

From: [Yingling, Gary L.](#)
To: [McMahon, Carrie](#)
Cc: [Vaughn, Jessica L.](#)
Subject: FW: Impossible Foods Letter Regarding Color Additive Regulations
Date: Wednesday, October 18, 2017 4:04:38 PM
Attachments: [\(93593290\) \(8\) FDA Response Letter - Soy Leghemoglobin and Color Additive Regulations \(2\).DOCX](#)
[Attachment 1 Impossible Foods Patent US9700067.pdf](#)

Dear Dr. McMahon: Attached is a letter to the Agency responding to the color additive question. Please accept my apology because it was suppose to have been sent last week. gary

Gary L. Yingling

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From: Vaughn, Jessica L.
Sent: Wednesday, October 18, 2017 3:32 PM
To: Yingling, Gary L.
Subject: Impossible Foods Letter Regarding Color Additive Regulations

Hi Gary,
Letter and Patent attached.

-Jessica

Jessica L. Vaughn, Ph.D.

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October 18, 2017

Carrie McMahon, Ph.D.
Consumer Safety Officer
Office of Food Additive Safety
U.S. Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

Re: Soy Leghemoglobin Protein as a Flavor Catalyst

Dear Dr. McMahon:

This letter responds to the Food and Drug Administration's ("FDA" or "Agency") color additive inquiry in a September 5, 2017 email to our client, Impossible Foods Inc. ("Impossible Foods"), concerning the use of soy leghemoglobin protein derived from *Pichia pastoris* ("soy leghemoglobin") in the Impossible Burger.

Soy leghemoglobin was originally sourced from soybean root nodules, and is produced by Impossible Foods via fermentation using *Pichia pastoris* that has been genetically modified to express soy leghemoglobin. Impossible Foods uses soy leghemoglobin as an ingredient of the Impossible Burger for the sole purpose of driving the chemistry that creates the flavor and aroma of meat. Impossible spent years researching how raw meat transforms from its bland, metallic taste into the complex profile of flavors and aromas that make cooked meat uniquely appealing and flavorful. Impossible Foods discovered that, when exposed to heat (i.e., cooking) a heme protein present in meat (myoglobin) catalyzes the various vitamins, minerals, amino acids and simple sugars found naturally in the surrounding meat tissues, thereby generating meat flavors and aromas. To create a plant-based analog to meat, Impossible Foods researched various plant-based heme proteins, and chose soy leghemoglobin as it actually performs the same function – as a flavor catalyst - as myoglobin in meat, and in some ways, performs even better than myoglobin (e.g., shelf life). The flavor chemistry made possible by soy leghemoglobin is what makes the plant-based Impossible Burger unique.

Because the flavor chemistry made possible by using soy leghemoglobin is so important and novel, Impossible Foods sought a patent covering the use of soy leghemoglobin as a

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flavor catalyst in plant-based foods. The Patent and Trademark Office recognized the inventiveness of Impossible Foods's use of soy leghemoglobin to drive flavor chemistry, and on July 11, 2017, granted to Impossible Foods U.S. Patent No. 9,700,067 B2, "Methods and Compositions for Affecting the Flavor and Aroma Profiles of Consumables" (Attachment 1). The patent discusses, at length, the use of the soy leghemoglobin to "modulate the flavor and/or aroma profile of other foods" and provides over 14 examples of various flavor profiles enabled by soy leghemoglobin (*see* pp. 21-34 of Attachment 1). Further supporting soy leghemoglobin's use solely for flavor, like many flavors, its use level in food is limited because it will impart an increasingly strong and disagreeable taste if used at levels above 0.8%.

The Agency noted that in posted material Impossible had referenced the red color of soy leghemoglobin, and the Agency asked whether the soy leghemoglobin was a color additive. Impossible does not believe the leghemoglobin is a color additive and consistent with this point, has removed all references in its materials to this inaccurate characterization of use of soy leghemoglobin. Impossible Foods provides further support of its conclusion through the following reasons.

The definition of the term "color additive" found in 21 U.S.C. § 321(t)(B) requires a material to be "added or applied to a food." Soy leghemoglobin is neither added nor applied to a food; rather, soy leghemoglobin is the most important functional ingredient of the food itself. The burger's other main ingredients are wheat protein, deflavored coconut oil and potato protein; the burger would taste entirely bland in the absence of the meaty flavor and aroma properties made possible by the soy leghemoglobin food ingredient. Without soy leghemoglobin, the Impossible Burger would be no different from any of the legion of "veggie burgers" on the market that may taste good, but would never be mistaken for meat. The flavor function of soy leghemoglobin is so important and integral that, as mentioned above, Impossible has secured its exclusive use by obtaining US Patent No. 9,700,067 B2.

Further, in the color additive regulations, 21 C.F.R. § 70.3(f), a food ingredient such as cherries, green or red peppers, chocolate and orange juice, which contribute their own natural color when mixed with other foods, are not regarded as color additives. Likewise, soy leghemoglobin is a food ingredient used for the sole purpose of flavor that will nonetheless impart its natural color to the food. Soy leghemoglobin drives the flavor chemistry of the burger, and is a critically necessary ingredient for that reason alone.

The food color additive ingredients listed in 21 C.F.R. § 73.1 to § 73.615, have a color property and limited, if any, impact other than color. That is not the case with soy leghemoglobin. Soy leghemoglobin has a critical impact by driving the chemistry that produces the distinctive flavor and aroma of meat. Impossible Foods uses soy leghemoglobin as a flavor catalyst. The heme molecule catalyzes flavor and has a natural color and it is not possible to extract the color from soy leghemoglobin without destroying the flavor properties.

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Thus, as stated at the outset, soy leghemoglobin is used for its ability to impart the flavor and aroma of meat in a plant-based product. It is not a color additive, pursuant to 21 C.F.R. § 70.3(f).

We hope we have addressed your questions.

Sincerely,

A rectangular area of the document is redacted with a solid grey fill. The redaction covers the signature of Gary L. Yingling. There are some faint, illegible marks around the edges of the redacted area, possibly from a scanner or the original document's texture.

Gary L. Yingling

GLY



US009700067B2

(12) **United States Patent**
Fraser et al.

(10) **Patent No.:** **US 9,700,067 B2**
(45) **Date of Patent:** **Jul. 11, 2017**

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

(52) **U.S. Cl.**
CPC . *A23J 3/14* (2013.01); *A23J 1/12* (2013.01);
A23J 1/14 (2013.01); *A23J 3/227* (2013.01);
(Continued)

(71) Applicant: **Impossible Foods Inc.**, Redwood City, CA (US)

(58) **Field of Classification Search**
CPC *A23L 1/221*; *A23L 1/231*; *A23L 1/31427*
See application file for complete search history.

(72) Inventors: **Rachel Fraser**, San Francisco, CA (US); **Patrick O'Reilly Brown**, Stanford, CA (US); **Jessica Karr**, San Francisco, CA (US); **Celeste Holz-Schietinger**, East Palo Alto, CA (US); **Elysia Cohn**, Mountain View, CA (US)

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(73) Assignee: **Impossible Foods Inc.**, Redwood City, CA (US)

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(Continued)

(21) Appl. No.: **14/797,006**

Primary Examiner — Elizabeth Gwartney

(22) Filed: **Jul. 10, 2015**

(74) *Attorney, Agent, or Firm* — Fish & Richardson P.C.

(65) **Prior Publication Data**

US 2015/0351435 A1 Dec. 10, 2015

(57) **ABSTRACT**

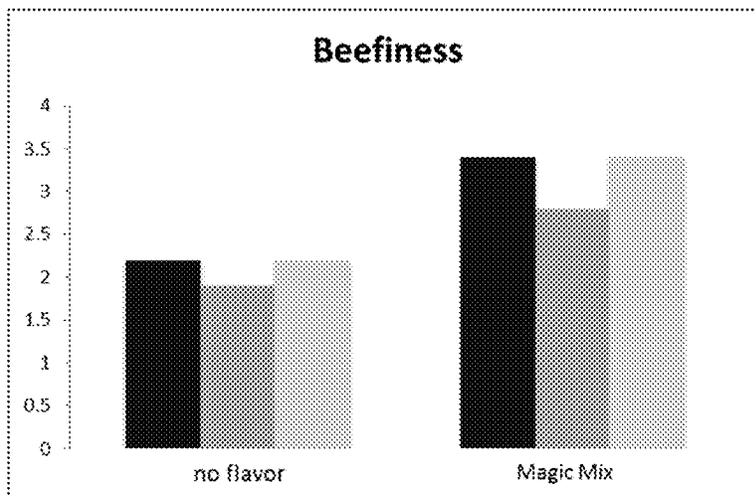
Related U.S. Application Data

(63) Continuation of application No. PCT/US2014/011347, filed on Jan. 13, 2014, which
(Continued)

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.

(51) **Int. Cl.**
A23J 1/14 (2006.01)
A23J 3/14 (2006.01)
(Continued)

23 Claims, 5 Drawing Sheets



Related U.S. Application Data

is a continuation of application No. 13/941,211, filed on Jul. 12, 2013.

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(51) **Int. Cl.**

- A23J 1/12* (2006.01)
- A23J 3/22* (2006.01)
- A23L 27/00* (2016.01)
- A23L 27/10* (2016.01)
- A23L 27/20* (2016.01)
- A23L 27/26* (2016.01)
- A23L 33/185* (2016.01)
- A23L 13/40* (2016.01)

(52) **U.S. Cl.**

- CPC *A23L 13/424* (2016.08); *A23L 13/426* (2016.08); *A23L 27/00* (2016.08); *A23L 27/10* (2016.08); *A23L 27/20* (2016.08); *A23L 27/26* (2016.08); *A23L 33/185* (2016.08)

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FIG. 1

SEQ ID NO:1 *Vigna radiata*

MTTTLERGFTEEQEALVVKSWNVMMKKNSGELGLKFFLKIIFEIAPSAQKLFSLFRDSTVPLEQNPK
LKPHAVSVFVMTCDASAVQLRKAGKVTVRESNLKLLGATHFRTGVANEHFEVTKFALLETIKEAVP
EMWSPAMKNAWGEAYDQLVDAIKYEMKPPSS

SEQ ID NO:2 *Methylococcus thermophilus*

MIDQKEKELIKESWKRIEPNKNEIGLLFYANLFKKEEPTVSVLFQNPFISSQSRKLMQVLGILVQGI
DNLEGLIPTLQDLGRRHKQYGVVDSHYPLVGDCLLKSITQEYLGQGFTEEAKAAWTKVYGIAAQVM
TAE

SEQ ID NO:3 *Aquifex aeolicus*

MLSEETIRVIKSTVPLLKEHGTEITARMYELLFSKYPKTKELFAGASEEQPKLANAIIAYATYI
DRLEELDNAISTIARSHVRRNVKPEHYPLVKECLLQAIIEVLNPGEEVLKAWEEAYDFLAKTLIT
LEKKLYSQP

SEQ ID NO:4 *Glycine max*

MGAFTEKQEALVSSSFEAFKANI PQYSVVFYTS ILEKAPAAKDLFSFLSNGVDPSNPKLTGHAEK
LFGGLVRDSAGQLKANGTVVADAALGSIHAQKAITDPQFVVVKEALLKTIKEAVGDKWSEDESSAW
EVAYDELAAAIAKKAF

SEQ ID NO:5 *Hordeum vulgare*

MSAAEGAVVFSEEKEALVLSWAIMKKDSANLGLRFFLKIIFEIAPSARQMFPFLRDSVPLETNP
KLKTHAVSVFVMTCEAAAQLRKAGKITVRETTLKRLLGGTHLKYGVADGHFEVTRFALLETIKEAL
PADMWGPENRANWGEAYDQLVAAIKQEMKPAE

SEQ ID NO:6 *Magnaporthe oryzae*

MDGAVRLDWTGLDLTGHEIHDGVP IASRVQVMVSFPLFKDQHIIMSSKESPSRKSSTIGQSTRNG
SCQADTQKQQLFPVGEKPKPVKENPMKKLKEMSQRPLPTQHGDTYPTTEKLLTGIGEDLKHIRGY
DVKTLLAMVKSCLKGKELKDDKTMMLERVMQLVARLPTESSKRAELTDSLINELWESLDHPPNLN
LGPEHSYRTPDGSYNHPFNPNQLGAAGSRYARSVIPTVTPPGALPDPGLIFDSIMGRTPNYSYRKH
NNVSSILWYATIIHDI FWTDPRDINTNKSSSYLDLAPLYGNSQEMQDS IRTFKDGRMKPDCYA
DKRLAGMPPGVSVLLIMFNRFHNVHAENLALINEGGFRFNKPSDLLEGEAREAAWKKYDNDLFQVA
RLVTSGLYINI TLVDYVRNIVNLNRVDTTWTLDPRQDAGAHVGTADGAERGTGNAVSAEFNLCYR
WHSCISEKDSKFVEAQFQNI FQKPASEVRPDEMWWKGFQAKMEQNTPADPGQRTFGGFKRGPDKGFD
DDDLVRCISEAVEDVAGAFGARNVPQAMKVETMGI IQGRKWNVAGLNEFRKHFHLKPYSTFEDI
NSDPGVAEALRRLYDHPDNVELYPGLVAEEDKQPMVPGVGIAPTYTISRVLSDAVCLVRGDRFY
TTDFTPRNLTNWGYKEVDYDLSVNHGCVFYKLFIRAFPNHFKQNSVYAHYPMVVPSENKRILEAL
GRADLDFEAPKYIPPRVNIITSYGAAYIILETQEKYKVTWHEGLGFLMGEGGLKFMLSGDDPLHA
QQRKCMAAQLYKDGWTEAVKAFYAGMMEELLVSKSYFLGNNKHRHVDI IRDVGNMVHVHFASQVF
GLPLKTAKNPTGVFTEQEMYGILAAIIFTTIFFDLDPKSFPLRKTREVCQKLAKLVEANVKLIN
KIPWSRGMFVVGKPAKDEPLSIYKTMIKGLKAHGLSDYDIAWVSHVPTSGAMVNPQAQVFAQAVD
YYLSPAGMHIYIPEIHMVALQPSTPETDALLLGYAMEGIRLAGTFGSYREAAVDDVVKEDNGRQVP
VKAGDRVFSFVDAARDPKHFPDFEVVNRPRPAKKYIHYGVGPHACLGRDASQIAIITEMFRCLFR
RRNVRVPGPQGELKKVPRPGGFYVYMRDWWGLFPFPVMTMRVMWDE

SEQ ID NO:7 *Fusarium oxysporum*

MKGSATLAFALVQFSAASQLVWPSKWDEVEDLLYMQGGFNKRGFADALRTCEFGSNVPGTQNTAE
WLRTAFHDAITHDAKAGTGGLDASIWESSRPNPAGAFNNTFGFFSGFHNPRATASDLTALGTV
LAVGACNGPRI PFRAGRIDAYKAGPAGVPEPSTNLKDTFAAFTKAGFTKEEMTAMVACGHAIGGV

FIG. 1-CONT.

HSVDFPEIVGIKADPNNDTNVFPQKDVSSFHNGIVTEYLAGTSKNPLVASKNATFHSDKRIFDND
KATMKKLSTKAGFNSMCADILTRMIDTVPKSVQLTPVLEAYDVRPYITELSLNNKNIHFTGSVR
VRITNNIRDNDLAINLIYVGRDGGKVTVPVTPQVTFQGGTSFGAGEVFANFEFD'TTMDAKNGITK
FFIQEVKPKSTKATVTHDNQKTGGYKVDVTVLYQLQSCAVLEKLPNAPLVVTAMVRDARAKDAL
LRVAHKKPVKGSIVPRFQTAITNFKATGKKSSGYTGFAKTMFEEQSTYFDIVLGGSPASGVQFL
TSQAMPSQCS

SEQ ID NO:8 *Fusarium graminearum*

MASATRQFARAATRATRNGFAIAPRQVIRQQGRRYSSSEPAQKSSSAWIWLTGAAVAGGAGYFYF
GNSASSATAKVFNPSKEDYQKVYNEIAARLEEKDDYDDGSYGPVLRVLAHWASGTYDKETGTGGS
NGATMRFAPESDHGANAGLAAARDFLQPVKEKFPWITYSDLWILAGVCAIQEMLGPAIPYRPGRS
DRDVSGCTPDGRLPDASKRQDHLRGIFGRMGFNDQEIVALSGAHALGRCHTDRSGYSGPWTFSP
VLTNDYFRLLVEEKWQKKWNGPAQYEDKSTKSLMMLPSDIALIEDKKFKPWVEKYAKDNDAFFK
DFSNVVLRLELGVPPFAQGTENQRWTFKPTHQE

SEQ ID NO: 9 *Chlamydomonas eugametos*

MSLFAKLGREAVEAAVDKFFYNKIVADPTVSTYFSNTDMKVQRSKQFAFLAYALGGASEWKGD
RTHAKDLVPHLSDVHFQAVARHLSDTLTELGVPPEDITDAMAVVASTRTEVLNMPQQ

SEQ ID NO:10 *Tetrahymena pyriformis*

MNKPQTIYEKLGGENAMKAAVPLFYKKVLADERVKHFFKNTDMDHQTKQQTDFLTMLLGGPNHYK
GKNMTEAHKGMNLQNLHFDAI IENLAATLKELGVTDAVINEAAKVIEHTRKDMLGK

SEQ ID NO:11 *Paramecium caudatum*

MSLFEQLGGQAAVQAVTAQFYANIQADATVATFFNGIDMPNQTNKTA AFLCAALGGPNAWTGRNL
KEVHANMGSNAQFTTVIGHLRSAITGAGVAAALVEQTVAVAETVRGDVVTV

SEQ ID NO:12 *Aspergillus niger*

MPLTPEQIKIKATVPVLQEYGTKITTA FYMNMSTVHPVPELNAVNTANQVKGHQARALAGALFAY
ASHIDDLGALGPAVELICNKHASLYIQADEYKIVGKYLLEAMKEVLGDACTDDILDAGGAYWAL
ADIMINREAAALYKQSQG

SEQ ID NO:13 *Zea mays*

MALAEADDGAVVFGEEQEALVLSWAVMCKDAANLGLRFFLKVFEIAPSAEQMF'SFLRDSVPLE
KNPKLKTAMSVFVMTCEAAAQLRKAGKVTVRETTLLKRLGATHLRYGVADGHFEVTFALLETIK
EALPADMWSLEMKKAWAEAYSQLVAAIKREMKPDA

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

MALVEGNGVSGGAVSFSEEQEALVLSWAIMKKDSANIGLRFLLKIFEVAPSASQMF'SFLRNSD
VPLEKNPKLKTAMSVFVMTCEAAAQLRKAGKVTVRD'TTLKRLGATHFKYGVGDAHFVTRFALL
ETIKEAVFVDMWS PAMKSAWSEAYNQLVAAIKQEMKPAE

SEQ ID NO:15 *Arabidopsis thaliana*

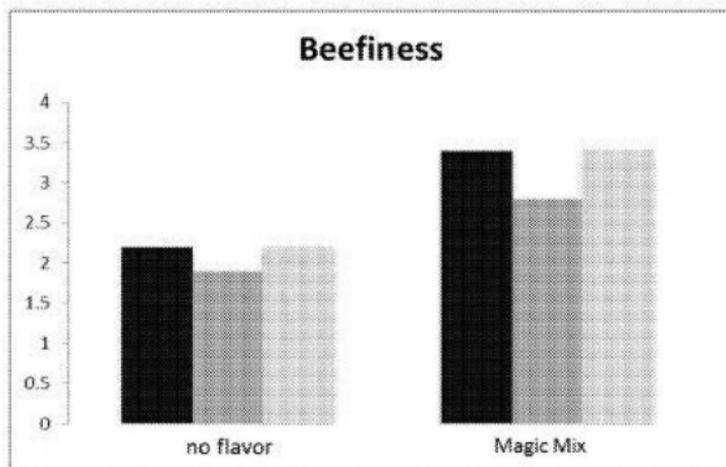
MESEGIKIVFTEEQEALVVKSWVMKNSAELGLKLFKIFEIFIAPT'TKKMF'SFLRDSPIPAEQNPK
LKPAMSVFVMTCEAAAQLRKAGKVTVRETTLLKRLGASHSKYGVVDEHFVAKYALLETIKEAVP
EMWSPKVAWGQAYDHLVAAIKAEMNLSN

FIG. 1-CONT.

SEQ ID NO:16 *Pisum sativum*MGFTDKQEALVNSSWESFKQNLNSGNSILFYTTIILEKAPAAKGLFSFLKDTAGVEDSPKLAHAHQ
VFGLVRDSAAQLRRTKGEVVLGNATLGAIHVQRGVTDPHFVVVKEALLQTIKKASGNNWSEELNTA
WEVAYDGLATAIKKAMTSEQ ID NO:17 *Vigna unguiculata*MVAFSDKQEALVNGAYEAFKANIPKYSVVFFYTTIILEKAPAAKNLFSFLANGVDATNPKLTGHAEK
LFGLVRDSAAQLRASGGVVADAALGAVHSQKAVNDAQFVVVKEALVKTLKEAVGDKWSDELGTAV
ELAYDELAAAIAKKAYSEQ ID NO:18 *Bos taurus*MGLSDGEWQVLVNAWGKVEADVAGHGQEVLIIRLFTGHPETLEKFDKFKHLKTEAEMKASEDLKHH
GNTVLTALGGILKKKGHHEAEVKHLAESHANKHKIPVKYLEFISDAI IHVLHAKHPSDFGADAQA
AMSKALELFRNDMAAQYKVLGFHGSEQ ID NO:19 *Sus scrofa*MGLSDGEWQVLVNVWGKVEADVAGHGQEVLIIRLFKGHPEKFDKFKHLKSEDEMASEDLKHH
GNTVLTALGGILKKKGHHEAELTPLAQSHATKHKIPVKYLEFISEAIIQVLQSKH
PGDFGADAQGAMSKALELFRNDMAAKYKELGFQGSEQ ID NO:20 *Equus caballus*MGLSDGEWQQVLVNVWGKVEADIAGHGQEVLIIRLFTGHPETLEKFDKFKHLKTEAEMKASEDLKHH
GTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAI IHVLHSHK
PGDFGADAQGAMTKALELFRNDIAAKYKELGFQGSEQ ID NO:21 *Nicotiana benthamiana*MSSFTEEQEALVVKSWDSMKNAGEWGLKFLKIFEIAPSARKLFSFLKDSNVPLEQNAKLPKPHS
KSVFVMTCEAAVQLRKAGKVVVRDSTLKKLGATHEFKYGVADHEHFEVTKFALLETIKEAVPEMWSV
DMKNAWGEAFDQLVNAIKTEMKSEQ ID NO:22 *Bacillus subtilis*MGQSFNAPYEAIGEELLSQLVDTFYERVASHPLLKPIFPDLTETARKQKQFLTQYLGPPPLYTE
EHGHPMLRARHLPPITNERADAWLSCMKDAMDHVGLEGEIREFLFGRLELTARHMVNQTEAEDR
SSSEQ ID NO:23 *Corynebacterium glutamicum*MTTSENFYDSVGGEEFTSLIVHRFYEQVPNDLILGPMYPPDDFEGAEQRLKMFLSQYWGGPKDYQ
EQRGHPRLMRHVNYPIGVTAERWLQLMSNALDGVDLTAEQREAIWEHVMVRAADMLINSNPDPH
ASEQ ID NO:24 *Synechocystis* PCC6803MSTLYEKLGGTTAVDLAVDKFYERVLQDDRIKHHFFADVDMAKQRAHQKAFITYAFGGTDKYDGRY
MREAHKELVENHGLNGEHFDAVAEDLLATLKEMGVPEDLIAEVAAVAGAPAHKRDVLNQSEQ ID NO:25 *Synechococcus* sp. PCC 7335MDVALLEKSFEQISPRAIIEFSASFYQNLFHHPHPELKLPLFAETSQTIQEKKLIFSLAAI IENLRNP
DILQPALKSLGARHAEVGTIKSHYPLVGQALIEFTFAEYLAADWTEQLATAWVEAYDVIASMTIEG
ADNPAAYLEPELTFYEWLDLYGEESPKVRNAIATLTHFYGEDPQDVQRDSRG

FIG. 1-CONT.SEQ ID NO:26 *Nostoc commune*MSTLYDNIGGQPAIEQVVDELHKRIATDSLLAPVVFAGTDMVKQRNHLVAFLAQIFEGPKQYGGRP
MDKTHAGLNLQQPHFDAIAKHLGERMAVRGVSAENTKAALDRVTNMKGAILNKSEQ ID NO:27 *Bacillus megaterium*MREKIHSPYELLGGEHTISKLVDAFYTRVGQHPHELAPIFPDNL TETARKQKQFLTQYLGGPSLYT
EEHGHPMLRARHLPFEITPSRAKAWLTCMHEAMDEINLEGPERDELYHRLIILTAQHMINSP EQTD
EKGFSH

FIG. 2



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

TECHNICAL FIELD

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

BACKGROUND

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

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

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

One or more flavor precursor molecules or compositions can be added along with a iron complex (e.g., ferrous

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

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

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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 bacteriochloro-

phyll, 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-butanal, 2-pentyl-furan, thiazole, (E,E)-2,4-decadienal, hexanoic acid, 1-ethyl-5-methylcyclopentene, (E,E)-2,4-nonadienal, (Z)-2-decenal, dihydro-5-pentyl-2(3H)-furanone, trans-3-nonen-2-one, (E,E)-3,5-octadien-2-one, (Z)-2-octen-1-ol, 5-ethyl-dihydro-2(3H)-furanone, 2-butanal, 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 phos-

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

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

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

TABLE 1

Flavor Precursor Molecules	
5	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, galactose, lactose, ribitol, gluconic acid and glucuronic acid, amylose, amylopectin, or xylose
10	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
15	Free fatty acids: caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic, oleic acid, linoleic acid, alpha linolenic acid, gamma linolenic acid, arachidic acid, arachidonic acid, behenic acid, or erucic acid
	Amino acids and derivatives thereof: cysteine, cystine, a cysteine sulfoxide,
20	allicin, selenocysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, 5-hydroxytryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, or tyrosine
25	Nucleosides and Nucleotides: inosine, inosine monophosphate (IMP), guanosine, guanoside monophosphate (GMP), adenosine, adenosine monophosphate (AMP)
	Vitamins: thiamine, vitamin C, Vitamin D, Vitamin B6, or Vitamin E
	Misc: phospholipid, lecithin, pyrazine, creatine, pyrophosphate
	Acids: acetic acid, alpha hydroxy acids such as lactic acid or glycolic acid,
30	tricarboxylic acids such as citric acid, dicarboxylic acids such as succinic acid or tartaric acid
	Peptides and protein hydrolysates: glutathione, vegetable protein hydrolysates, soy protein hydrolysates, yeast protein hydrolysates, algal protein hydrolysates, meat protein hydrolysates
35	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 Fe²⁺ state of a heme-containing protein can be similar to that of myoglobin or can exceed it by 10%, 20%, 30% 50%, 100% or more under conditions in which the heme-protein-containing consumable is manufactured, stored, handled or prepared for consumption

Non-limiting examples of heme-containing polypeptides can include an androglobin, a cytoglobin, a globin E, a globin X, a globin Y, a hemoglobin, a myoglobin, an erythrocyruorin, a beta hemoglobin, an alpha hemoglobin, a protoglobin, a cyanoglobin, a cytoglobin, a histoglobin, a neuroglobins, a chlorocruorin, a truncated hemoglobin (e.g., 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*, *Myceliophthera thermophile*, *Kluyvera lactis*, or *Fusarium oxysporum*. Heme-containing proteins can be isolated from bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Synechocystis* sp., *Aquifex aeolicus*, *Methylococcus thermophilus*, 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 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 *Methylococcus thermophilus* (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 (B12seq) program from the stand-alone version of BLASTZ containing BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from Fish & Richardson's web site (e.g., www.fr.com/blast/) or the U.S. government's National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov). Instructions explaining how to use the B12seq program can be found in the readme file accompanying BLASTZ. B12seq performs a comparison between two amino acid sequences using the BLASTP algorithm. To compare two amino acid sequences, the options of B12seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two amino acid sequences: C:\B12seq -i 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 250 g/L.

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

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

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

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

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TABLE 2-continued

Flavor Types	
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	acetoneitrile	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	fomic 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-dimethyl-ethyl)-benzene
1-heptanol	octanoic acid	heptane
dimethyl trisulfide	2-ethyl-5-methyl-pyrazine	4,7-dimethyl-undecane

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TABLE 3-continued

Compounds Produced		
2-nonanone	3-ethyl-2,5-dimethyl-pyrazine	acetophenone
2-pentanone	1,3,5-cycloheptatriene	tridecane
2-heptanone	2-ethyl-1-hexanol	thiophosphoramidate, s-methyl ester
2,3-butanedione	4-methyl-octanoic acid	2-methyl-thiazole
heptanal	m-aminophenylacetylene	3-(1-methylethoxy)-propanenitrile,
10 nonanal	benzene	2,4-bis(1,1-dimethyl-ethyl)-phenol
2-octanone	thiophene	3-ethyl-2,2-dimethyl-pentane
2-butanone	2-methyl-furan	3-ethyl-pentane
15 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
20 2,4-Heptadienal, (E,E)-(Z)-2-heptenal	Furan, 2-hexyl-:2	4-penten-2-one
6-methyl-2-heptanone	3-methyl-butanol	3-methylthiazole
	2-methyl-butanol	2-methyl-3-pentanone
		2,3-pentanedione
25 (Z)-4-heptenal	methacrolein	(E)-2-tridecen-1-ol
(E,Z)-2,6-nonadienal	octane	2-thio-phenemethanamine
3-methyl-2-butenal	ethanol	(Z)-2-nonenal,
		methyl thiolacetate
2-pentyl-furan	2-methyl-propanal	methyl ethanoate
thiazole	acetone	isothiazole
(E,E)-2,4-decadienal	propanal	3,3-dimethyl-hexane
30 hexanoic acid	methyl-thiirane	
1-ethyl-5-methylcyclopentene	acetaldehyde	
(E,E)-2,4-nonadienal		
(Z)-2-decenal	2-propenal	4-methyl-heptane
dihydro-5-pentyl-2(3h)-furanone	2-propyl-furan	2,4-dimethyl-heptane
35 trans-3-nonen-2-one	dihydro-5-propyl-2(3H)-furanone	2,3,4-trimethyl-heptane
	dihydro-3-(2H)-thiophenone	2-methyl-heptane
	(E,E)-3,5-octadien-2-one	2-methyl-3-furanthiol
		4-amino-1,2,5-oxadiazole-3-carbonitrile
	(Z)-2-octen-1-ol	1,2-benzisothiazol-3(2H)-one
40 5-ethyl-dihydro-2(3h)-furanone	1-heptene	1,2-benzisothiazol-3(2H)-one
2-butenal		2-acetyl-propen-2-ol,
1-penten-3-ol	1,3-octadiene	1-decen-3-one
1-(ethylthio)-2-(methylthio)-buta-1,3-diene	1-nonene	

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-thio-

phenecarboxaldehyde, 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 gluconate produced a sulfurous, odor, but addition of heme-containing proteins reduced the sulfurous odor and replaced it with flavors including but not limited to chicken

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

In some embodiments, an iron complex (e.g., a ferrous chlorophyllin or a heme-cofactor such as a heme-containing protein) described herein can be heated in the 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 epigallocatechin gallate attached to a solid support such as a bead or resin. See, e.g., WO2013138793. In addition, depending on the protein component of the food product, certain combinations of antioxidants and/or LOX inhibitors can be used as effective agents to minimize off-flavor or off-odor generation especially in the presence of fats and oils. Such compounds can include, for example, one or more of 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, meat-loaf, bacon, strips, fingers, nuggets, cutlets, or cubes.

In addition, food products described herein can be used to modulate the taste and/or aroma profile of other food products (e.g., meat replicas, meat substitutes, tofu, mock duck

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

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

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

Food products described herein also can include a natural coloring agent such as turmeric or beet juice, or an artificial coloring agent such as azo dyes, triphenylmethanes, 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 as beefiness, texture, and flavor. Panelists can be encouraged to rinse their mouths with water between samples, and given opportunity to comment on each sample.

In some embodiments, a food product described herein can be compared to another food product (e.g., meat or meat substitute) 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 Jan. 11, 2013. Control burgers were prepared as in Table 4 with precursors cysteine (10 mM), glutamic acid (10 mM), glucose (10 mM), and thiamine (1 mM) except 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

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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
<u>Beefyness</u>			
mean	5.33	1.30	3.20
STDEV	1.58	0.67	0.79
<u>Bloody</u>			
mean	4.00	1.10	2.78
STDEV	1.32	0.32	1.64
<u>Savory</u>			
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

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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)
ribose (10 mM)	cysteine (10 mM)	bread crust with beef drippings, sweet, grassy, umami
ribose (10 mM)	cysteine (10 mM)	lactic acid (1 mM)
ribose (10 mM)	cysteine (10 mM)	breadly, malty, browned, breadcrumb
ribose (10 mM)	cysteine (10 mM)	lysine (5 mM)
ribose (10 mM)	cysteine (10 mM)	savory, beefy, little grassy, brothy, bread
ribose (10 mM)	cysteine (10 mM)	alanine (5 mM)
ribose (10 mM)	cysteine (10 mM)	savory, weak beefy, brothy, little metallic
ribose (10 mM)	cysteine (10 mM)	I + G (2 mM)
ribose (10 mM)	cysteine (10 mM)	savory, weak beefy, brothy, sweet

TABLE 7-continued

Flavors generated by addition of precursors to LegH (1%)						
Precursor (concentration)				Flavor Description		
	ribose (10 mM)	cysteine (10 mM)		methionine	cooked potato	
	ribose (10 mM)	cysteine (10 mM)	glutamic acid (5 mM)		little meaty, pretzel, brothy, savory, sweet, chocolate	
glucose (10 mM)	ribose (10 mM)	cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	slight beefy, browned, grassy,	
glucose (10 mM)	ribose (10 mM)	cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	IMP (2 mM)	bacon, very umami, savory, brothy, slight beef
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)		beef jerky, bloody, meaty, brothy
glucose (10 mM)		cysteine (10 mM)	thiamine (2 mM)	glutamic acid (5 mM)	lactic acid (1 mM)	savory, beefy, bloody, meaty, savory, gravy
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

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)	
35 benzaldehyde	X	X	
2-butanone	X	X	
dimethyl trisulfide	X	X	
2-pentyl-furan	X	X	
2-methyl-propanal	X	X	
thiazole	X	X	
40 butyrolactone	X	X	
2-acetylthiazole	X	X	
pentanal	X	X	
3-methyl-butanal	X	X	
methyl-thiirane	X	X	
nonanal	X	X	
45 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	
50 2-ethyl-furan	X	X	
2-octanone	X	X	
propanal	X	X	
trichloromethane	X	X	
2-methyl-furan	X	X	
furan	X	X	
55 pyrazine	X	X	
thiophene	X	X	
1,3-dimethyl-benzene	X	X	
octane		X	
octanal	X	X	
thiazole	X	X	
60 2-pentanone		X	
furfural	X	X	
2-nonanone	X	X	
(Z)-2-heptenal	X	X	
(E)-2-heptenal	X	X	
1-octene	X	X	
65 formic acid, heptyl ester	X	X	
2-pentyl-thiophene		X	

TABLE 8-continued

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)
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 150 C for

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

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

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

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

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

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

The addition of lysine in mixes with heme protein ribose, and cysteine controlled the formation of flavor compounds dimethyl trisulfide, nonanal, 2-pentyl-thiophene, furfural, 2-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.

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Reactions containing heme protein at 1%, cysteine (10 mM), and ribose (20 mM), and lysine (1 mM), at pH 6.0, were prepared and heated to 150 C for 3 minutes. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Characteristic flavor and fragrance components were mostly produced during the cooking process where precursors could react with the heme-protein. These samples were evaluated by GCMS for the flavor compounds generated and evaluated for the sensory experience. Volatile chemicals were isolated from the head space around the flavor reaction. Dimethyl trisulfide, nonanal, 2-pentyl-thiophene, furfural, 2-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	<i>A. aeolicus</i>
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

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TABLE 9-continued

Flavor compounds created by different heme-protein when heated with ribose and cysteine.				
Name	LegH	Barley	B. myoglobin	<i>A. aeolicus</i>
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.

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TABLE 10

Legend showing components of each sample		
Sample Name	Solution	Additives
MRM_None	Maillard Reaction Mix	None
MRM_Linoleic 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_Linoleic Acid	PPB, pH 6	1% linoleic acid
KPhos6_Oleic Acid	PPB, pH 6	1% oleic acid
KPhos6_C14	PPB, pH 6	1% C14:0 free fatty 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 abun-

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dance 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_OleicAcid	herbal, plastic, slight cheesy, brothy
K-Phos6_Linoleic Acid	light plastic
MRM_Linoleic 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

TABLE 12-continued

Compounds in Beef	MRM only	MRM_BeefHeart	MRM_NatCholinePC40	MRM_Linoleic acid
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-ethyl-dihydro-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 acetaldehyde	Y	N	N	N
acetic acid	N	N	N	N
acetic acid ethenyl ester	Y	N	N	N

TABLE 12-continued

Compounds in Beef	MRM only	MRM_BeefHeart	MRM_NatCholinePC40	MRM_Linoleic acid
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

TABLE 12-continued

Compounds in Beef	MRM only	MRM_BeefHeart	MRM_NatCholinePC40	MRM_Linoleic acid
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

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

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_BeefHeart, MRM_BioLipon95, MRM_Canola, and MRM_Coconut. Thiazole was created in samples with MRM_Coconut, MRM_BeefHeart, MRM_BioLipon95, MRM_C14, MRM_C18, MRM_Canola, MRM_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_BeefHeart, 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_

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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.

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TABLE 13

Chemical entities added to the Magic Mix		
	Chemical entity	mM
5	Alanine	5.6
	Arginine	0.6
	Asparagine	0.8
	Aspartate	0.8
	Cysteine	0.8
10	Glutamic acid	3.4
	Glutamine	0.7
	Glycine	1.3
	Histidine	0.6
	Isoleucine	0.8
	Leucine	0.8
15	Lysine	0.7
	Methionine	0.7
	Phenylalanine	0.6
	Proline	0.9
	Threonine	0.8
	Tryptophan	0.5
20	Tyrosine	0.6
	Valine	0.9
	glucose	5.6
	Ribose	6.7
	Maltodextrin	5.0
	Thiamine	0.5
25	GMP	0.24
	IMP	0.6
	Lactic acid	1.0
	creatine	1.0
30	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	
40	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
45	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
50	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
55	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-butanol	L	M
	390.6	isopropyl alcohol	0	S
	399.6	ethanol	L	M
60	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
65	453.2	pentanal/2,3-butanedione	L	0
	453.8	2,3-butanedione	L	M

TABLE 14-continued

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
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-disilacyclobutane	S	M
789.4	3-methyl-2-butenal	M	0
793.4	4-methyl-2-heptanone	M	0
810.4	pyrazine	M	0
818.8	isothiazole	S	0
827.1	acetyl valeryl	M	0
831.8	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

TABLE 14-continued

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
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 (8 L) was added with mixing and the material was allowed to extract for 1 hour. The material was filtered through Whatman filter paper and the acetone was removed on a rotary evaporator (Buchi). To the residual green suspension (500 mL) was added 2 mL of 10 M HCl, causing the suspension to turn brown. To this was added 1 g of FeCl₂·4H₂O in 10 mL H₂O. The solution was shaken then left at 4° C. for 16 hours. This suspension was extracted with diethyl ether (3×50 mL) to give a bright green organic phase, the combined organics were washed with saturated sodium chloride solution, dried over sodium sulfate, filtered and evaporated to leave a black paste (1.1 g). The pellet was dissolved in chloroform for fractionation.

Chlorophyll and Ferrous chlorin crude fractions were stored at -20° C. Crude extracts were fractionated by

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

Preparation of Flavor Reaction Containing Ferrous Chlorin or Leghemoglobin

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

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

TABLE 16

Flavor Compounds created by both Ferrous 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

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TABLE 16-continued

Flavor Compounds created by both Ferrous Chlorin and LegH with Magic Mix.		
5	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
10	(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

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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	large	large
2-propenal	large	large
3-methyl-2-butenal	large	medium
2-methyl-3-(methylthio) furan	large	large
ethyl acetate	large	medium
methacrolein	large	medium
methyl-thiirane	large	large
methional	large	large
methyl alcohol	large	medium
2-butanone	large	Low
2,3-butanedione	large	medium
acetone	large	large
furan	large	medium
benzaldehyde	large	medium
methyl thioacetate	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

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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-acetyl-1-propene		low	low
2-methyl-butanal		low	medium
1,3-dimethyl-benzene		low	low
octane		low	low
benzene		low	low
benzaldehyde			very high
2-butanone			very high
furfural			very high
thiazole			high
nonanal			high
thiazole			high
2-acetylthiazole			medium
3-methyl-butanal			medium
(Z)-2-heptenal			medium
heptanal			medium
methyl-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

TABLE 18-continued

Flavor molecules created by the combination of LegH and precursor mix.			
Compound	Precursor mix	LegH	Precursor mix + Leg H
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.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 27

<210> SEQ ID NO 1

<211> LENGTH: 161

<212> TYPE: PRT

<213> ORGANISM: *Vigna radiata*

<400> SEQUENCE: 1

```

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

<210> SEQ ID NO 2

<211> LENGTH: 133

<212> TYPE: PRT

<213> ORGANISM: *Methylobacterium thermophilum*

<400> SEQUENCE: 2

```

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

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<210> SEQ ID NO 3
<211> LENGTH: 139
<212> TYPE: PRT
<213> ORGANISM: Aquifex aeolicus

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<400> SEQUENCE: 3

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```

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

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<210> SEQ ID NO 4
<211> LENGTH: 145
<212> TYPE: PRT
<213> ORGANISM: Glycine max

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<400> SEQUENCE: 4

```

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

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-continued

115					120					125						
Ala	Trp	Glu	Val	Ala	Tyr	Asp	Glu	Leu	Ala	Ala	Ala	Ala	Ile	Lys	Lys	Ala
	130					135							140			

Phe
145

<210> SEQ ID NO 5
<211> LENGTH: 162
<212> TYPE: PRT
<213> ORGANISM: Hordeum vulgare

<400> SEQUENCE: 5

Met	Ser	Ala	Ala	Glu	Gly	Ala	Val	Val	Phe	Ser	Glu	Glu	Lys	Glu	Ala
1				5					10					15	

Leu	Val	Leu	Lys	Ser	Trp	Ala	Ile	Met	Lys	Lys	Asp	Ser	Ala	Asn	Leu
		20						25					30		

Gly	Leu	Arg	Phe	Phe	Leu	Lys	Ile	Phe	Glu	Ile	Ala	Pro	Ser	Ala	Arg
		35				40					45				

Gln	Met	Phe	Pro	Phe	Leu	Arg	Asp	Ser	Asp	Val	Pro	Leu	Glu	Thr	Asn
	50					55					60				

Pro	Lys	Leu	Lys	Thr	His	Ala	Val	Ser	Val	Phe	Val	Met	Thr	Cys	Glu
65					70					75					80

Ala	Ala	Ala	Gln	Leu	Arg	Lys	Ala	Gly	Lys	Ile	Thr	Val	Arg	Glu	Thr
			85					90						95	

Thr	Leu	Lys	Arg	Leu	Gly	Gly	Thr	His	Leu	Lys	Tyr	Gly	Val	Ala	Asp
			100					105						110	

Gly	His	Phe	Glu	Val	Thr	Arg	Phe	Ala	Leu	Leu	Glu	Thr	Ile	Lys	Glu
		115					120						125		

Ala	Leu	Pro	Ala	Asp	Met	Trp	Gly	Pro	Glu	Met	Arg	Asn	Ala	Trp	Gly
	130					135					140				

Glu	Ala	Tyr	Asp	Gln	Leu	Val	Ala	Ala	Ile	Lys	Gln	Glu	Met	Lys	Pro
145					150					155					160

Ala Glu

<210> SEQ ID NO 6
<211> LENGTH: 1153
<212> TYPE: PRT
<213> ORGANISM: Magnaporthe oryzae

<400> SEQUENCE: 6

Met	Asp	Gly	Ala	Val	Arg	Leu	Asp	Trp	Thr	Gly	Leu	Asp	Leu	Thr	Gly
1				5					10					15	

His	Glu	Ile	His	Asp	Gly	Val	Pro	Ile	Ala	Ser	Arg	Val	Gln	Val	Met
		20					25						30		

Val	Ser	Phe	Pro	Leu	Phe	Lys	Asp	Gln	His	Ile	Ile	Met	Ser	Ser	Lys
		35				40						45			

Glu	Ser	Pro	Ser	Arg	Lys	Ser	Ser	Thr	Ile	Gly	Gln	Ser	Thr	Arg	Asn
	50					55					60				

Gly	Ser	Cys	Gln	Ala	Asp	Thr	Gln	Lys	Gly	Gln	Leu	Pro	Pro	Val	Gly
65					70					75					80

Glu	Lys	Pro	Lys	Pro	Val	Lys	Glu	Asn	Pro	Met	Lys	Lys	Leu	Lys	Glu
			85					90						95	

Met	Ser	Gln	Arg	Pro	Leu	Pro	Thr	Gln	His	Gly	Asp	Gly	Thr	Tyr	Pro
			100					105						110	

Thr	Glu	Lys	Lys	Leu	Thr	Gly	Ile	Gly	Glu	Asp	Leu	Lys	His	Ile	Arg
				115				120					125		

-continued

Gly Tyr Asp Val Lys Thr Leu Leu Ala Met Val Lys Ser Lys Leu Lys
 130 135 140

Gly Glu Lys Leu Lys Asp Asp Lys Thr Met Leu Met Glu Arg Val Met
 145 150 155 160

Gln Leu Val Ala Arg Leu Pro Thr Glu Ser Lys Lys Arg Ala Glu Leu
 165 170 175

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

Leu Asn Tyr Leu Gly Pro Glu His Ser Tyr Arg Thr Pro Asp Gly Ser
 195 200 205

Tyr Asn His Pro Phe Asn Pro Gln Leu Gly Ala Ala Gly Ser Arg Tyr
 210 215 220

Ala Arg Ser Val Ile Pro Thr Val Thr Pro Pro Gly Ala Leu Pro Asp
 225 230 235 240

Pro Gly Leu Ile Phe Asp Ser Ile Met Gly Arg Thr Pro Asn Ser Tyr
 245 250 255

Arg Lys His Pro Asn Asn Val Ser Ser Ile Leu Trp Tyr Trp Ala Thr
 260 265 270

Ile Ile Ile His Asp Ile Phe Trp Thr Asp Pro Arg Asp Ile Asn Thr
 275 280 285

Asn Lys Ser Ser Ser Tyr Leu Asp Leu Ala Pro Leu Tyr Gly Asn Ser
 290 295 300

Gln Glu Met Gln Asp Ser Ile Arg Thr Phe Lys Asp Gly Arg Met Lys
 305 310 315 320

Pro Asp Cys Tyr Ala Asp Lys Arg Leu Ala Gly Met Pro Pro Gly Val
 325 330 335

Ser Val Leu Leu Ile Met Phe Asn Arg Phe His Asn His Val Ala Glu
 340 345 350

Asn Leu Ala Leu Ile Asn Glu Gly Gly Arg Phe Asn Lys Pro Ser Asp
 355 360 365

Leu Leu Glu Gly Glu Ala Arg Glu Ala Ala Trp Lys Lys Tyr Asp Asn
 370 375 380

Asp Leu Phe Gln Val Ala Arg Leu Val Thr Ser Gly Leu Tyr Ile Asn
 385 390 395 400

Ile Thr Leu Val Asp Tyr Val Arg Asn Ile Val Asn Leu Asn Arg Val
 405 410 415

Asp Thr Thr Trp Thr Leu Asp Pro Arg Gln Asp Ala Gly Ala His Val
 420 425 430

Gly Thr Ala Asp Gly Ala Glu Arg Gly Thr Gly Asn Ala Val Ser Ala
 435 440 445

Glu Phe Asn Leu Cys Tyr Arg Trp His Ser Cys Ile Ser Glu Lys Asp
 450 455 460

Ser Lys Phe Val Glu Ala Gln Phe Gln Asn Ile Phe Gly Lys Pro Ala
 465 470 475 480

Ser Glu Val Arg Pro Asp Glu Met Trp Lys Gly Phe Ala Lys Met Glu
 485 490 495

Gln Asn Thr Pro Ala Asp Pro Gly Gln Arg Thr Phe Gly Gly Phe Lys
 500 505 510

Arg Gly Pro Asp Gly Lys Phe Asp Asp Asp Asp Leu Val Arg Cys Ile
 515 520 525

Ser Glu Ala Val Glu Asp Val Ala Gly Ala Phe Gly Ala Arg Asn Val
 530 535 540

-continued

Pro Gln Ala Met Lys Val Val Glu Thr Met Gly Ile Ile Gln Gly Arg
 545 550 555 560
 Lys Trp Asn Val Ala Gly Leu Asn Glu Phe Arg Lys His Phe His Leu
 565 570 575
 Lys Pro Tyr Ser Thr Phe Glu Asp Ile Asn Ser Asp Pro Gly Val Ala
 580 585 590
 Glu Ala Leu Arg Arg Leu Tyr Asp His Pro Asp Asn Val Glu Leu Tyr
 595 600 605
 Pro Gly Leu Val Ala Glu Glu Asp Lys Gln Pro Met Val Pro Gly Val
 610 615 620
 Gly Ile Ala Pro Thr Tyr Thr Ile Ser Arg Val Val Leu Ser Asp Ala
 625 630 635 640
 Val Cys Leu Val Arg Gly Asp Arg Phe Tyr Thr Thr Asp Phe Thr Pro
 645 650 655
 Arg Asn Leu Thr Asn Trp Gly Tyr Lys Glu Val Asp Tyr Asp Leu Ser
 660 665 670
 Val Asn His Gly Cys Val Phe Tyr Lys Leu Phe Ile Arg Ala Phe Pro
 675 680 685
 Asn His Phe Lys Gln Asn Ser Val Tyr Ala His Tyr Pro Met Val Val
 690 695 700
 Pro Ser Glu Asn Lys Arg Ile Leu Glu Ala Leu Gly Arg Ala Asp Leu
 705 710 715 720
 Phe Asp Phe Glu Ala Pro Lys Tyr Ile Pro Pro Arg Val Asn Ile Thr
 725 730 735
 Ser Tyr Gly Gly Ala Glu Tyr Ile Leu Glu Thr Gln Glu Lys Tyr Lys
 740 745 750
 Val Thr Trp His Glu Gly Leu Gly Phe Leu Met Gly Glu Gly Gly Leu
 755 760 765
 Lys Phe Met Leu Ser Gly Asp Asp Pro Leu His Ala Gln Gln Arg Lys
 770 775 780
 Cys Met Ala Ala Gln Leu Tyr Lys Asp Gly Trp Thr Glu Ala Val Lys
 785 790 795 800
 Ala Phe Tyr Ala Gly Met Met Glu Glu Leu Leu Val Ser Lys Ser Tyr
 805 810 815
 Phe Leu Gly Asn Asn Lys His Arg His Val Asp Ile Ile Arg Asp Val
 820 825 830
 Gly Asn Met Val His Val His Phe Ala Ser Gln Val Phe Gly Leu Pro
 835 840 845
 Leu Lys Thr Ala Lys Asn Pro Thr Gly Val Phe Thr Glu Gln Glu Met
 850 855 860
 Tyr Gly Ile Leu Ala Ala Ile Phe Thr Thr Ile Phe Phe Asp Leu Asp
 865 870 875 880
 Pro Ser Lys Ser Phe Pro Leu Arg Thr Lys Thr Arg Glu Val Cys Gln
 885 890 895
 Lys Leu Ala Lys Leu Val Glu Ala Asn Val Lys Leu Ile Asn Lys Ile
 900 905 910
 Pro Trp Ser Arg Gly Met Phe Val Gly Lys Pro Ala Lys Asp Glu Pro
 915 920 925
 Leu Ser Ile Tyr Gly Lys Thr Met Ile Lys Gly Leu Lys Ala His Gly
 930 935 940
 Leu Ser Asp Tyr Asp Ile Ala Trp Ser His Val Val Pro Thr Ser Gly
 945 950 955 960
 Ala Met Val Pro Asn Gln Ala Gln Val Phe Ala Gln Ala Val Asp Tyr

-continued

965					970					975					
Tyr	Leu	Ser	Pro	Ala	Gly	Met	His	Tyr	Ile	Pro	Glu	Ile	His	Met	Val
			980					985					990		
Ala	Leu	Gln	Pro	Ser	Thr	Pro	Glu	Thr	Asp	Ala	Leu	Leu	Leu	Gly	Tyr
		995					1000					1005			
Ala	Met	Glu	Gly	Ile	Arg	Leu	Ala	Gly	Thr	Phe	Gly	Ser	Tyr	Arg	Glu
	1010					1015					1020				
Ala	Ala	Val	Asp	Asp	Val	Val	Lys	Glu	Asp	Asn	Gly	Arg	Gln	Val	Pro
1025					1030					1035					1040
Val	Lys	Ala	Gly	Asp	Arg	Val	Phe	Val	Ser	Phe	Val	Asp	Ala	Ala	Arg
			1045						1050						1055
Asp	Pro	Lys	His	Phe	Pro	Asp	Pro	Glu	Val	Val	Asn	Pro	Arg	Arg	Pro
			1060					1065					1070		
Ala	Lys	Lys	Tyr	Ile	His	Tyr	Gly	Val	Gly	Pro	His	Ala	Cys	Leu	Gly
		1075					1080					1085			
Arg	Asp	Ala	Ser	Gln	Ile	Ala	Ile	Thr	Glu	Met	Phe	Arg	Cys	Leu	Phe
1090						1095					1100				
Arg	Arg	Arg	Asn	Val	Arg	Arg	Val	Pro	Gly	Pro	Gln	Gly	Glu	Leu	Lys
1105				1110						1115					1120
Lys	Val	Pro	Arg	Pro	Gly	Gly	Phe	Tyr	Val	Tyr	Met	Arg	Glu	Asp	Trp
			1125						1130						1135
Gly	Gly	Leu	Phe	Pro	Phe	Pro	Val	Thr	Met	Arg	Val	Met	Trp	Asp	Asp
			1140						1145					1150	

Glu

<210> SEQ ID NO 7

<211> LENGTH: 530

<212> TYPE: PRT

<213> ORGANISM: Fusarium oxysporum

<400> SEQUENCE: 7

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

-continued

180					185					190					
Gly	Gly	Val	His	Ser	Val	Asp	Phe	Pro	Glu	Ile	Val	Gly	Ile	Lys	Ala
	195						200					205			
Asp	Pro	Asn	Asn	Asp	Thr	Asn	Val	Pro	Phe	Gln	Lys	Asp	Val	Ser	Ser
210						215					220				
Phe	His	Asn	Gly	Ile	Val	Thr	Glu	Tyr	Leu	Ala	Gly	Thr	Ser	Lys	Asn
225					230					235					240
Pro	Leu	Val	Ala	Ser	Lys	Asn	Ala	Thr	Phe	His	Ser	Asp	Lys	Arg	Ile
				245					250					255	
Phe	Asp	Asn	Asp	Lys	Ala	Thr	Met	Lys	Lys	Leu	Ser	Thr	Lys	Ala	Gly
	260							265					270		
Phe	Asn	Ser	Met	Cys	Ala	Asp	Ile	Leu	Thr	Arg	Met	Ile	Asp	Thr	Val
	275						280					285			
Pro	Lys	Ser	Val	Gln	Leu	Thr	Pro	Val	Leu	Glu	Ala	Tyr	Asp	Val	Arg
	290					295					300				
Pro	Tyr	Ile	Thr	Glu	Leu	Ser	Leu	Asn	Asn	Lys	Asn	Lys	Ile	His	Phe
305					310					315					320
Thr	Gly	Ser	Val	Arg	Val	Arg	Ile	Thr	Asn	Asn	Ile	Arg	Asp	Asn	Asn
				325					330					335	
Asp	Leu	Ala	Ile	Asn	Leu	Ile	Tyr	Val	Gly	Arg	Asp	Gly	Lys	Lys	Val
	340							345					350		
Thr	Val	Pro	Thr	Gln	Gln	Val	Thr	Phe	Gln	Gly	Gly	Thr	Ser	Phe	Gly
		355					360						365		
Ala	Gly	Glu	Val	Phe	Ala	Asn	Phe	Glu	Phe	Asp	Thr	Thr	Met	Asp	Ala
	370					375					380				
Lys	Asn	Gly	Ile	Thr	Lys	Phe	Phe	Ile	Gln	Glu	Val	Lys	Pro	Ser	Thr
385					390					395					400
Lys	Ala	Thr	Val	Thr	His	Asp	Asn	Gln	Lys	Thr	Gly	Gly	Tyr	Lys	Val
				405					410					415	
Asp	Asp	Thr	Val	Leu	Tyr	Gln	Leu	Gln	Gln	Ser	Cys	Ala	Val	Leu	Glu
		420						425					430		
Lys	Leu	Pro	Asn	Ala	Pro	Leu	Val	Val	Thr	Ala	Met	Val	Arg	Asp	Ala
	435						440						445		
Arg	Ala	Lys	Asp	Ala	Leu	Thr	Leu	Arg	Val	Ala	His	Lys	Lys	Pro	Val
	450					455					460				
Lys	Gly	Ser	Ile	Val	Pro	Arg	Phe	Gln	Thr	Ala	Ile	Thr	Asn	Phe	Lys
465					470					475					480
Ala	Thr	Gly	Lys	Lys	Ser	Ser	Gly	Tyr	Thr	Gly	Phe	Gln	Ala	Lys	Thr
				485					490					495	
Met	Phe	Glu	Glu	Gln	Ser	Thr	Tyr	Phe	Asp	Ile	Val	Leu	Gly	Gly	Ser
		500						505					510		
Pro	Ala	Ser	Gly	Val	Gln	Phe	Leu	Thr	Ser	Gln	Ala	Met	Pro	Ser	Gln
		515					520						525		
Cys	Ser														
	530														

<210> SEQ ID NO 8

<211> LENGTH: 358

<212> TYPE: PRT

<213> ORGANISM: Fusarium graminearum

<400> SEQUENCE: 8

Met	Ala	Ser	Ala	Thr	Arg	Gln	Phe	Ala	Arg	Ala	Ala	Thr	Arg	Ala	Thr
1				5					10					15	

-continued

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

<210> SEQ ID NO 10
 <211> LENGTH: 121
 <212> TYPE: PRT
 <213> ORGANISM: Tetrahymena pyriformis

<400> SEQUENCE: 10

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

<210> SEQ ID NO 11
 <211> LENGTH: 117
 <212> TYPE: PRT
 <213> ORGANISM: Paramecium caudatum

<400> SEQUENCE: 11

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

-continued

Asp Val Val Thr Val
115

<210> SEQ ID NO 12
<211> LENGTH: 147
<212> TYPE: PRT
<213> ORGANISM: *Aspergillus niger*

<400> SEQUENCE: 12

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

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

<400> SEQUENCE: 13

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

-continued

Met Lys Pro Asp Ala
165

<210> SEQ ID NO 14
<211> LENGTH: 169
<212> TYPE: PRT
<213> ORGANISM: *Oryza sativa* subsp. *japonica*

<400> SEQUENCE: 14

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

<210> SEQ ID NO 15
<211> LENGTH: 160
<212> TYPE: PRT
<213> ORGANISM: *Arabidopsis thaliana*

<400> SEQUENCE: 15

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

-continued

Tyr Asp His Leu Val Ala Ala Ile Lys Ala Glu Met Asn Leu Ser Asn
145 150 155 160

<210> SEQ ID NO 16
<211> LENGTH: 147
<212> TYPE: PRT
<213> ORGANISM: Pisum sativum

<400> SEQUENCE: 16

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

<210> SEQ ID NO 17
<211> LENGTH: 145
<212> TYPE: PRT
<213> ORGANISM: Vigna unguiculata

<400> SEQUENCE: 17

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

-continued

<210> SEQ ID NO 18
 <211> LENGTH: 154
 <212> TYPE: PRT
 <213> ORGANISM: Bos taurus

<400> SEQUENCE: 18

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

<210> SEQ ID NO 19
 <211> LENGTH: 154
 <212> TYPE: PRT
 <213> ORGANISM: Sus scrofa

<400> SEQUENCE: 19

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

<210> SEQ ID NO 20

-continued

<211> LENGTH: 154

<212> TYPE: PRT

<213> ORGANISM: Equus caballus

<400> SEQUENCE: 20

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

<210> SEQ ID NO 21

<211> LENGTH: 152

<212> TYPE: PRT

<213> ORGANISM: Nicotiana benthamiana

<400> SEQUENCE: 21

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

<210> SEQ ID NO 22

<211> LENGTH: 132

<212> TYPE: PRT

<213> ORGANISM: Bacillus subtilis

-continued

<400> SEQUENCE: 22

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

<210> SEQ ID NO 23

<211> LENGTH: 131

<212> TYPE: PRT

<213> ORGANISM: *Corynebacterium glutamicum*

<400> SEQUENCE: 23

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

<210> SEQ ID NO 24

<211> LENGTH: 124

<212> TYPE: PRT

<213> ORGANISM: *Synechocystis* sp.

<400> SEQUENCE: 24

Met Ser Thr Leu Tyr Glu Lys Leu Gly Gly Thr Thr Ala Val Asp Leu
 1 5 10 15
 Ala Val Asp Lys Phe Tyr Glu Arg Val Leu Gln Asp Asp Arg Ile Lys
 20 25 30

-continued

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Ala Phe Leu Ala Gln Ile Phe Glu Gly Pro Lys Gln Tyr Gly Gly Arg
 50                               55                               60

Pro Met Asp Lys Thr His Ala Gly Leu Asn Leu Gln Gln Pro His Phe
65                               70                               75                               80

Asp Ala Ile Ala Lys His Leu Gly Glu Arg Met Ala Val Arg Gly Val
85                               90                               95

Ser Ala Glu Asn Thr Lys Ala Ala Leu Asp Arg Val Thr Asn Met Lys
100                              105                              110

Gly Ala Ile Leu Asn Lys
115

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<210> SEQ ID NO 27
<211> LENGTH: 136
<212> TYPE: PRT
<213> ORGANISM: Bacillus megaterium

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<400> SEQUENCE: 27

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Met Arg Glu Lys Ile His Ser Pro Tyr Glu Leu Leu Gly Gly Glu His
 1                               5                               10                               15

Thr Ile Ser Lys Leu Val Asp Ala Phe Tyr Thr Arg Val Gly Gln His
20                               25                               30

Pro Glu Leu Ala Pro Ile Phe Pro Asp Asn Leu Thr Glu Thr Ala Arg
35                               40                               45

Lys Gln Lys Gln Phe Leu Thr Gln Tyr Leu Gly Gly Pro Ser Leu Tyr
50                               55                               60

Thr Glu Glu His Gly His Pro Met Leu Arg Ala Arg His Leu Pro Phe
65                               70                               75                               80

Glu Ile Thr Pro Ser Arg Ala Lys Ala Trp Leu Thr Cys Met His Glu
85                               90                               95

Ala Met Asp Glu Ile Asn Leu Glu Gly Pro Glu Arg Asp Glu Leu Tyr
100                              105                              110

His Arg Leu Ile Leu Thr Ala Gln His Met Ile Asn Ser Pro Glu Gln
115                              120                              125

Thr Asp Glu Lys Gly Phe Ser His
130                              135

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What is claimed is:

1. A ground beef-like food product comprising:
 - a) 0.1%-5% by weight of a heme-containing protein comprising an amino acid sequence having at least 80% sequence identity to the polypeptide set forth in SEQ ID NO:4;
 - b) a compound selected from glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, and galactose, and mixtures of two or more thereof;
 - c) at least 10 mM of a compound selected from cysteine, cystine, selenocysteine, thiamine, methionine, and mixtures of two or more thereof; and
 - d) 10% or more by weight of one or more plant proteins, wherein the ground beef-like food product contains no animal products, and wherein cooking the ground beef-like food product results in the production of at least two volatile compounds which have a beef-associated aroma.
2. The ground beef-like food product of claim 1, wherein the heme-containing protein comprises an amino acid sequence having at least 85% sequence identity to the polypeptide set forth in SEQ ID NO:4.
3. The ground beef-like food product of claim 1, wherein the heme-containing protein comprises an amino acid sequence having at least 90% sequence identity to the polypeptide set forth in SEQ ID NO:4.
4. The ground beef-like food product of claim 1, wherein the heme-containing protein comprises an amino acid sequence having at least 95% sequence identity to the polypeptide set forth in SEQ ID NO:4.
5. The ground beef-like food product of claim 1, wherein the heme-containing protein comprises a polypeptide as set forth in SEQ ID NO:4.
6. The ground beef-like food product of claim 1, further comprising one or more of inosine, inosine monophosphate (IMP), guanosine, guanosine monophosphate (GMP), and adenosine monophosphate (AMP).
7. The ground beef-like food product of claim 1, further comprising one or more of beta-carotene, alpha-tocopherol, caffeic acid, propyl gallate, and epigallocatechin gallate.
8. The ground beef-like food product of claim 1, further comprising one or more of a vegetable oil, an 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, canola oil, flaxseed oil, coconut oil, and mango oil.

9. The ground beef-like food product of claim 1, further comprising coconut oil.

10. The ground beef-like food product of claim 1, further comprising lactic acid.

11. The ground beef-like food product of claim 1, comprising a textured vegetable protein.

12. The ground beef-like food product of claim 1, wherein the ground beef-like food product has a pink to red color before cooking to indicate a raw or uncooked state.

13. The ground beef-like food product of claim 1, wherein at least a portion of the ground beef-like food product, upon cooking, transitions in color from a pink to red color in a raw or uncooked state to a lighter pink to brown color in a partially cooked to fully cooked state.

14. The ground beef-like food product of claim 1, comprising about 5.6 to about 20 mM of the compound selected from glucose, ribose, fructose, lactose, xylose, arabinose, glucose-6-phosphate, maltose, and galactose, and mixtures of two or more thereof.

15. The ground beef-like food product of claim 1, comprising about 0.8 mM to about 10 mM cysteine.

16. The ground beef-like food product of claim 1, comprising about 0.1 mM to about 2 mM thiamine.

17. The ground beef-like food product of claim 1, wherein the at least two volatile compounds are selected from 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, dihydro-3-(2H)-thiophenone, 5-methyl-2-thiophenecarboxaldehyde, 3-methyl-2-thiophenecarboxaldehyde, 2-methyl-thiazole, dimethyl sulfide,

decanal, 5-ethylidihydro-2(3H)-furanone, dihydro-5-pentyl-2(3H)-furanone, 2-octanone, 3,5-octadien-2-one, p-Cresol, and hexanoic acid.

18. The ground beef-like food product of claim 1, wherein cooking comprises heating the ground beef-like food product at 150° C. for about 3 to about 5 minutes.

19. The ground beef-like food product of claim 1, further comprising one or more of acetic acid, lactic acid, glycolic acid, citric acid, succinic acid, tartaric acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha linolenic acid, gamma linolenic acid, arachidic acid, arachidonic acid, behenic acid, and erucic acid.

20. The ground beef-like food product of claim 1, wherein cooking the ground beef-like food product results in the production of at least five volatile compounds which have a beef-associated aroma.

21. The ground beef-like food product of claim 1, wherein cooking the ground beef-like food product results in the production of at least ten volatile compounds which have a beef-associated aroma.

22. The ground beef-like food product of claim 1, wherein cooking the ground beef-like food product results in the production of at least twenty volatile compounds which have a beef-associated aroma.

23. The ground beef-like food product of claim 1, wherein the at least two volatile compounds are 2-methyl-furan and bis(2-methyl-3-furyl)disulfide.

* * * * *

From: [Yingling, Gary L.](#)
To: [McMahon, Carrie](#)
Cc: [Vaughn, Jessica L.](#)
Subject: FW: FDA request
Date: Wednesday, November 29, 2017 2:04:20 PM
Attachments: [94561475_2 Memorandum to Carrie McMahon Regarding Requested Publication Information.docx](#)
[Jin et al-2017-Molecular Nutrition & Food Research.pdf](#)
[Fraser et al-2017-BioRxIV.pdf](#)

Carrie: Here is a memo on the publications and a copy of publication that is available in the public file. gary

Gary L. Yingling
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1111 Pennsylvania Avenue, NW | Washington, DC 20004-2541
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Morgan Lewis

MEMORANDUM

TO: Carrie McMahon
FROM: Gary L. Yingling
DATE: November 29, 2017
SUBJECT: Requested Publication Information

Manuscript #1

Manuscript describing the allergenicity and toxicity assessment of LegH Prep performed by Dr. Richard Goodman was published in the peer reviewed journal *Molecular Nutrition and Food Research* on October 17, 2017. The electronic publication was available online ahead of print on September 18, 2107.

Article citation:

Y. Jin, X. He, K. Andoh-Kumi, R. Z. Fraser, M. Lu, R. E. Goodman, Evaluating potential Risks of Food Allergy and Toxicity of Soy Leghemoglobin Expressed in *Pichia pastoris*. *Mol. Nutr. Food Res.* 2017, 1700297. <https://doi.org/10.1002/mnfr.201700297>

Manuscript #2

Manuscript describing the genotoxicology evaluation and rat feeding studies performed on LegH Prep is under review in the peer reviewed *International Journal of Toxicology*. Impossible has received and responded to reviewer comments and expects a decision by the end of the year. Concurrent with submission to the *International Journal of Toxicology*, a pre-print on the article (prior to peer review) was posted online at bioRxiv.org to make available to the public (citation below)

Manuscript submitted to *International Journal of Toxicology* (IJT): 10/1/17

Manuscript posted on bioRxiv: 10/1/17

Received peer review from IJT: 10/25/17

Submitted revised manuscript and point-by-point response addressing reviewer comments to IJT: 11/23/17

Current status (as of 11/27/17): Under review at IJT

Article citation @ bioRxiv

Rachel Z. Fraser, Mithila Shitut, Puja Agrawal, Odete Mendes, Sue Klapholz. Safety evaluation of soy leghemoglobin protein preparation derived from *Pichia pastoris*, intended for use as a flavor catalyst in plant-based meat. bioRxiv 196766; doi: <https://doi.org/10.1101/196766>

62 pages have been removed in accordance with copyright laws. The removed reference citations are:

Y.Jin, X. He, K. Andoh-Kumi, R. Z. Fraser, M. Lu, R. E. Goodman, Evaluating potential Risks of FoodAllergy and Toxicity of Soy Leghemoglobin Expressed in *Pichia pastoris*. *Mol. Nutr. Food Res.* 2017,1700297. <https://doi.org/10.1002/mnfr.201700297>

Rachel Z. Fraser, Mithila Shitut, Puja Agrawal, Odete Mendes, Sue Klapholz. Safety evaluation of soy leghemoglobin protein preparation derived from *Pichia pastoris*, intended for use as a flavor catalyst in plant-based meat. *bioRxiv* 196766; doi: <https://doi.org/10.1101/196766>

From: [Yingling, Gary L.](#)
To: [McMahon, Carrie](#)
Cc: [Vaughn, Jessica L.](#)
Subject: translation of handwritten notes for GRAS
Date: Tuesday, December 05, 2017 2:10:09 PM
Attachments: [Translation 43166 CAL 5 Dec 17.pdf](#)
[43166 Clarification COW 5 Dec 17.pdf](#)
[Translation 44856.pdf](#)

The three attachments are memos from the PSI Study providing a typed version of the handwritten language. The pages from the GRAS Notice 737 are 184, 228 and 733. Please contact us if you need any additional information. gary

Gary L. Yingling

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Product Safety Labs

STUDY NOTES

PSL Study# 43166 (report page 111)

Translation of Handwritten Notes: Water Analysis 2nd Quarter 2016:

SOP 508 Dev, testing not repeated on sipper tube (CAL 7/6/16)

Room 13 faucet not analyzed due to room being out of service (CAL 7/7/16)

Animals no longer given water from unfiltered source (Nonedstrom), missing sampling of rom has no impact.

Sipper bottled tested OK, no need to test sipper top (tube) (CAL 7/7/17)

CML 12/5/17

Product Safety Labs

STUDY NOTES

PSL Study# 43166 (report page 67)

Translation of Handwritten Notes: Eye Exam Letter

Clarification of Eurofins Study No.: 43166, PSL No. 160720-5R (COW 11/18/16)

COW 12/3/17

Product Safety Labs

STUDY NOTES

PSL Study# 44856 (report page 67)

Translation of Handwritten Notes: Water Analysis 1st Quarter 2017:

Room #7 unavailable for testing due to special procedure in place for guinea pigs. Room #4 is the next room

in line.

CMC 12/5/2017

From: [Yingling, Gary L.](#)
To: [McMahon, Carrie](#)
Cc: [Vaughn, Jessica L.](#)
Subject: RE: GRN 737 - publication status of Fraser et al manuscript
Date: Tuesday, February 27, 2018 5:37:49 PM

Dear Dr. McMahon: I left you a voice mail today that the International Journal of Toxicology has agreed that it will publish the paper by Dr Frazer et al on "Safety Evaluation of Soy Leghemoglobin Protein Preparation Derived from Pichia pastorised Intended for Use as a Flavor Catalyst in Plant-based Meat. Please contact me if you have any questions. gary

Gary L. Yingling
Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW | Washington, DC 20004-2541
Direct: +1.202.739.5610 | Main: +1.202.739.3000 | Fax: +1.202.739.3001
gary.yingling@morganlewis.com | www.morganlewis.com

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From: [Vaughn, Jessica L.](#)
To: [McMahon, Carrie](#)
Cc: [Yingling, Gary L.](#)
Subject: RE: GRN 737 - publication status of Fraser et al manuscript
Date: Tuesday, March 06, 2018 10:46:02 AM
Attachments: [image007.png](#)
[Manuscript ID UITO-2017-0326.R2.pdf](#)
[Decision Letter.pdf](#)
[Manuscript ID UITO-2017-0326.R2 Supplement.pdf](#)

Dr. McMahon,

Please find attached the final manuscript and supplementary figures, and the letter of acceptance, on behalf of Impossible Foods. The letter of acceptance indicates that typesetting will be complete in 4-6 weeks, but Impossible Foods has not yet received a formal publication date.

Please let us know if you need anything further.

Regards,
Jessica Vaughn

Jessica L. Vaughn, Ph.D.

Regulatory Scientist

Morgan, Lewis & Bockius LLP

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Direct: 202.739.5332 | Main: 202.739.3000 | Fax: 202.739.3001

jessica.vaughn@morganlewis.com | www.morganlewis.com

Assistant: Anne-Marie J. Drakes | 202.739.5747 | anne-marie.drakes@morganlewis.com

From: McMahon, Carrie [mailto:Carrie.McMahon@fda.hhs.gov]

Sent: Monday, March 05, 2018 5:45 PM

To: Yingling, Gary L. <gary.yingling@morganlewis.com>

Cc: Vaughn, Jessica L. <jessica.vaughn@morganlewis.com>

Subject: RE: GRN 737 - publication status of Fraser et al manuscript

[EXTERNAL EMAIL]

Dear Mr. Yingling,

REGARDING: GRN 737, publication status of manuscript

Thank you for notifying us that the Fraser *et al* manuscript has been accepted by IJT for publication.

- Did IJT provide the authors with written confirmation of the manuscript acceptance? If so, we would appreciate a copy of the acceptance confirmation.
- Impossible Foods submitted a copy of the manuscript to FDA in support of its GRAS conclusion. If changes were made to the manuscript in response to IJT reviewer comments, please provide a copy of the final accepted version of the manuscript.
- Is there a projected issue/date for the manuscript's publication?

Regards,

Carrie McMahon, Ph.D.

Consumer Safety Officer

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
U.S. Food and Drug Administration
Tel: 240-402-1202
Carrie.McMahon@fda.hhs.gov



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International Journal of Toxicology

Decision Letter (UITO-2017-0326.R3)

From: marybeth.genter@uc.edu

To: rachel.fraser@impossiblefoods.com

CC:

Subject: International Journal of Toxicology - Decision on Manuscript ID UITO-2017-0326.R3

Body: 27-Feb-2018

Dear Dr Fraser:

****Apologies for the delay in responding, but the reviewer did accelerate her review, and it's all good!****

Ref: Safety evaluation of soy leghemoglobin protein preparation derived from *Pichia pastoris*, intended for use as a flavor catalyst in plant-based meat

I am pleased to accept your paper in its current form for publication in International Journal of Toxicology. The manuscript will now be forwarded to the publisher for copy editing and typesetting.

You will receive proofs in approximately 4-6 weeks. Please watch for a follow-up email which will guide you through our online Copyright Transfer process.

Thank you for your contribution to International Journal of Toxicology, and we look forward to receiving further submissions from you.

Sincerely,
Mary Beth Genter, PhD, DABT, ATS
Editor-in-Chief, International Journal of Toxicology
marybeth.genter@uc.edu

Date Sent: 27-Feb-2018

 Close Window

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69 pages have been removed in accordance with copyright laws. The removed reference citation is:

Fraser RZ, Shitut M, Agrawal P, Mendes O, Klapholz S. Safety Evaluation of Soy Leghemoglobin Protein Preparation Derived From *Pichia pastoris*, Intended for Use as a Flavor Catalyst in Plant-Based Meat. *Int J Toxicol*. 2018 May/Jun;37(3):241-262. doi: 10.1177/1091581818766318. Epub 2018 Apr 11. PMID: 29642729; PMCID: PMC5956568.

From: [Yingling, Gary L.](#)
To: [McMahon, Carrie](#)
Cc: [Vaughn, Jessica L.](#)
Subject: tox article published
Date: Thursday, April 19, 2018 9:36:10 AM

Carrie: I realize that you do not “need” this reference to the online publication but since I had it, am passing it along. gary

Tox paper is now published online and available to the general public via open access:

<http://journals.sagepub.com/eprint/3FNzWrRdPanXheUdTigx/full>

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McMahon, Carrie

From: Yingling, Gary L. <gary.yingling@morganlewis.com>
Sent: Friday, June 29, 2018 4:13 PM
To: McMahon, Carrie
Subject: : Amendment to GRN 000737
Attachments: GRN 000737 Amended Language.pdf

Dear Ms. McMahon:

On behalf of Impossible Foods, we are providing an updated Intended Conditions of Use section with clarified language that is intended to replace p. 4 of GRN 000737. The changes are shown in redline in the attached document.

[gary](#)

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PART 1: SIGNED STATEMENTS AND CERTIFICATIONS

1. This GRAS notice is submitted in accordance with 21 C.F.R. Part 170, Subpart E.
2. Name and Address of Submitting Company:
Impossible Foods Inc.
525 Chesapeake Drive
Redwood City, CA 94063
Phone: (650) 461-4385
3. Name of Notified Substance: Soy leghemoglobin protein preparation
4. Intended Conditions of Use:
 - a. List of foods and/or drinking water to be added to: Ground beef analogue products intended to be cooked to optimize flavor.
 - b. Proposed levels of use: Soy leghemoglobin protein preparation will be added to the ground beef analogue product to deliver not more than 0.8% soy leghemoglobin protein.
 - c. Purpose of substance in the food product: The primary purpose of the characterizing component of soy leghemoglobin protein preparation, soy leghemoglobin protein, is to ~~create a~~optimize flavor ~~impact~~ in ground beef analogue products intended to be cooked. In addition, soy leghemoglobin protein has a nutritive value as a source of iron, analogous to the role of myoglobin as an iron source in meat.
 - d. Subpopulation expected to consume product: (if appropriate): No subpopulations are anticipated.
5. Statutory Basis for GRAS Conclusion:

The statutory basis for the GRAS conclusion for soy leghemoglobin protein preparation is scientific procedures. Impossible Foods has assembled the scientific data to conclude that soy leghemoglobin protein preparation is generally recognized as safe for use as a component of ground beef analogue products.
6. It is the view of Impossible Foods that the substance is not subject to premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Impossible Foods' conclusion that soy leghemoglobin preparation is GRAS for the intended use as a component of ground beef analogue products.
7. Availability of Information for FDA Review: The data and information that are the basis for Impossible Foods GRAS determination are available for FDA's review, and copies

From: [Yingling, Gary L.](#)
To: [McMahon, Carrie](#)
Cc: [Vaughn, Jessica L.](#)
Subject: FDA's question on allergen in fermentation media
Date: Tuesday, July 10, 2018 8:53:41 AM

Carrie: Here is the statement from Impossible. I hope this statement responds to what FDA needs. If not, please get back to me. gary

We confirm there are no components in the fermentation media derived from one of the eight most common allergenic foods under the Food Allergen Labeling and Consumer Protection Act (FALCPA): milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat and soybeans. The soy leghemoglobin is produced during the fermentation.

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