indicated Attorney Docket Number.

Respectfully submitted,

Date: /May 2, 2018/

/M. Angela Parsons/
M. Angela Parsons, Ph.D.
Reg. No. 44,282

Customer Number 26191
Fish & Richardson P.C.
Telephone: (612) 335-5070
Facsimile: (877) 769-7945

61675261.doc

PTO/SB/44 (09-07)
Approved for use through 08/31/2013. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 9,943,096

APPLICATION NO.: 15/398,479

ISSUE DATE : April 17, 2018

INVENTOR(S) : Rachel Fraser, Patrick O'Reilly Brown M.D., Ph.D., Jessica Karr, Celeste Holz-Schietinger and Elysia P. Cohn

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In Column 1, Line 8, delete “10, 2105” and insert --10, 2015--, therefor.

In Column 73, Line 65, In Claim 2, delete “histoglobin,” and insert --histaglobin-- therefor.

In Column 75, Line 11, In Claim 10, after “glucose,” insert --ribose,--.

In Column 75, Line 12, In Claim 10, delete “glactose,” and insert --galactose,--, therefor.

In Column 75, Line 13, In Claim 10, delete “thereof” and insert --thereof;--, therefor.

In Column 75, Line 14, In Claim 10, delete “cysteine,” and insert --cystine,--, therefor.

In Column 75, Line 18, In Claim 10, delete “a” and insert --the--, therefor.

In Column 75, Line 28, In Claim 11, after “flavoring” delete “wherein the food flavoring”.

In Column 76, Line 7, In Claim 15, delete “histoglobin,” and insert --histaglobin,--, therefor.

In Column 76, Line 40, In Claim 22, delete “and or” and insert --and/or--, therefor.

MAILING ADDRESS OF SENDER (Please do not use customer number below):
Fish & Richardson P.C.
P.O. Box 1022
Minneapolis, MN 55440-1022

This collection of information is required by 37 CFR 1.322, 1.323, and 1.324. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1.0 hour to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Attention Certificate of Corrections Branch, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and selection option2.

Electronic Patent Application Fee Transmittal

Application Number:	15398479
Filing Date:	04-Jan-2017
Title of Invention:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES
First Named Inventor/Applicant Name:	Rachel Fraser
Filer:	M. Angela Parsons/Judith Witzel
Attorney Docket Number:	38767-0015002

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
CERTIFICATE OF CORRECTION	1811	1	150	150

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**150**

Electronic Acknowledgement Receipt

EFS ID:	32507320
Application Number:	15398479
International Application Number:	
Confirmation Number:	2358
Title of Invention:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES
First Named Inventor/Applicant Name:	Rachel Fraser
Customer Number:	26191
Filer:	M. Angela Parsons/Judith Witzel
Filer Authorized By:	M. Angela Parsons
Attorney Docket Number:	38767-0015002
Receipt Date:	02-MAY-2018
Filing Date:	04-JAN-2017
Time Stamp:	09:51:59
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$150
RAM confirmation Number	050218INTEFSW00008013061050
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Certificate of Correction	TransCOC.pdf	135007 59e248bbd8fa2d2ae383eb32ba384f11ce37df7b	no	2
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30529 eb33378b1676fbfc767276976dd3ff17266b7910	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	165536		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.
National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.
New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,943,096 B2
APPLICATION NO. : 15/398479
DATED : April 17, 2018
INVENTOR(S) : Rachel Fraser et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

In Column 1, Line 8, delete "10, 2105" and insert --10, 2015--, therefor.

In the Claims

In Column 73, Line 65, In Claim 2, delete "histoglobin," and insert --histaglobin--, therefor.

In Column 75, Line 11, In Claim 10, after "glucose," insert --ribose,--.

In Column 75, Line 12, In Claim 10, delete "glactose," and insert --galactose,--, therefor.

In Column 75, Line 13, In Claim 10, delete "thereof" and insert --thereof;--, therefor.

In Column 75, Line 14, In Claim 10, delete "cysteine," and insert --cystine,--, therefor.

In Column 75, Line 18, In Claim 10, delete "a" and insert --the--, therefor.

In Column 75, Line 28, In Claim 11, after "flavoring" delete "wherein the food flavoring".

In Column 76, Line 7, In Claim 15, delete "histoglobin," and insert --histaglobin,--, therefor.

In Column 76, Line 40, In Claim 22, delete "and or" and insert --and/or--, therefor.

Signed and Sealed this
Twenty-ninth Day of May, 2018

Andrei Iancu
Director of the United States Patent and Trademark Office

AO 120 (Rev. 08/10)

TO:	REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK
Mail Stop 8 Director of the U.S. Patent and Trademark Office P.O. Box 1450 Alexandria, VA 22313-1450	

In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court for the District of Delaware on the following

☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
22-cv-00311-CFC	3/9/2022	for the District of Delaware

PLAINTIFF	DEFENDANT
IMPOSSIBLE FOODS INC.	MOTIF FOODWORKS, INC.

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	10,863,761	12/15/2020	IMPOSSIBLE FOODS INC.
2			
3			
4			
5			

In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY
7/25/2022	☑ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	9,943,096	4/17/2018	IMPOSSIBLE FOODS INC.
2	10,039,306	8/7/2018	IMPOSSIBLE FOODS INC.
3	11,013,250	5/25/2021	IMPOSSIBLE FOODS INC.
4	11,224,241	1/18/2022	IMPOSSIBLE FOODS INC.
5			

In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE

Copy 1—Upon initiation of action, mail this copy to Director **Copy 3—Upon termination of action, mail this copy to Director**
Copy 2—Upon filing document adding patent(s), mail this copy to Director **Copy 4—Case file copy**

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1	9,943,096	4/17/2018	IMPOSSIBLE FOODS INC. (included by amendment on 7/25/22)
2	10,039,306	8/7/2018	IMPOSSIBLE FOODS INC. (included by amendment on 7/25/22)
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5	See Exhibit A		

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