Supplementary material for Nei *et al.* (February 20, 2001) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.051611498

Table 3. 104 protein sequence data from five vertebrate species (data from Hovergen)

	Protein	Estimate (a) of gamma parameter	Number of amino acids used	Accession number*
1	Insulin-like growth factor I	0.588	112	FAM00007
2	Prepro-insulin	0.811	106	FAM00008
3	Heparin cofactor II	1.139	453	FAM000037
4	Muscarinic acetylcholine receptor M1	0.454	459	FAM000059d
5	Angiotensin II receptor type I	0.994	354	FAM000084
6	Alcohol dehydrogenase-1 (Adh-1)	0.938	228	FAM000111
7	Gastric H+, K+ ATPase beta-subunit	1.144	281	FAM000133
8	Na+, K+ ATPase beta-subunit	0.718	301	FAM000135
9	Acetylcholine receptor delta-subunit	0.886	508	FAM000152a
10	Acetylcholine gamma-subunit	1.307	500	FAM000152b
11	Acetylcholine receptor alpha-subunit	0.409	456	FAM000152e
12	Serum albumin	4.916	596	FAM000192
13	Gap junction protein connexin-4	0.23	377	FAM000242
14	Alpha-A-crystallin	0.887	163	FAM000248a
15	Cu-Zn superoxide dismutase (SOD1)	0.853	150	FAM000256
16	Neural cadherin (N-cadherin)	0.355	900	FAM000258
17	Basic fibroblast growth factor (bFGF)	0.369	152	FAM000274
18	Preproglucagon	1.358	131	FAM000286
19	Bone morphogenetic protein-4 (BMP-4)	0.392	394	FAM000306a
20	Bone morphogenetic protein-2 (BMP-2)	0.581	351	FAM000306b
21	Tissue inhibitor of metalloproteinase-3 (TIMP-3)	0.512	209	FAM000330b
22	Aldehyde dehydrogenase I (ALDH-1)	0.595	499	FAM000337
23	Ornithine decarboxylase (ODC)	0.489	448	FAM000348
24	Osteonectin (Sparc)	0.367	292	FAM000350
25	Poly (ADP-ribose) synthetase	0.634	990	FAM000356
26	Phosphatase 2A-beta (PP2A-beta)	0.039	309	FAM000362
27	Preprosomatostatin	0.398	115	FAM000371
28	ABC transporter (TAP2)	2.661	656	FAM000385
29	Tryptophan hydroxylase (Tph)	0.668	437	FAM000388b
30	Iron binding protein (ferretin) heavy chain	0.403	176	FAM000533
31	Glutamine synthetase	0.46	368	FAM000534
32	Retinol-binding protein (RBP)	0.924	189	FAM000555
33	Type IIA activin receptor	0.286	513	FAM000561a
34	Alpha-enolase (2-phospho-D-glycerate hydrolase)	0.252	434	FAM000627
35	Cyclin D1	0.711	290	FAM000646a
36	Complement component C	1.96	1556	FAM000664

37	Zona pellucida glycoprotein-3 (ZP3)	3.254	397	FAM000667
38	Helix-loop-helix protein (Id-2)	0.243	133	FAM000769
39	Cardiac troponin	0.74	162	FAM000868a
40	Hepatic nuclear factor 1-alpha unit	0.608	585	FAM001004b
41	Retinal degeneration slow (RDS) protein	0.602	343	FAM001091
42	Hepatocyte growth factor (HGF)	0.883	706	FAM001247a
43	Hepatocyte growth factor-like protein	1.243	692	FAM001247c
44	Vimentin (intermediate filament)	0.365	455	FAM001327a
45	Middle weight neurofilament (NF-M)	0.838	748	FAM001328b
46	Cardiac calsequestrin	1.301	380	FAM001355
47	Steroidogenic factor-1 SF-1	0.505	451	FAM001365a
48	Thyroid hormone receptor alpha (c-erbA)	0.288	408	FAM001369a
49	Thyroid hormone receptor beta subunit	0.192	368	FAM001369b
50	Very low density lipoprotein receptor (VLDLR)	0.398	830	FAM001390b
51	Domain transcription factor LIM-1	0.187	401	FAM001396
52	Cyclin-dependent kinase (CDC2)	0.422	296	FAM001432b
53	Neural cell-adhesion molecule (N-CAM)	0.576	716	FAM001444
54	Neural cell surface protein F	0.665	991	FAM001446a
55	Focal adhesion kinase (FAK)	0.182	1051	FAM001463
56	Protein-tyrosine (src) kinase (CSK)	0.224	450	FAM001467
57	Neural receptor protein-tyrosine kinase (trkB)	0.508	797	FAM001471b
58	Insulin-like growth factor I receptor	0.434	1301	FAM001471e
59	c-met oncogene	0.881	1366	FAM001472
60	c-kit receptor tyrosine kinase	0.957	924	FAM001478b
61	Oocyte maturation factor Mos (c-mos)	1.456	324	FAM001493
62	Fibronectin (FN1)	0.839	1069	FAM001519
63	Zinc finger protein slug (SLUG)	0.665	148	FAM001601
64	Wilms tumor protein	0.35	375	FAM001607
65	Integrin beta-1 subunit	0.376	789	FAM001621
66	Muscle regulatory factor (MRF4)	0.38	239	FAM001622c
67	Achaete-scute homologue	0.293	189	FAM001624
68	Collagenase-3 (matrix metalloproteinase-13)	1.02	378	FAM001631c
69	Fos-related antigen-2 (fra-2)	0.336	313	FAM001679a
70	Follistatin (activin antagonist)	0.354	315	FAM001730
71	Structure-specific recognition protein 1 (SSRP1)	0.305	529	FAM001987
72	Gonadotrophin-releasing hormone (GnRH)	3.093	87	FAM002205
73	Alpha 2,8-sialyltransferase-1 (pst-1)	0.577	353	FAM002490
74	Syndecan-3 (Syn-3)	2.598	317	FAM002852
75	Distal-less 3 (Dlx-5) homeobox	0.331	278	FAM002888a
76	Homeobox protein Nkx-2.5 subunit	1.097	285	FAM002890b
77	Corticotrophin-releasing factor type1 receptor	0.375	410	FAM003058b

78	T-cell leukemia (LERK-2)	0.439	326	FAM003670
79	Delta-1 protein	0.584	705	FAM003932a
80	Conductin/axin (AXIN)	0.667	818	FAM006148
81	Transforming growth factor – beta3	0.653	389	FAM000027†
82	Na, K-ATPase beta-2 subunit (ATP1B2)	1.61	271	FAM000134†
83	Embryonic beta-type globin	1.364	137	FAM000214†
84	Alpha globin	1.277	142	FAM000215†
85	Heat shock protein	0.138	650	FAM000300†
86	Lactate dehydrogenase-A	0.616	330	FAM000364a†
87	Histone H2A	0.149	129	FAM000427†
88	bcl-x regulator of cell death	0.764	200	FAM000506†
89	c-ets-1 (p54) protein	0.202	437	FAM000514†
90	Glutamine synthetase	0.471	368	FAM000534a†
91	Bone morphogenetic receptor	0.28	522	FAM000306d†
92	Cyclin (D2, D3)	0.291	288	FAM000646†
93	Furin	0.522	768	FAM000747†
94	Hepatocyte growth factor (HGF)	1.261	686	FAM001247†
95	Liver glyceraldehyde-3-phosphate dehydrogenase	0.243	332	FAM001297†
96	Nucleolin	1.793	605	FAM001318†
97	Vimentin	0.361	454	FAM001327†
98	Lamin B1	0.675	578	FAM001331†
99	Nucleolar phosphoprotein B23 (NPM1)	1.022	285	FAM001359†
100	N-myc protein	0.698	398	FAM001383†
101	CDC2 involved in cell cycle control	0.332	296	FAM001432†
102	Protein tyrosine phosphatase (PTPase-alpha)	0.280	778	FAM001447†
103	Phosphotyrosil protein phosphatase	0.084	591	FAM001456†
104	M-sam fibroblast growth factor	0.314	807	FAM001479†

*Gene family names from the Hovergen database (pbil.univ-lyon1.fr/search/query.html).
†Entries 81--104 from Hovergen 1996 Release.

Tree of Life Reveals Clock-Like Speciation and Diversification

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Associate editor: Emma Teeling

Abstract

Genomic data are rapidly resolving the tree of living species calibrated to time, the timetree of life, which will provide a framework for research in diverse fields of science. Previous analyses of taxonomically restricted timetrees have found a decline in the rate of diversification in many groups of organisms, often attributed to ecological interactions among species. Here, we have synthesized a global timetree of life from 2,274 studies representing 50,632 species and examined the pattern and rate of diversification as well as the timing of speciation. We found that species diversity has been mostly expanding overall and in many smaller groups of species, and that the rate of diversification in eukaryotes has been mostly constant. We also identified, and avoided, potential biases that may have influenced previous analyses of diversification including low levels of taxon sampling, small clade size, and the inclusion of stem branches in clade analyses. We found consistency in time-to-speciation among plants and animals, ~2 My, as measured by intervals of crown and stem species times. Together, this clock-like change at different levels suggests that speciation and diversification are processes dominated by random events and that adaptive change is largely a separate process.

Key words: **biodiversity, diversification, speciation, timetree, tree of life.**

Introduction

The evolutionary timetree of life (TTOL) is needed for understanding and exploring the origin and diversity of life (Hedges and Kumar 2009; Nei 2013). For this reason, scientists have been leveraging the genomics revolution and major statistical advances in molecular dating techniques to generate divergence times between populations and species. Collectively, tens of thousands of species have been timed, and new divergence time estimates are appearing in hundreds of publications each year (supplementary fig. S1, Supplementary Material online). A global synthesis of these results will allow direct comparison of the TTOL with the fossil record and Earth history and provide new opportunities for discovery of patterns and processes that operated in the past. The TTOL is also essential for studying the multidimensional nature of biodiversity and predicting how anthropogenic changes in our environment will impact the distribution and composition of biodiversity in the future (Hoffmann et al. 2010). A robust TTOL will provide a framework for research in diverse fields of science and medicine and a stimulus for science education. Data now exist for building a synthetic species-level TTOL of substantial size from the growing knowledge (supplementary fig. S1, Supplementary Material online).

There are challenges in synthesizing a global TTOL. The most common approach for constructing a large timetree using a sequence alignment or super alignment is possible (Smith and O'Meara 2012; Tamura et al. 2012), but not generally practical because of data matrix sparseness. For example, genes appropriate for closely related species are unalignable at higher levels, and those appropriate for higher levels are too conserved for resolving relationships of species. Disproportionate attention to some species, such as model organisms and groups of general interest (e.g., mammals and birds), also results in an uneven distribution of knowledge. In addition, computational limits are reached for Bayesian timing methods involving more than a few hundred species (Battistuzzi et al. 2011; Jetz et al. 2012).

Here, we have taken an approach to build a global TTOL by means of a data-driven synthesis of published timetrees into a large hierarchy. We have synthesized timetrees and related information in 2,274 molecular studies, which we collected and curated in a knowledgebase (Hedges et al. 2006) (supplementary Materials and Methods, Supplementary Material online). We mapped timetrees and divergence data from those studies on a robust and conservative guidetree based on community consensus (National Center for Biotechnology Information 2013) and used those times to resolve polytomies and derive nodal times in the TTOL (supplementary fig. S2, Supplementary Material online). We present this synthesis here, for use by the community, and explore how it bears on evolutionary hypotheses and mechanisms of speciation and diversification.

Results

A Global Timetree of Species

Our TTOL contains 50,632 species (fig. 1). Nearly all (~99.5%) of the 1.9 million described species of living organisms are

Article

Fast Track

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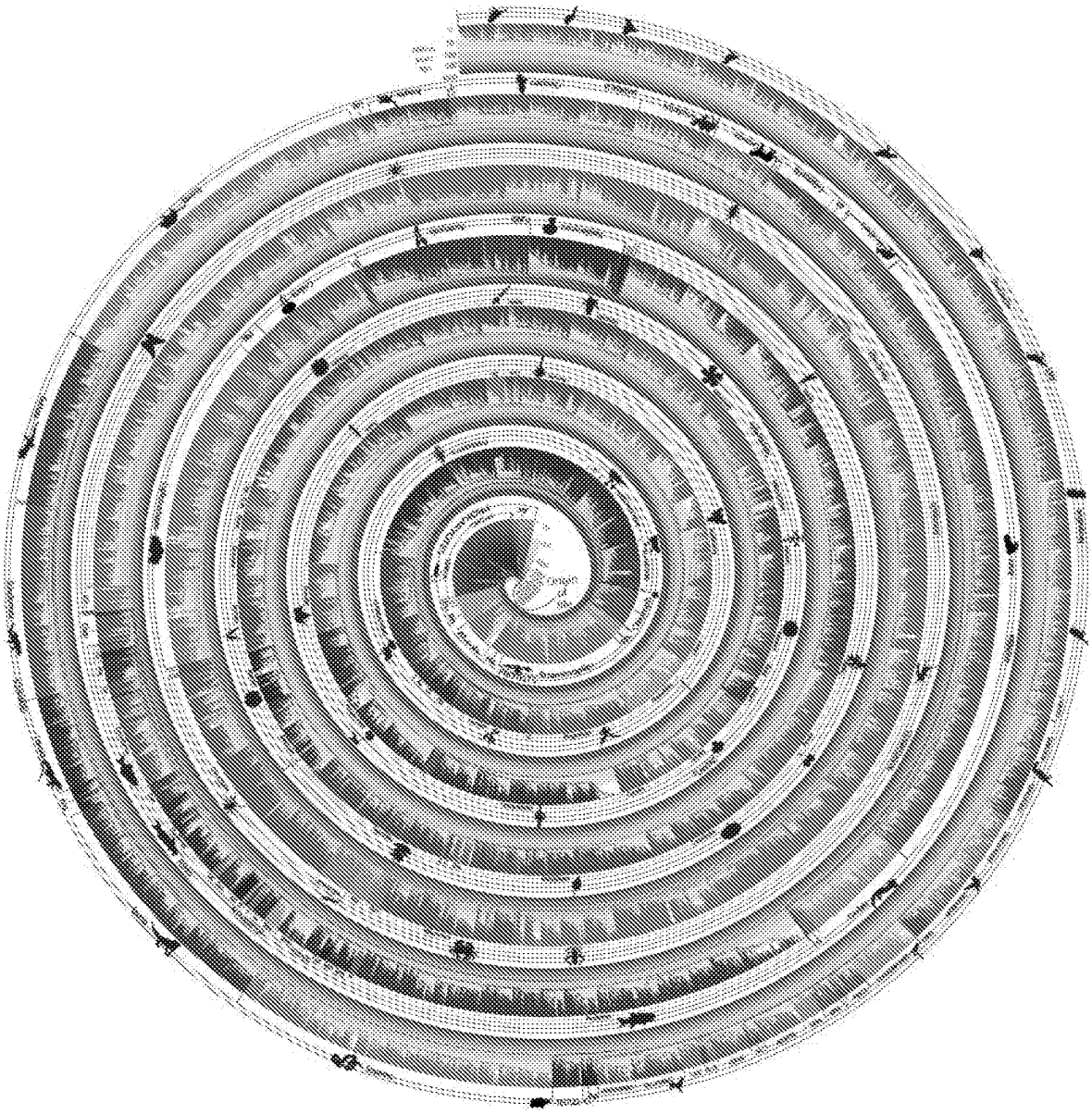

Fig. 1. (2 columns) A timetree of 50,632 species synthesized from times of divergence published in 2,274 studies. Evolutionary history is compressed into a narrow strip and then arranged in a spiral with one end in the middle and the other on the outside. Therefore, time progresses across the width of the strip at all places, rather than along the spiral. Time is shown in billions of years on a log scale and indicated throughout by bands of gray. Major taxonomic groups are labeled and the different color ranges correspond to the main taxonomic divisions of our tree.

eukaryotes (Costello et al. 2013), and the proportion is similar (99.7%) in our TTOL (fig. 1). The naturally unbalanced shape of the TTOL, for example, with more eukaryote than prokaryote species, allowed us to present it in a unique spiral format to accommodate its large size. A "timeline" can be envisioned for each species in the TTOL, based on its sequence of branching events back in time to the origin of life. For example, a timeline from humans (fig. 2) captures evolutionary events that have received the most queries (74%) by users of the TimeTree knowledgebase (Hedges et al. 2006) (supplementary Materials and Methods, Supplementary Material online).

Linnaean taxonomic ranks exhibit temporal inconsistencies (fig. 3) as has been suggested in smaller surveys (Hedges and Kumar 2009; Avise and Liu 2011). For example, a class (higher rank) of angiosperm averages younger than an order (lower rank) of fungus, and an order of animal averages younger than a genus (low rank) of basidiomycete fungus. Ranks for prokaryotes are all older than the corresponding ranks of eukaryotes, with a genus of eubacteria averaging 715.7 ± 139.4 Ma compared with 12.6 ± 1.2 Ma for a genus of eukaryotes (fig. 3 and supplementary Materials and Methods, Supplementary Material online). These inconsistencies will cause difficulties in any scientific study comparing

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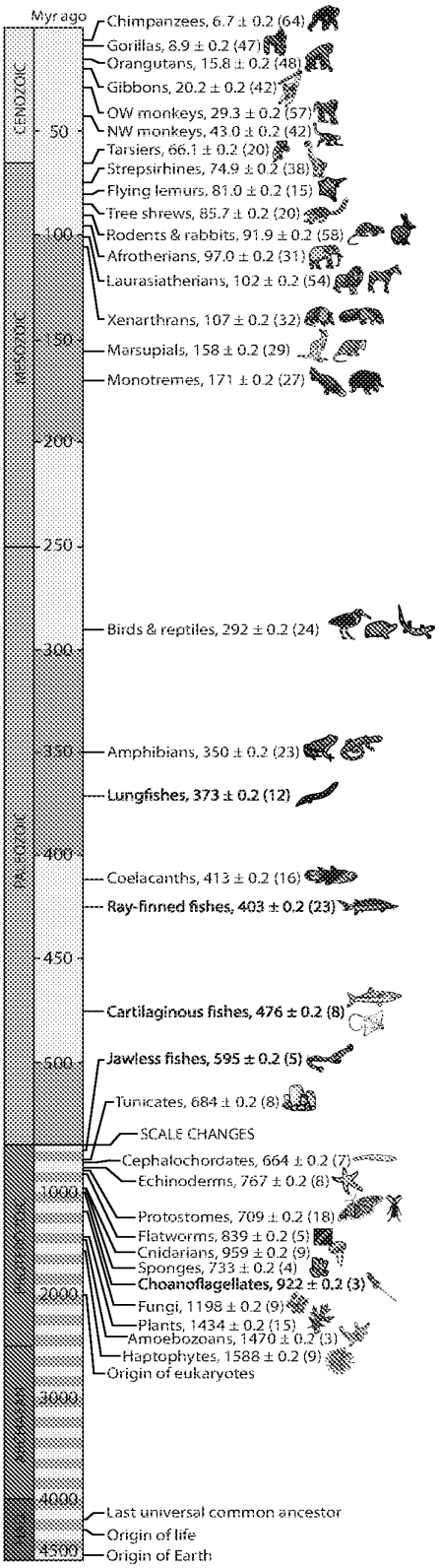

FIG. 2. (1 column) A timeline from the perspective of humans, showing divergences with other groups of organisms. In each case, mean ± standard error (among studies) is shown, with number of studies in parentheses. Also shown are the times for the origin of life, eukaryotes, and last universal common ancestor (Hedges and Kumar 2009).

different types of organisms, where Linnaean rank is assumed to have a temporal equivalence.

Diversification

The large TTOL afforded us the opportunity to examine patterns of lineage splitting across the diversity of eukaryotes (we omit prokaryotes in our TTOL analyses because they have an arbitrary species definition). Under models of "expansion," diversity will continue to expand, either at an increasing diversification rate (hyper-expansion), the same rate (constant expansion), or decreasing rate (hypo-expansion). Saturation, on the other hand, refers to a drop in rate to zero as diversity reaches a plateau (equilibrium), possibly because of density-dependent biotic factors such as species interactions (Morlon 2014). Most recent analyses, but not all (Venditti et al. 2010; Jetz et al. 2012), have suggested that hypo-expansion is the predominant pattern in the tree of life, although there has been considerable debate as to the importance of timescales, biotic or abiotic factors, and potential biases in the analyses (Sepkoski 1984; Benton 2009; Morlon et al. 2010; Rabosky et al. 2012; Cornell 2013; Rabosky 2013).

Instead, we find that constant expansion and hyper-expansion are the dominant patterns of lineage diversification in the TTOL (fig. 4*a*). Our result was statistically significant irrespective of the method used, including diversification rate tests and simulations, a coalescent approach (not conducted for the TTOL), gamma tests, a clade age-size relationship (see Materials and Methods), and branch length distribution analysis (supplementary Materials and Methods, Supplementary Material online). The rate of diversification did not decrease over the history of eukaryotes (fig. 4). The same results were retrieved with another method (supplementary fig. S3, Supplementary Material online); we did not interpret the data before 1,000 Ma because only 37 lineages were present at this time, also explaining the large confidence intervals for this period. The observed diversification closely matched a simulated pure birth–death (BD) model, increasing slightly during the last 200 My (fig. 4*a* and *b*). The terminal drop in rate at ~1 Ma is a normal characteristic of diversification plots (Etienne and Rosindell 2012) related to the taxonomic level selected for the study, in this case species. This "taxonomic bias" (rate drop) occurs at different times (Hedges and Kumar 2009) when genera, families, or other taxa are selected for study, because lower level clades (in this case, populations destined to become species) are omitted.

The TTOL was partitioned into 58 diverse Linnaean clades (Actinopterygii, Afrotheria, *Amazona*, *Ambystoma*, Amphibia, Amphibolurinae, Amphisbaenia, angiosperms, Anguimorpha, *Anolis*, Anseriformes, birds, Boidae, *Bolitoglossa*, Canidae, Cetardiodactyla, *Chamaeleo*, Chondrichthyes, Ciconiiformes, Columbiformes, Cracidae, Cuculidae, *Eleutherodactylus*, Erinaceidae, *Falco*, Feliformia, Gymnophiona, gymnosperms, *Hylarana*, Hyloidea, *Hynobius*, *Hyperolius*, Iguanidae, Kinyongia, Lacertidae, *Liolaemus*, mammals, Megapodiidae, Metatheria, Moniliformopses, Musophagidae, Palaeognathae, Pelobatoidea, Perissodactyla, Phaeophyceae, Phasianidae, *Plethodon*, Podicipedidae,

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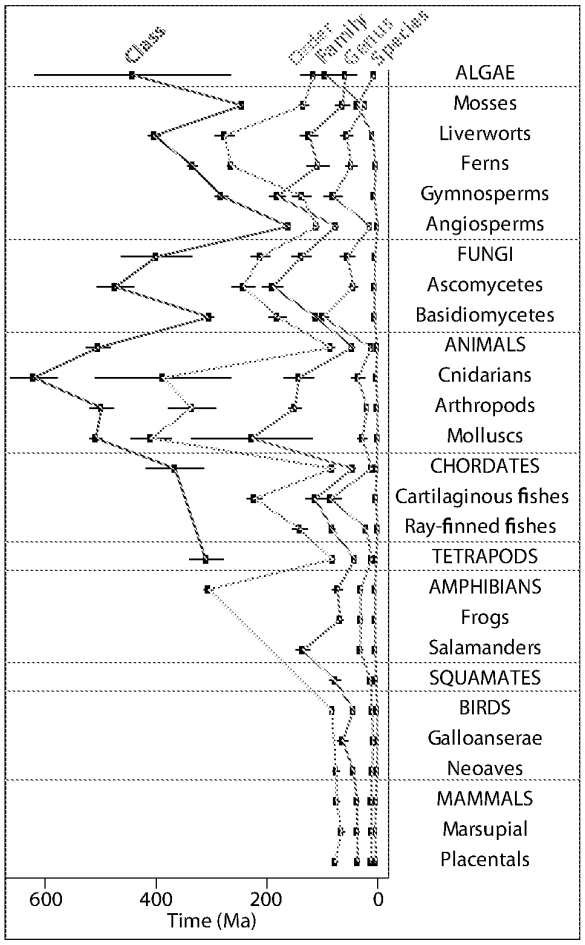

Fig. 3. (1 column) Temporal relationships of Linnaean ranks of eukaryotes, showing mode and 95% confidence intervals. Prokaryotes are not shown because of large differences in scale (supplementary Materials and Methods, Supplementary Material online).

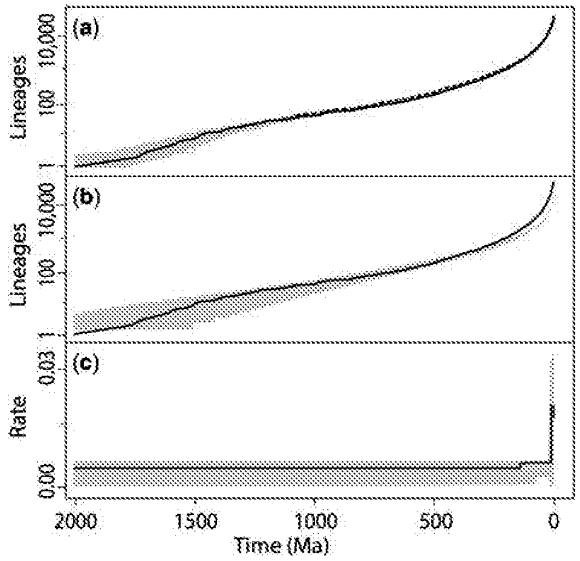

Fig. 4. (1 column) Patterns of lineage diversification. (*a*) Cumulative lineages-through-time (LTT) curve for eukaryotes (50,455 sp.), in black, showing the number of lineages through time (unsmoothed, dashed; smoothed, solid) and variance (red, 500 replicates). (*b*) Same LTT curve (black line), but compared with a simulated constant-expansion LTT curve (λ (speciation rate) = 0.073 and μ (extinction rate) = 0.070) shown as ± 99% confidence intervals (red). (*c*) Diversification rate plot of same data showing only significant changes in rate as determined in maximum-likelihood tests; variance (red, 500 replicates) shown as ± 99% intervals.

Primates, Ranoidea, Squamata, *Suncus*, Thamnophis, Tupaiidae, Ursidae, *Varanus*, Xenarthra, and *Xenopus*) for coalescence and gamma tests encompassing 44,958 total species (supplementary Materials and Methods, Supplementary Material online). The clades were chosen to represent taxonomic diversity and a range of clade size, clade age, and taxonomic sampling. Species sampling levels were high, with a median clade size of 66% of known species and 84% of the clades having 40% or more of known species. Among the 10 largest clades (Actinopterygii, Amphibia, angiosperms, birds, Ciconiiformes, Hyloidea, mammals, Moniliformopses, Ranoidea, Squamata), only one (angiosperms) showed hypo-expansion using the coalescent method, while the others were exclusively hyper-expanding. Low levels of sampling are known to bias toward hypo-expansion (Cusimano and Renner 2010; Moen and Morlon 2014), and the same clade (angiosperms) was also the most poorly sampled (5%). Also, there was no support in those clades for a decline in diversification rate using the gamma test. Although some methods can account for incomplete random sampling, the nonrandom sampling typically used by systematists (e.g., selecting for deeply branching lineages) is not accounted for by current methods and will bias results in favor of hypo-expansion (Cusimano and Renner 2010).

Focusing on the 48 smaller clades (e.g., genera and families) of tetrapods, we found that these likewise favored the expanding models (84% of clades showing significant results) rather than the saturation model (see Materials and Methods). Of those, most (87%) were either expanding or hyper-expanding, with only 13% hypo-expanding. Concerning the gamma test, 67% of these smaller clades did not show a decline in diversification rate through time, as was true for eukaryotes as a whole (fig. 4; gamma statistic = 136.4, $P = 1$). Hyper-expanding clades were significantly larger (fig. 5*a*; $x^{\text{Pleasechecktrpezium}}$, 831 vs. 91 species; $P < 0.05$) and older (fig. 5*b*; $x^{\text{Pleasechecktrpezium}}$, 104 vs. 57 Ma; $P < 0.001$) than other clades (see Materials and Methods). We also found that TTOL branch-length distribution fits an exponential distribution significantly better than other models (see Materials and Methods), agreeing with an earlier study (Venditti et al. 2010) using a small data set and approach.

Diversification by expansion predicts a significant correlation between clade age and clade size (see Materials and Methods). Therefore, we evaluate the expansion hypothesis in two densely sampled groups: Mammals (5,363 species) and birds (9,879 species). We also tested the effect of stem versus crown age (fig. 5*c*) on clade sizes (supplementary Materials and Methods, Supplementary Material online); stem age

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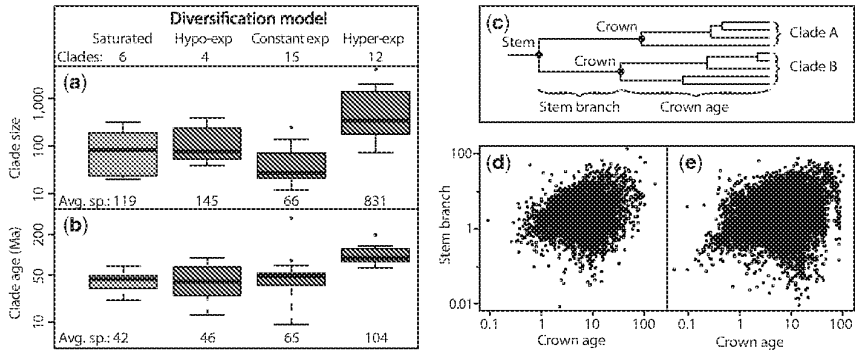

Fig. 5. (1 column) Diversification analyses of major Linnaean clades in the timetree of life. (*a*–*b*) Results of coalescent analyses testing models of diversification. Of 48 tetrapod clades, 37 showed significant model selected and they were used in these analyses. (*a*) Effect of clade size (number of described species). (*b*) Effect of clade age. (*c*) Diagram illustrating difference between stem and crown age for two clades. (*d*–*e*) Relationship of stem branch and crown age in mammals (*d*; $r^2 = 0.07$) and birds (*e*; $r^2 = 0.04$).

includes the time elapsed on the branch leading to the crown. We examined 1,990 clades in two separate analyses: Families (153 and 113 nonnested clades for birds and mammals, respectively) and genera (1,115 and 609 nonnested clades for birds and mammals, respectively). In each case, the relationship was highly significant for crown age ($r = 0.43$–0.47, $P < 0.001$) but nonsignificant or weakly significant ($r = 0.02$–0.09, $P = 0.01$–0.82) using stem age (see Materials and Methods). These results demonstrate a significant relationship between clade age and clade size in major groups of vertebrates, and provide an explanation why this was not observed in past studies, as they used stem ages (crown age + stem branch time).

Our results show that it is best to avoid stem branch time, because the length of any (stem) branch in the tree should not be related to the time depth of the descendant node (crown age; fig. 5*d* and *e*). Therefore, the use of stem branch time will introduce large statistical noise and make the test extremely conservative. For example, when considering every node in a timetree of species, the coefficient of variation of stem branch length relative to crown age is over 200% in the best sampled groups, mammals (208%) and birds (224%). That noise is further weighted by the pull of the present (Nee et al. 1994), which, we determined, adds 40% time (median) to crown age at any given node (if stem age is used instead of crown age), in separate analyses of birds, mammals, and all eukaryotes. This is because the pull of the present creates longer internal branches deeper in a tree, as more lineages are pruned by extinction. Therefore, the use of stem branches in diversification analyses adds noise (variance) and gives increased weight to that noise. We believe that the stronger signal of constant expansion in our results, compared with earlier studies that have supported hypo-expansion and saturation, is in part because we have identified and avoided some biases (e.g., sampling effort, clade size, and stem age) that can impact diversification analyses.

Speciation

The rate of formation of new species is the primary input into the diversification of life over time. This process starts as two or more lineages split from an existing species at a node in the tree. To learn more about the timing of speciation, we assembled a separate data set of studies containing timetrees that included populations and species of eukaryotes (supplementary Materials and Methods, Supplementary Material online). Considering a standard model of speciation based on geographic isolation, in the absence of gene flow (Sousa and Hey 2013), we estimated the time required for speciation to occur. For a given species, the time-to-speciation (TTS), after isolation, must fall between the crown and the stem age of the species (fig. 6*a*). All lineages younger than the crown age of a species lead to populations that are still capable of interbreeding and, therefore, must be younger than the TTS. On the other hand, the stem age is the time when a species joins its closest relative and, therefore, it must be older than the TTS. A sampling omission of either a population or species could lower the crown age estimate or raise the stem age estimate, but the resulting interval would still contain the TTS. Although coalescence of alleles may lead to overestimates of time (Nei and Kumar 2000), those times were interpreted as population and species splits, not allelic splits, in the published studies reporting them. Also, calibrations can ameliorate the effect of allelic coalescence because they are usually based on population and species divergences, not coalescence events. We conducted a simulation to test this approach (supplementary Materials and Methods, Supplementary Material online) finding that the true TTS is estimated precisely with a mode because of skewness of the statistical distribution and it is robust to undersampling of lineages, either younger (populations) or older (species). We also found that the true TTS was not affected by the addition of 10% noise (intervals that do not include the TTS) and only weakly affected (2.5%) by adding a large amount (50%) of noise.

Analyses of three disparate taxonomic groups (vertebrates, arthropods, and plants) with greatly varying generation times and life histories produced similar TTS of ~2 My (fig. 6*b*). For comparison, divergence times among closely related species of prokaryotes, which are defined in practical terms (Cohan 2002), are about 50–100 times older (supplementary Materials and Methods, Supplementary Material online).

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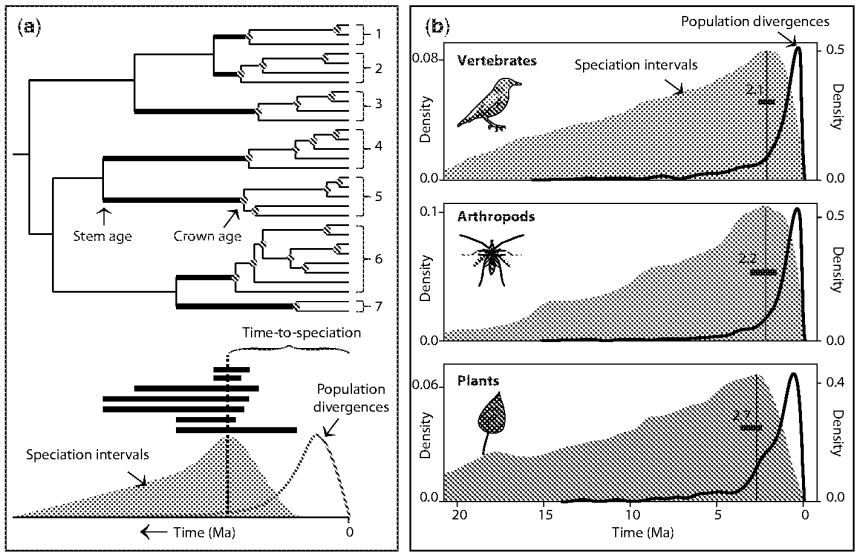

FIG. 6. (2 columns) Estimation of time-to-speciation. (*a*) Analytical design showing expected results. Intervals between stem age and crown age of seven species contain time-to-speciation (dashed line represents the modal time-to-speciation). Divergences among populations, and corresponding histogram, are in red. (*b*) Colored histograms of observed time-to-speciation, showing modes (vertical lines) and confidence intervals (bars) in vertebrates ($N = 213$, 2.1 Ma, 1.74–2.55 Ma), arthropods ($N = 85$, 2.2 Ma, 1.57–3.07 Ma), and plants ($N = 55$, 2.7 Ma, 2.37–3.63 Ma). Black curves to the right are histograms of population divergences.

The observed similarity of TTS in diverse groups of multicellular eukaryotes suggests a model of speciation that places importance on the time that two populations are isolated (fig. 7). If speciation is an outcome of the buildup of genic incompatibilities (GIs) between isolated populations (Coyne and Orr 2004; Matute et al. 2010), then the continuous fixation of selectively neutral mutations in two populations (Zuckerkandl and Pauling 1965; Kimura 1968) will accumulate with time and eventually lead to a number, or fraction, of GIs (here, "S-value") that will cause postzygotic reproductive isolation (fig. 7*c*). In essence, speciation—in the strictest sense—can be defined as this specific moment in time, a "point of no return," because reproductive incompatibility and isolation are inevitable at that point. The diverging lineages will remain independent forever or until they become extinct. If they do come back into contact (fig. 7*a*), they might hybridize briefly but would then undergo reinforcement, leading to prezygotic reproductive isolation. We focus here on the major model, geographic isolation, but time constraints should be similar with ecological speciation, and other models exist (Coyne and Orr 2004).

A speciation clock has been suggested previously (Coyne and Orr 1989), where GIs buildup linearly over time in postzygotic reproductive isolation. However, current evidence indicates a faster-than-linear ("snowball") rate of buildup of GIs in postzygotic reproductive isolation (Matute et al. 2010). The snowball pattern of increase in GIs is not a problem for a speciation clock as long as the time of attainment of the S-value is similar in different species. Our evidence from the TTS analysis (fig. 7*b*) suggests this to be the case.

Relatively few populations will remain isolated until the point of no return because environments often change rapidly in the short term, raising and lowering barriers to gene flow (fig. 7*a*). This is amplified by a preponderance of low incline landscapes (Strahler 1952), resulting in a greater impact of climate and sea level change. Therefore, the buildup of GIs will be reset many times before a successful speciation event occurs (fig. 7*c*). This resetting, which results in failed isolates, may explain "barcode gaps" (Puillandre et al. 2011), which we interpret as prespeciation loss of potential lineages. We estimate that branches between nodes in well-sampled (> 20% coverage) groups of the TTOL are, on average, 4–5 My long using a slope method (4.92 ± 0.32 My) and a coalescent method (4.21 ± 0.76 My) (supplementary Materials and Methods, Supplementary Material online). This indicates that branch time is longer than TTS, and includes a significant "lag time," referring to any time along a branch that is not part of a successful speciation event, such as the resetting of the speciation clock by reversal of the buildup of GIs. The high variance of branch length in trees (fig. 5*d* and *e*) may reflect the stochastic nature of environmental change and isolation of populations, contributing to a "diversification clock" (fig. 4). This is consistent with the absence of a strong correlation between reproductive isolation and diversification rate in some taxa (Rabosky and Matute 2013).

In relating this speciation model to a phylogeny (fig. 7*d*), it is evident that the split of two lineages in a tree is not the speciation event but rather the moment of isolation of two or more populations that will remain isolated until the point of no return. For example, if we assume a TTS of 2 My, then two populations that only recently became species would have split ~2 Ma (fig. 7*d*, species 1–2). Yet, much deeper in a tree, where branches may be tens of millions of years long, the TTS is a relatively small fraction of total branch time, which makes

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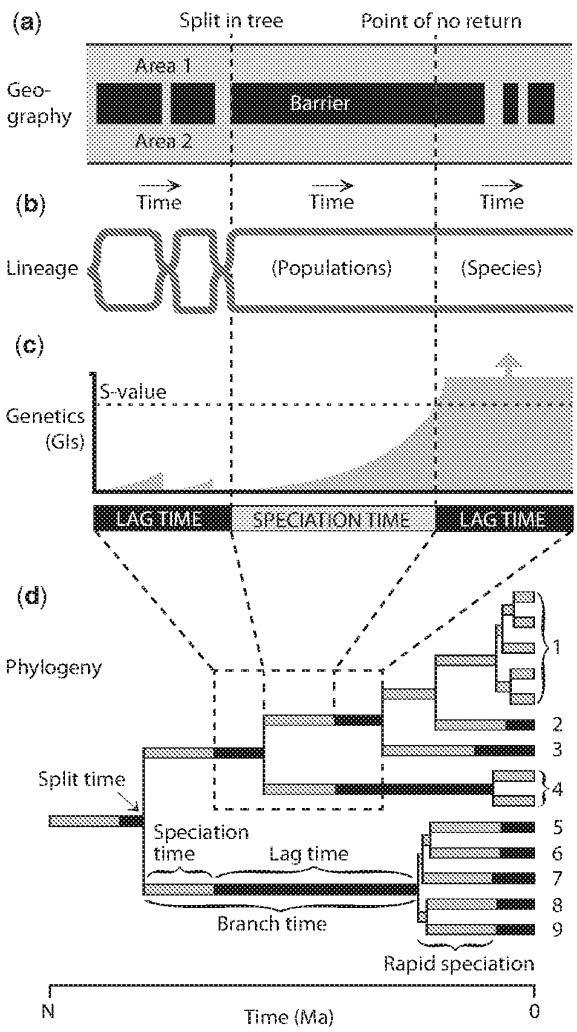

Fig. 7. (1 column) Summary model of speciation. (*a*) Biogeographic history showing the contact and isolation of areas occupied by the two populations. (*b*) Phylogenetic lineages showing times of independence (two lineages) and times of interbreeding (one lineage). (*c*) Genic incompatibilities between the two populations, showing how they accrue at a time-dependent rate during geographic isolation, reset to zero during contact (interbreeding), increase to the S-value (the number of GIs that will cause speciation, the point of no return), and continue increasing beyond the S-value despite later contact of the newly formed species. (*d*) Hypothetical phylogeny, with numbered species, illustrating parameters of speciation in (*a*–*c*) to splits and branches in a tree.

a splitting event more-or-less equivalent to the speciation event. Furthermore, branch splitting leading to eventual species formation can happen simultaneously, in many lineages, as long as the resulting populations remain isolated until the point of no return (fig. 7*d*, species 5–9).

Discussion

These results have implications for patterns of species diversification and the interpretation of timetrees. If adaptation is largely decoupled from speciation, we should not expect it to be a driver of speciation as is frequently assumed. Also, we should not expect to see major diversification rate increases following mass extinction events, even though large adaptive changes took place at those times. That expectation is realized in our analyses (fig. 4) where we see constant splitting through time across the two major Phanerozoic extinction events (251 and 66 Ma). Likewise, our diversification analyses of smaller groups, such as birds and mammals (supplementary fig. S4, Supplementary Material online), and past studies of those groups (Bininda-Emonds et al. 2007; Meredith et al. 2011; Jetz et al. 2012) have not found rate increases immediately following the end-Cretaceous mass extinction event (66 Ma). Rate decreases from the extinction events themselves are not expected because of the inability to detect, in trees, a large proportion of species extinctions that occurred (Nee and May 1997).

The consistency in TTS among groups found here suggests that the time-based acquisition of GIs, and not adaptive change, is driving reproductive isolation, almost in a neutral process. By implication, geographically isolated populations, even if morphologically different and diagnosable, are not expected to be species until they have reached the point of no return. This is because some differentiation and adaptive change should occur in isolation, and many isolates will be ephemeral (Rosenblum et al. 2012), merging with other isolates or disappearing and never becoming species that enter the tree of life. More population data are needed before it is possible to identify the point of no return with precision. Nonetheless, these data suggest that, in most cases, described species separated by only tens of thousands of years are not real species. The Linnaean rank of subspecies, which has declined in use for decades, might be appropriate for such diagnosable isolates that have not yet reached the point of no return. Oversplitting of species by taxonomists may explain the sharp peak in diversification (hyper-expansion) in the last 10 My of eukaryote history (fig. 4*b*). On the other hand, it could also result from statistical (small sample) artifacts that were published in many different studies (summarized in the TTOL). Therefore, further analysis of that unusual rate spike is warranted.

In summary, the diversification of life as a whole is expanding at a constant rate and only in some small clades (<500 species) is there evidence of decline or saturation in diversification rate. We believe these results can be explained as many different groups of organisms undergoing expansion and contraction through time, with those patterns captured in different stages (expanding, slowing, or in equilibrium). If true, the predominant pattern of expansion in large clades is expected from the law of large numbers, where such smaller, random events would average to a constant rate. Constant expansion also follows from random environmental changes leading to isolation and speciation. Rate constancy is consistent with the fossil record (Benton 2009) and does not deny the importance of biotic factors in evolution (Ricklefs 2007), but it suggests an uncoupling of speciation and adaptation. Cases where the phenotype has changed little (e.g., cryptic species) or greatly during the TTS are interpreted here as evidence of uncoupling. The lineage splitting seen in trees probably reflects, in most instances, random environmental events leading to isolation of populations,

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and potentially many in a short time. However, the relatively long TTS (~2 My), a process resulting from random genetic events, will limit the number of isolates that eventually become species. Under this model, diversification is the product of those two random processes, abiotic and genetic, and rate increases (bursts of speciation) are more likely to be associated with long-term changes in the physical environment (e.g., climate, sea level) causing extended isolation rather than with short-term ecological interactions. However, reductions in extinction rate could also explain increases in diversification rate. Adaptive change that characterizes the phenotypic diversity of life would appear to be a separate process from speciation. Although a full understanding of these processes remains a challenge, determining how speciation and adaptation are temporally related would be an important "next step."

Materials and Methods

Detailed methods are described in supplementary Materials and Methods, Supplementary Material online.

TTOL Data Collection

We synthesized the corpus of scientific literature where the primary research on the TTOL is published. We first identified and collected all peer-reviewed publications in molecular evolution and phylogenetics that reported estimates of time of divergence among species. These included phylogenetic trees scaled to time (timetrees) and occasionally tables of time estimates and regular text. We assembled timetree data from 2,274 studies (http://www.timetree.org/reference_list.php) that have been published between 1987 and April 2013, as well as two timetrees estimated herein (supplementary table S1, Supplementary Material online). Most (96%) of nodal times used were published in the last decade.

TTOL Analytics and Synthesis

We used a hierarchical average linkage method of estimating divergence times (T_s) of clade pairs to build a Super Timetree, along with a procedure for testing and updating topological partitions to ensure the highest degree of consistency with individual timetrees in every study. For the TTOL, uncertainty derived from individual studies is available for each node (supplementary table S2, Supplementary Material online). Branch time modes of different Linnaean categories were estimated (supplementary table S3, Supplementary Material online).

Diversification Analyses

All diversification analyses were performed in R (http://www.r-project.org/) with the APE package (Paradis et al. 2013). The gamma statistic was employed to detect decrease in speciation rate over the history of the tree. To test if diversification is saturated or expanding, we used a coalescent method as well as the relationship between the ages of different clades and their sizes (numbers of species). The number and location of shifts in diversification rates were also tested. We also estimated among-study uncertainty in both the lineages-through-time (LTT) curve and rate test by producing 500 replicates of the TTOL, sampling actual study times at each node randomly. These replicates were then used in the analysis of rate shifts to estimate and test rate change. Results of all diversification analyses were summarized (supplementary tables S4–S10, Supplementary Material online).

Gamma Test

The gamma statistic (Pybus and Harvey 2000) was employed on 59 clades (all eukaryotes plus 58 clades listed in supplementary table S5, Supplementary Material online) to detect decrease in speciation rate over the history of the tree (LASER package; Rabosky and Schliep 2013). These 58 groups were chosen to have an overview of the diversification processes on a diversity of clades across life, in terms of size, sampling effort (above 5%; median 66%, with 84% of the clades having more than 40% coverage), and clade age. Included in the 58 clades are 10 nonnested groups over the major Linnaean groups, which allowed us to draw conclusions about high-level and low-level clades. We further selected 10 nonnested clades from within each of the four well-sampled groups of tetrapods (amphibians, birds, mammals, and squamates). We also analyzed separately the Eukaryote clade because all the other groups are nested within this one.

A negative gamma value reveals a concentration of branching times near the root, meaning a decelerated diversification. Incomplete lineage sampling can affect the result of this test because some tips will be pruned, thus lengthening the branch length in the more recent past (Nee et al. 1994). An incomplete sampling could therefore lead to an erroneous detection of slowdown. To avoid this bias, the Monte Carlo constant rate test (MCCR test) (Pybus and Harvey 2000) was also performed on clades with a significant negative gamma value (LASER package). Here, the critical value for rejecting a constant rate ($\alpha = 0.05$) was calculated by examining the distribution of gamma for simulated trees with the same incomplete lineage sampling proportion as the observed tree. We conducted 5,000 Monte Carlo simulations for each MCCR test. If the MCCR test finds that the detection of slowdown is erroneous, then there is no support for decline in speciation rate for this clade.

Models of Diversification

To test if diversification is saturated or expanding, we used a coalescent method (Morlon et al. 2010) on 58 clades (supplementary table S5, Supplementary Material online). We defined two sets of models: Saturated diversity (models 1 and 2) and expanded diversity (models 3, 4a, 4b, 4c, 4d, 5, and 6). As recommended by the author of the method, we first selected the model with the lowest second-order Akaike's Information Criterion in each subset (saturated vs. expanding models). We then evaluated the relative probability of these two models based of their Akaike weights. The relative probabilities of two models (l and k) were calculated as $\frac{wl}{wl+wk}$ and $\frac{wk}{wl+wk}$, with wl (the model's l Akaike weight) as

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$wl = \frac{\exp(-\triangle l/2)}{\sum_{r=1}^{R}\exp(-\triangle r/2)}$ and R the number of candidate models. This method can account for missing species. We therefore used the number of described species (supplementary table S5, Supplementary Material online) as the number of tips of the phylogenies (N_0). When neither of the two relative probabilities obtained exceeded 0.6, we did not draw a conclusion on this specific clade (referred as "nonsignificant" in supplementary table S5, Supplementary Material online) and the mean λ (between the best expanding and saturating model) was used (supplementary table S4, Supplementary Material online). Statistical tests were performed (supplementary table S6, Supplementary Material online) to compare the four models, saturated (sat), hyper-expanding (exp+), expanding (exp), and hypo-expanding (exp-), at the same time (Kruskall–Wallis rank-sum test) and by pair (Wilcoxon rank-sum test). Another test was performed (*t*-test) to compare the means (described number of species and crown age) between the clades under hyper-expansion and the others (saturated, hypo-expanding, and expanding). We confirmed for all clades that the BD polytomy resolution did not change the results, although a different model was selected for 3 of the 58 clades (5%). This method was computationally unfeasible with the entire TTOL.

Clade Age and Clade Size Analyses

To test, in another way, for expansion (hyper-, constant-, and hypo-) versus saturation of the diversification curve, we evaluated the relationship between the ages of different clades and their sizes (numbers of species) (Cornell 2013). Under the saturated diversity model, no relationship should be observed, in contrast to a positive relationship under models of expanding diversity. For this test, we chose the two best sampled groups, birds and mammals, and performed comparative analyses on clades (all nonnested) of genera (1,115 and 609 for birds and mammals respectively) and families (153 and 113 for birds and mammals, respectively), accounting for phylogeny (phylogenetic generalized linear models) with the CAPER package (Orme et al. 2013). In one set of analyses, we used stem age and in another set, crown age (supplementary table S7, Supplementary Material online) was used.

Detection of Shifts in Diversification Rates

The TREEPAR package (Stadler 2013) was used to estimate the number of changes in diversification rate under a BD model and to obtain estimates of the corresponding diversification rate ($\lambda-\mu$) and the extinction fraction (μ/λ). To analyze our eukaryote timetree, as well as the individual component timetrees of birds and mammals, we used the greedy approach, as described in Stadler (2011). We estimated rates in 0.1 My steps between 2 and 70 Ma and in 10 My steps between 2 and 3,500 My for eukaryotes. The BD shift model (without mass extinction) was used and we obtained maximum-likelihood rate estimates for the different data sets for zero, one, two, three, four, five, six, and seven rate shifts. The likelihood ratio test was used to select the best model in each case at the 99% level.

For the TTOL, we estimated node time uncertainty in both the LTT curve and rate test by producing 500 replicates of the TTOL, sampling a time between the confidence intervals at each node under a uniform distribution. These replicates were then used in the TREEPAR analysis to estimate and test rate change, with the uncertainty shown in figure 4 and the significant shifts by time interval (20 My) shown in supplementary table S8, Supplementary Material online.

Our diversification results for birds are similar to an earlier analysis (Jetz et al. 2012), with a strong increase in diversification from ~45 Ma to the present. The mammal results are also generally similar to past mammal analyses in showing the absence of any rate increase immediately after the end-Cretaceous extinctions (Bininda-Emonds et al. 2007; Meredith et al. 2011). We did not find a sharp mid-Cenozoic increase in rate that was found in one other analysis (Stadler 2011), although we determined that the cause of that rate increase was from a polytomy in the mammal timetree used in that study (Stadler 2011); otherwise our results are comparable.

For the bird tree (supplementary fig. S4*a*, Supplementary Material online), the models with zero, one, two, three, four, and five rate shifts are rejected in favor of a model with six rate shifts ($P < 0.05$) (supplementary table S9, Supplementary Material online). A model with six rate shifts is not rejected in favor of a model with seven rate shifts ($P = 0.091$). The 6 shifts are detected at 1, 3.4, 14.4, 48.2, 73.3, and 84.4 Ma (supplementary table S10, Supplementary Material online). The parameters obtained for the 6 shifts model between 73.3 and 48.2 Ma ($\lambda-\mu = 0.04058$ and $\mu/\lambda = 0.0395$) were used to plot the confidence interval of the null distribution (supplementary fig. S4*a*, Supplementary Material online).

For the mammal tree (supplementary fig. S4*b*, Supplementary Material online), the models with zero, one, two, or three rate shifts are rejected in favor of a model with four rate shifts ($P < 0.05$) (supplementary table S9, Supplementary Material online). A model with four rate shifts is not rejected in favor of a model with five rate shifts ($P = 0.123$). The 4 shifts are detected, at 2.1, 9.6, 42.4 and 105 Ma (supplementary table S10, Supplementary Material online). The parameters obtained for the 4 shifts model between 105 and 42.4 Ma ($\lambda-\mu = 0.05186$ and $\mu/\lambda = 0.3528$) were used to plot the confidence interval of the null distribution (supplementary fig. S4*b*, Supplementary Material online).

For the eukaryote tree (fig. 4), the models with zero, one, two, three, four, and five rate shifts are rejected in favor of a model with six rate shifts ($P < 0.05$) (supplementary table S9, Supplementary Material online). A model with six rate shifts is not rejected in favor of a model with seven rate shifts ($P = 0.055$). Six shifts are thus detected at 1, 11, 21, 121, 14,1 and 151 Ma (supplementary table S10, Supplementary Material online). The parameters obtained for the 6 shifts model between 2,100 and 151 Ma ($\lambda-\mu = 0.00307$ and $\mu/\lambda = 0.9581$) were used to plot the confidence interval of the null distribution (fig. 4).

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In addition, we used the BAMMtools package (Rabosky 2014) in order to detect the diversification rate differences across lineages. The function "setBAMMpriors" was used to generate a prior block that matched the "scale" (e.g., depth of the tree) of our data. Both the speciation and the extinction rate were allowed to vary through time and across lineages, and 50,000,000 generations of MCMC simulation were performed. The sampling fraction (0.02635422) was specified by setting the "globalSamplingFraction" parameter. A burnin of 0.5 was applied and a diversification rate plot was obtained with the function "plotRateThroughTime" (supplementary fig. S3, Supplementary Material online).

Time-to-Speciation Analyses

In addition to our species-level TTOL data collection described above, we collected a separate data set on TTS from published molecular timetrees that included timed nodes among populations and closely related species of three major groups: Vertebrates, arthropods, and plants (supplementary tables S11–S13, Supplementary Material online). To test the robustness of our approach for estimating TTS, we used simulations. A BD tree was simulated using the function "sim.bd.taxa" (TreeSim in R).

Supplementary Material

Supplementary figures S1–S4, tables S1–S13, and Materials and Methods are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

Acknowledgments

The work was supported by grants from the U.S. National Science Foundation (0649107, 0850013, 1136590, 1262481, 1262440, 1445187, 1455761, 1455762), the Science Foundation of Arizona, the U.S. National Institutes of Health (HG002096-12), and from the NASA Astrobiology Institute (NNA09DA76A). We thank K. Boccia, J.R. Dave, S.L. Hanson, A. Hippenstiel, M. McCutchan, Y. Plavnik, A. Shoffner, and L.-W. Wu for database assistance; K.R. Hargreaves, S.G. Loelius, and K.M. Wilt for population data collection assistance; B.A. Gattens, A.Z. Kapinus, L.M. Lee, A.B. Marion, J. McKay, A. Nikolaeva, M.J. Oberholtzer, M.A. Owens, V.L. Richter, and L. Stork for illustration assistance; R. Chikhi for programming assistance; J. Hey for comments on the manuscript; Penn State and Arizona State universities; and authors of studies for contributing timetrees.

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EXHIBIT A

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Education

Ph.D. Biochemistry, **University of California** at Santa Barbara *2007-2012*
from the Biomolecular Science and Engineering Program

B.S. Molecular Biology & Evolution, minor in Chemistry, *2001- 2005*
The Evergreen State College, Olympia, WA

Research

Principal Scientist, *Impossible Foods, Dr. Pat Brown* *2015- Current*

Senior Scientist, *Impossible Foods, Dr. Peg Scott, Dr. Chris Davis* *2013- 2015*

Scientist, *Impossible Foods, Dr. Chris Davis* *2012-2013*

- R&D scientist and flavor department leader at Impossible Foods, directing flavor technology research and flavor product integration.
- Manage a team of scientists to understand the fundamental biology and chemistry of flavor perception and flavor creation. We identify the catalysis and precursors that create aroma compounds, determine how aroma compounds create particular flavors profiles, and then how to recreate these flavors in products.
- Lead chemist on the identification, characterization and manipulation of biological flavor catalysis, including heme-proteins, microbes, and enzymes.
- Support off flavor identification and removal in products and feedstock with product developers, protein biochemist and process engineers.
- Flavor product integration, leading efforts for creating meat replicas from plants that taste and smell like animal based meats, working closely with manufacturing to scale the process, and marketing to meet consumers desires.
- Previous roles also included but not limited to sensory panel leader, lipid group leader, and product developer creating nut-base cheese (Kite Hill Cheese).

Enzymology Research Associate, *UC Santa Barbara, Dr. Norbert Reich* *2007 –2012*

- Researched the underlying chemical mechanisms that regulate how the genetic code is translated to creates phenotypic variation in human cells; particularly, understanding the developmental enzymes (DNMT3B and DNMT3A) and their modulators.
- Identified the mechanisms and structural changes that occurred with mutated forms of DNMT3A, highly associated with diseased states that change gene expression.

- Particular specialty was the development and execution of kinetic and biophysical assays to characterize the enzymology and regulation of the *de novo* DNA methyltransferase (DNMT3A).
- Mentored and lead 6 undergraduate researchers.

Biochemistry Visiting Associate, *Stanford University, Dr. Pat Brown (collaboration 2008-2010) 06-11/2009*

- Global identification of RNA associated with developmental enzymes (DNMT3B and DNMT3A) through cellular differentiation and correlated to gene expression and DNA methylation patterns. Developed RNA library construction protocols for next generation sequencing, and became skilled in microarray, hES cell culture, and fluorescence microscopy.

Scientist in Endocrinology, *U.S. Geological Survey* *05-09/2006*

- Conducted research on the stress response of translocation on the desert tortoise, though ELISA-based assays, leading team of 5 interns.

Molecular Biology NSF Fellow, *University of Georgia, Dr. Travis Glenn* *05-09/2005*

- Conducted independent research on pair bonding in alligators and determining mutation rates by carrying out ABI Prism sequencing for SNP and microsatellites, multiplexed PCR, and sequence analyzes for genotyping.

Genetics Research Assistant, *Evergreen State College, Dr. Erik Thusen* *2004–2005*

- Independent research on the population genetics of moon jellies, gaining experience in genomic DNA purification, PCR for species identification, and phylogenetic analyzes.

Developmental Biology Research Assistant, *Evergreen State College, Dr. Donald Morisato* *2003–2004*

- Investigated the molecular mechanisms of generating the anterior-posterior axis in *Drosophila*, by carrying out *lac z* staining, *in situ* hybridization, and genetic crosses.

Environmental Science Research Assistant, *Pacific Crest Community School, Dr. Tigerin Peare* *2000–2001*

- Independent research on the *E. coli* levels in the Willamette River, Portland, OR. Data collected results in new city testing and regulations on the city water treatment facilities.

Teaching/Mentoring Experience

Research Mentor for Undergraduate Students, *UC Santa Barbara* *2008-2011*

- Lead undergraduate students in research projects; taught techniques and guided experiments.

Teaching Assistant for epigenetics, developmental & biology laboratory, *UCSB, intermittently 2007-2010*

- Prepared lectures, gave lectures, and instructed laboratory sections.

Teaching Assistant, *Santa Barbara City College,* *2006 - 2007*

- Molecular biology, botany and general biology laboratories.

Scientific Computing Specialist, *Evergreen State College* *2003 –2006*

- Taught and helped students with science software - including biological modeling, statistics, phylogenetic, mathematical, and graphical programs.

Publications

Holz-Schietinger, C., Matje, D.M., Reich, N.O. Mutations in DNA methyltransferase (DNMT3A) observed in Acute Myeloid Leukemia patients disrupt methylation of clustered CpG sites. *J. Biol. Chem, 287: 30941*-30951 (2012).

Holz-Schietinger, C., and Reich, N.O. RNA modulation of the human *de novo* methyltransferase 3A (DNMT3A). *Nucleic Acids Res.* 40: 8550-8557 (2012).

Holz-Schietinger, C., Matje, D.M., Flexer Harrison, M. and Reich, N.O. DNA oligomerization controls the mechanism of *de novo* DNA methylation. *J. Biol. Chem.* 286, 41479-41488 (2011).

Holz-Schietinger, C. & Reich, N.O. The inherent processivity of the human *de novo* methyltransferase 3A (DNMT3A) is enhanced by DNMT3L. *J. Biol. Chem.* 285, 29091–29100 (2010).

Holz-Schietinger, C., Matje, D.M. and Reich, N.O. *De novo* DNA methyltransferase DNMT3A: regulation of oligomeric state and mechanism of action in response to physiological pH changes. *Biochimica et Biophysica Acta (BBA). 1850:1131-1139 (2015).*

Purdy, M.M., **Holz-Schietinger, C.** & Reich, N.O. Identification of a second DNA binding site in human DNA methyltransferase 3A by substrate inhibition and domain deletion. *Arch. Biochem. Biophys.* 498, 13-22 (2010).

Lance, S., Tuberville, T., Dueck, L., **Holz-Schietinger, C.,** Trosclair, P., Elsey, R., Glenn, T. Multi-year multiple paternity and mate fidelity in the American alligator, *Alligator mississippiensis. Molecular Ecology.* 2009. 18, 4508-4520 (2009).

Awards/Fellowships

- 2010-2011 Philip & Aida Siff Fellowship for excellent undergraduate student mentoring
- 2010, 2011 Best graduate presentation for USCB BMSE and MCDB departments
- 2009 Honorable Mention NSF Graduate Research Fellowship
- 2007-2008 Amgen Fellowship
- 2005 NFS Undergraduate Research Fellowship
- 2005 Evergreen foundation grant (research funding)
- 2002-2004 Evergreen State College foundation scholarship for academic excellence
- 2001-2002 USAA All-American Scholar, Philip S. Wilder Award for Academic Distinction, National Deans List

Training Courses /Research Conferences

- 14th Weurman Flavour Research Symposium, (2014) Cambridge, UK
- FKS Flavor Reaction Course, (2014), University of Missouri St Louis
- American Oil Chemistry Society (AOCS) conference, Lecithin Functions in Technology and Nutrition Course, (2013) AOCS Montreal QC, Canada
- Enzymes, Coenzymes & Metabolic Pathways Gordon conference, (2011, 2010) Waterville Valley, NH

EXHIBIT B

Compound	Signal intensity (ND = Not detected (signal to noise of 50, ~ signal of > 20,000))			Fold Increase or Novel ((LegH + Ribose + Cysteine) - (LegH))/ (Ribose + Cysteine)	References
	LegH	Ribose + Cysteine	LegH + Ribose + Cysteine		
O- and N-Heterocyclic compounds					
Furan, 2-methyl-	ND	ND	162,329	Novel	Song et al. (2012, J. Chromatog. A, 1254:115-24); Kerth et al. (2013, White Paper: Product Quality, Texas A&M University Dept of Animal Sciences); Moon et al. (2006, Food Res. Int., 39:294-308); www.thegoodscentscompany.com
Furan, 2-pentyl-	ND	ND	3,574,602	Novel	Song et al. (2012, J. Chromatog. A, 1254:115-24); Moon et al. (2006, Food Res. Int., 39:294-308); Brewer (2006, "The Chemistry of Beef Flavor -- Executive Summary," National Cattlemen's Beef Association, #12814); Karahadian & Lindsay (1989, J. Amer. Oil Chem. Soc., 66:1302-8); Leduc et al. (2012, J. Sci. Food Agric., 92:2560-8); www.thegoodscentscompany.com
bis(2-methyl-3-furyl) disulfide*	ND	ND	1,215,514	Novel	Mottram (1998, Food Chem., 62:415-24); Macleod (1994, The flavour of beef in "The falvour of Meat and Meat Products," ed Shahidi); Moon et al. (2006, Food Res. Int., 39:294-308); Song et al. (2012, J. Chromatog. A, 1254:115-24); www.thegoodscentscompany.com
Pyrazine, 2,5-dimethyl-	ND	ND	224,117	Novel	Brewer (2006, "The Chemistry of Beef Flavor – Executive Summary," National Cattlemen's Beef Association, #12814); Morita et al. (2003, J. Sci. Food Agric., 83:289-97); www.thegoodscentscompany.com
Pyrazine, methyl-	1049328	ND	12,559,346	Novel	Song et al. (2012, J. Chromatog. A, 1254:115-24); Brewer (2006, "The Chemistry of Beef Flavor -- Executive Summary," National Cattlemen's Beef Association, #12814); Morita et al. (2003, J. Sci. Food Agric., 83:289-97)
Pyrazine, 2-ethyl-5-methyl-	40894	ND	87,684	Novel	Van Ba et al. (2012, "Principle of meat aroma flavors and future prospect," Ch 7 in Latest Research into Quality Control, InTech Open Access Publisher)
S-Heterocyclic compounds					
2-Methyl-3-furanthiol	ND	ND	793,456	Novel	Song et al. (2012, J. Chromatog. A, 1254:115-24); Mottram (1998, Food Chem., 62:415-24); Moon et al. (2006, Food Res. Int., 39:294-308); www.thegoodscentscompany.com;
2-Acetylthiazole	ND	153,183	436,716,305	2851	Song et al. (2012, J. Chromatog. A, 1254:115-24); McGorrin et al. (2001, Special Publication - Royal Soc. Chem., 274:67-84); Kerth et al. (2013, White Paper: Product Quality, Texas A&M University Dept of Animal Sciences); Karahadian & Lindsay (1989, J. Amer. Oil Chem. Soc., 66:1302-8); www.thegoodscentscompany.com;
Dihydro-3-(2H)-thiophenone	ND	ND	1,168,594	Novel	www.thegoodscentscompany.com;
Thiophene, 3-methyl-	ND	1,127,036	2,650,365	2	Shibamoto (1980, J. Agric. Food Chem., 28:237-43)

3-Thiophenemethanol	ND	ND	430,476	Novel	Chen et al. (2000, J. Agric. and Food Chem., 48:3512-6)
Thiophene, 2,3-dihydro-5-methyl-	ND	ND	11,799,570	Novel	Elmore et al. (1999, J. Agric. Food Chem., 47:1619-25)
Thiophene	ND	2,422,423	216,296,040	89	Shibamoto (1980, J. Agric. Food Chem., 28:237-43); Macleod (1994, The flavour of beef in "The falvour of Meat and Meat products" ed Shahidi); www.thegoodscentscompany.com
3(2H)-Thiophenone, dihydro-2-methyl-	ND	ND	204,409	Novel	www.thegoodscentscompany.com
5-Methyl-2-thiophenecarboxaldehyde	ND	ND	3,046,036	Novel	Shibamoto (1980, J. Agric. Food Chem., 28:237-43); www.thegoodscentscompany.com;
3-Methyl-2-thiophenecarboxaldehyde	ND	ND	657,464	Novel	Shibamoto (1980, J. Agric. Food Chem., 28:237-43); www.thegoodscentscompany.com
Ethanone, 1-(4,5-dihydro-2-thiazolyl)-	ND	ND	1,304,816	Novel	www.thegoodscentscompany.com;
Thiazole, 2-methyl-	ND	ND	18,835,504	Novel	Shibamoto (1980, J. Agric. Food Chem., 28:237-43)
Sulfides					
Dimethyl sulfide	ND	ND	1,601,935	Novel	Van Ba et al. (2012, "Principle of meat aroma flavors and future prospect," Ch 7 in Latest Research into Quality Control, InTech Open Access Publisher); Kerth et al. (2013, White Paper: Product Quality, Texas A&M University Dept of Animal Sciences); Grigorakis et al. (2003, Aquaculture, 225:109-19); Leduc et al. (2012, J. Sci. Food Agric., 92:2560-8); www.thegoodscentscompany.com
Aldehydes					
2,4-Hexadienal, (E,E)-	ND	ND	9,495,304	Novel	Rochet & Chaintreau (2005, J. Agric. Food Chem., 53:9578-85); Karahadian & Lindsay (1989, J. Amer. Oil Chem. Soc., 66:1302-8); Grigorakis et al. (2003, Aquaculture, 225:109-19); www.thegoodscentscompany.com;
Benzaldehyde, 4-ethyl-	ND	ND	24,320,418	Novel	Morita et al. (2003, J. Sci. Food Agric., 83:289-97); www.thegoodscentscompany.com
Decanal	ND	ND	26,163	Novel	Van Ba et al. (2012, "Principle of meat aroma flavors and future prospect," Ch 7 in Latest Research into Quality Control, InTech Open Access Publisher); Morita et al. (2003, J. Sci. Food Agric., 83:289-97); Moon et al. (2006, Food Res. Int., 39:294-308); Song et al. (2012, J. Chromatog. A, 1254:115-24); Leduc et al. (2012, J. Sci. Food Agric., 92:2560-8); www.thegoodscentscompany.com
Undecanal	ND	ND	813,152	Novel	Moon et al. (2006, Food Res. Int., 39:294-308); Rochet & Chaintreau (2005, J. Agric. Food Chem., 53:9578-85); Van Ba et al. (2012, "Principle of meat aroma flavors and future prospect," Ch 7 in Latest Research into Quality Control, InTech Open Access Publisher); Morita et al. (2003, J. Sci. Food Agric., 83:289-97)
Lactones					
2(3H)-Furanone, 5-ethyldihydro-	ND	ND	72,377,175	Novel	Song et al. (2012, J. Chromatog. A, 1254:115-24)
2(3H)-Furanone, dihydro-5-pentyl-	ND	ND	31,360,192	Novel	Song et al. (2012, J. Chromatog. A, 1254:115-24)
Ketones					

2-Octanone	ND	ND	1,242,778	Novel	Moon et al. (2006, Food Res. Int., 39:294-308); Song et al. (2012, J. Chromatog. A, 1254:115-24); Van Ba et al. (2012, "Principle of meat aroma flavors and future prospect," Ch 7 in Latest Research into Quality Control, InTech Open Access Publisher); Karahadian & Lindsay (1989, J. Amer. Oil Chem. Soc., 66:1302-8); Grigorakis et al. (2003, Aquaculture, 225:109-19)
3-Nonanone	ND	ND	650,546	Novel	Leduc et al. (2012, J. Sci. Food Agric., 92:2560-8); www.thegoodscentscompany.com
3,5-Octadien-2-one, (E,E)-	ND	ND	537,770	Novel	Karahadian & Lindsay (1989, J. Amer. Oil Chem. Soc., 66:1302-8); Grigorakis et al. (2003, Aquaculture, 225:109-19); Leduc et al. (2012, J. Sci. Food Agric., 92:2560-8); www.thegoodscentscompany.com;
Alcohols					
p-Cresol	ND	ND	536,008	Novel	Kerth et al. (2013, White Paper: Product Quality, Texas A&M University Dept of Animal Sciences)
Carboxylic Acids					
Hexanoic acid	ND	ND	14,935	Novel	Moon et al. (2006, Food Res. Int., 39:294-308); Song et al. (2012, J. Chromatog. A, 1254:115-24); Brewer (2006, "The Chemistry of Beef Flavor – Executive Summary," National Cattlemen's Beef Association, #12814); Karahadian & Lindsay (1989, J. Amer. Oil Chem. Soc., 66:1302-8); Selli et al. (2009, Food Chem., 114:317-22)
Hexadecanoic acid	ND	ND	1,959,921	Novel	Song et al. (2012, J. Chromatog. A, 1254:115-24); www.thegoodscentscompany.com;
* in addition to being categorized as an O- and N-containing heterocyclic, bis(2-methyl-3-furyl) disulfide also can be categorized as a sulfide and as a S-containing heterocyclic compound.					

EXHIBIT A

Keith R. Cadwallader

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and Human Nutrition
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4005 Turnberry Dr.
Champaign, IL 61822

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102 ABL, MC-640

Education

University of Florida, Gainesville, FL	Fall, 1990	Ph.D. Food Science (Chem. minor)
University of Florida	Summer. 1987	M.S. Food Science
University of Georgia, Athens, GA	Spring, 1985	B.S. Food Science

Professional Experience

2007-present. Professor of Food Chemistry, Department of Food Science & Human Nutrition, University of Illinois. **Research Interests:** Chemistry and analysis of food flavor. Food flavor (aroma) characterization and flavor quality determination. Use of instrumental and sensory methods to identify and quantify character-impact flavor compounds in foods/agricultural/industrial materials. Development of process flavors. Recovery of flavor constituents from food processing by-products. Measurement and prediction of flavor diffusion, release and binding. Development of methods to stabilize labile flavors.
1999-2007. Associate Professor of Food Chemistry, University of Illinois.
1996-1999. Associate Professor of Food Science, Food Science & Technology, Mississippi State Univ.
1994-1996. Assistant Professor of Food Science, Mississippi State University
1991-1994. Assistant Professor of Food Science, Dept. Food Science, Louisiana State University.
1985-1990. Pre-doctoral Research Fellow, Dept. Food Science & Human Nutrition, Univ. Florida.

Current Appointment (UIUC)

Research – 60%; Teaching – 40%

Awards/Honors

- 2016 College of ACES Senior Faculty Award for Excellence in Research, University of Illinois,
- 2010 Elected Fellow, Agricultural and Food Chemistry Division of the American Chemical Society.
- Co-recipient, 2008 Donald A. Holt Achievement Award, Illinois Council on Food and Agricultural Research, presented to the Illinois Center for Soy Foods leadership team.
- 2007 Stephen S. Chang Award for Excellence in Lipid or Flavor Science, Institute of Food Technologists.
- Co-recipient, 2007 College of ACES Team Award for Excellence, University of Illinois, presented to the Illinois Center for Soy Foods leadership team
- Co-recipient, 2005 Bor S. Luh International Award, Institute of Food Technologists
- UIUC Incomplete list of teachers rated as excellent (Spr00, Fall00, Fall01, Spr03, Spr04, Spr05, Fall05; Spr06; Spr08; Spr10; Fall12; Fall14)
- 1996 Alpha Zeta Outstanding Teaching Award
- 1985-1990 FMC Corp. Research Fellowship Recipient
- 1984 U. Georgia Food Science Department Faculty Scholarship Recipient

Professional Memberships

Institute of Food Technologists & IFT, Chicago Section
American Chemical Society & ACS, Division of Agricultural and Food Chemistry

Teaching Interests and Experience

Teaching interests include food analysis, food chemistry, flavor chemistry and analysis, and sensory evaluation of foods. Courses taught are listed below.

University of Illinois

FSHN 418, *Food Analysis*: *2 h lecture: 6 h lab.* Principles and application of the chemical, physical, and instrumental methods used to determine the constituents of foods; special considerations applicable to the analysis of certain foods.

FSHN 519, Flavor Chemistry and Analysis: *2 h lecture: 6 h lab.* a graduate level course that involves the discussion/demonstration of the chemistry, manufacture/delivery and analysis (sensory, instrumental, and physical) of food flavor.

Mississippi State University

FST 4114/6114 *Analysis of Food Products*: *2 h. lecture; 6 h lab.* Principles, methods and techniques for quantitative, physical, chemical, and/or biological analysis of food products and ingredients.

FST 4241/6241 *Applied Food Chemistry*: *2 h lab.* Basic laboratory experiments to provide understanding of the function and interactions of chemical components of food.

FST 8163 *Flavor and Food Acceptance*: *2 h lecture; 1 h demonstration.* Theory and application of sensory evaluation and analytical chemistry techniques for the analysis of food flavor.

Louisiana State University

FDSC 4050 *Food Composition and Analysis*: *2 h. lecture; 6 h lab.* Principles, methods and techniques for quantitative, physical, chemical, and/or biological analysis of food products and ingredients.

FDSC 7040 *Flavor and Colors of Foods*: *2 h lecture; 3 lab.* Methods of chemical, physical, and instrumental analysis of food colors and flavors; natural and synthetic flavorings and colorants.

Research Publications

Books Edited (5)

Cadwallader, K.R. and Chang, S.K.C. (Eds.). 2010. *Chemistry, Texture and Flavor of Soy.* ACS Symposium Series 1059, American Chemical Society, Washington, DC.

Cadwallader, K.R., Drake, M.A. and McGorrin, R.J. (Eds.) 2007. *Flavor of Dairy Products.* ACS Symposium Series 971, American Chemical Society, Washington, DC.

Cadwallader, K.R. and Weenen, H. (Eds.). 2003. *Freshness and Shelf Life of Foods.* ACS Symposium Series 836, American Chemical Society, Washington, DC.

Rouseff, R.L. and Cadwallader, K.R. (Eds.). 2001. *Headspace Analysis of Foods and Flavor: Theory and Practice.* Kluwer Academic/Plennum Publishers, New York.

Shahidi, F. and Cadwallader, K.R. (Eds.). 1997. *Flavor and Lipid Chemistry of Seafoods.* ACS Symposium Series 674. American Chemical Society, Washington, DC.

Patent Applications (1)

Cadwallader, K.R. and Fang, M.-C. 2014. Stabilized compositions and methods of manufacture. US Patent Application US 2014/0275543 A1.

Book Chapters (57)

Cadwallader, K.R. 2016. Flavor challenges in functional beverages. Chapter 3. In *Handbook of Functional Beverages and Human Health*. Alasalvar, C. and Shahidi, F. (Eds.), CRC Press, pp. 27-34.

Yin, Y., Cadwallader, K.R. and Fang, M.-C. 2015. A novel method for the stabilization of the potent odorant 2-acety-1-pyrroline. In *Flavour Science: Proceedings of the XIV Weurman Flavour Research Symposium*, Taylor, A.J. and Mottram, D.S (Eds.), Context Products Ltd, Leicestershire, UK, pp. 571-574.

Burnside, E. and Cadwallader, K.R. 2014. Aroma components of commercial and self-prepared rum ethers. In *Current Topics in Flavor Chemistry and Biology: Proceedings of the 9th Wartburg Symposium on Flavor Chemistry & Biology*, T. Hofmann, W. Krautwurst, D., and Schieberle, P. (Eds.), Eigenverlag Deutsche Forschungsanstalt für Lebensmittelchemie, Freising, Germany, pp. 244-249.

Moran, L., Yin, Y. Cadwallader, K.R. and Padua, G.W. 2014. Testing tools and physical, chemical, and microbiological characterization of microencapsulated systems. In *Microencapsulation in the Food Industry: A Practical Implementation Guide*. Gaonkar, A., Vasisht, N, Khare, A. and Sobel, R. (Eds.). Academic Press/Elsevier Inc., Oxford, UK, pp. 323-352.

Lorjaroenphon, Y., Hausch, B.J. and Cadwallader, K.R. 2014. Formation and stability of 2,3-dehydro-1,8-cineol in a model carbonated beverage system. In *Flavor Science: Proceedings from the XIII Weurman Flavour Research Symposium*, Elsevier, Inc. Oxford, UK.

Fang, M. and Cadwallader, K.R. 2012. Improved synthesis of deuterium-labeled alkyl pyrazines for use in stable isotope dilution analysis. In *Recent Advances in the Analysis of Foods and Flavor*. Mussinan, C. et al. (Eds.). ACS Symposium Series 1098, American Chemical Society, Washington, DC, pp. 57-69.

Lahne, J. and Cadwallader, K.R. 2012. Streamlined analysis of potent odorants in distilled alcoholic beverages. In *Chemistry and Flavor of Alcoholic Beverages.* Qian, M. et al., (Eds.). ACS Symposium Series 1104, American Chemical Society, Washington, DC, pp. 37-53.

Cadwallader, K.R., Potts, D., Brisske-BeVier, L. and Mirarefi, S. 2011. Contribution of volatile sulfur compounds to the characteristic aroma of roasted garlic. In *Volatile Sulfur Compounds in Food.* Qian, M.C., Fan, X. and Mahattanatawee, K. (Eds.). ACS Symposium Series 1068, American Chemical Society, Washington, DC, pp. 137-151.

Genthner, L.R. and Cadwallader, K.R. 2011. Structure-odor relationships of selected esters with (Z,Z)-3,6-nonadiene backbone structures. In *Advances and Challenges in Flavor Chemistry & Biology: Proceedings of the 9th Wartburg Symposium on Flavor Chemistry & Biology*, T. Hofmann, W. Meyerhof, P. Schieberle, Eds., Eigenverlag Deutsche Forschungsanstalt für Lebensmittelchemie, Garching, Germany, pp. 123-128.

Kim, H.K. and Cadwallader, K.R. 2011. Instrumental analysis of seafood flavor. In *Handbook of Seafood Quality, Safety and Health Applications.* Alasalvar, C., Shahidi, F., Miyashita, K. and Wanasundara, U. (Eds.). Blackwell Publishing Ltd., UK, pp. 50-67.

Cadwallader, K.R., Moore, J.J., Zhang, Z. and Schmidt, S.J. 2010. Comparison of spray drying and Refractance Window™ drying technologies for the encapsulation of orange oil. In *Recent Advances in Food and Flavor Chemistry: Food Flavors and Encapsulation, Health Benefits, Analytical Methods, and Molecular Biology of Functional Foods.* Ho C.-T., Mussinan, C.J., Shahidi, F. and Tratras Contis, E. (Eds.). The Royal Society of Chemistry, Cambridge, UK, pp. 246-254.

Sun, C., Cadwallader, K.R. and Kim, H. 2010. Comparison of key aroma components between soymilks prepared by cold and hot grinding methods. In *Chemistry, Texture and Flavor of Soy.* Cadwallader, K.R. and Chang, S.K.C. (Eds.). ACS Symposium Series 1059, American Chemical Society, Washington, DC, pp. 361-373.

Suppavorasatit, I. and Cadwallader, K.R. 2010. Flavor-soy protein interactions. In *Chemistry, Texture and Flavor of Soy.* Cadwallader, K.R. and Chang, S.K.C. (Eds.). ACS Symposium Series 1059, American Chemical Society, Washington, DC, pp. 339-359.

Cadwallader, K.R., Tamamoto, L.C. and Sajuti, S.C. 2010. Aroma components of fresh and stored pomegranate (*Punica granatum* L.) juice. In *Flavors in Noncarbonated Beverages.* Da Costa, N.C. and Cannon, R.J. (Eds.). ACS Symposium Series 1036. American Chemical Society, Washington, DC, pp. 93-101.

Liu, G.-L. and Cadwallader, K.R. 2010. Aroma-impact components of "Carlos" muscadine grape juice. In *Flavor and Health Benefits of Small Fruits.* Qian, M.C. and Rimando, A.M. (Eds.). ACS Symposium Series 1035, American Chemical Society, Washington, DC, pp. 63-77.

Cadwallader, K.R. and Singh, T. 2009. Flavours and off-flavours in milk and dairy products. In *Advanced Dairy Chemistry-Volume 3. Lactose, Water, Salts and Minor Constituents.* McSweeny, P.L.H. and Fox, P.F. (Eds). Springer Science+Business Media, LLC, New York, pp. 631-690.

Cadwallader, K.R. and Puangpraphant, S. 2008. Volatile compounds in tree nuts. In Tree Nut s: Composition, Phytochemicals, and Health Effects. Alasalvar, C. and Shahidi, F. (Eds.) Taylor and Francis, LLC, Boca Raton, FL, pp. 109-125.

Singh, T. and Cadwallader, K.R. 2008. Cheese. In *Dairy Processing and Quality Assurance.* Chandan, R.C., Kilara, A. and Shah, N. (Eds.), Wiley-Blackwell, Danvers, MA, pp. 273-308.

Lorjaroenphon, Y., Cadwallader, K.R., Kim, H. and Lee, S-Y. 2008. Evaluation of key odorants in chipotle pepper by quantitative analysis, calculation of odor activity values and omission studies. In *Recent Highlights in Flavor Chemistry and Biology: Proceedings of the 8th Warburg Symposium on Flavor Chemistry & Biology,* Hofmann, T., Meyerhof, W. and Schieberle, P. (Eds.). Deutche Forschungsanstalt für Lebensmittelchemie, Garching, Germany, pp. 191-200.

Zhou, Q. and Cadwallader, K.R. 2008. Measurement of flavor-soy protein interactions in low-moisture solid food systems by inverse gas chromatography. Ch. 4. In *Food Flavor: Chemistry, Sensory Evaluation and Biological Activity*. Tamura, H., Ebeler, S.E., Kubota, K. and Takeoka, G.R. (Eds.). ACS Symposium Series 988. American Chemical Society, Washington, DC., pp. 45-54.

Cadwallader, K.R. 2007. Wood smoke flavor. In *Handbook of Meat, Poultry & Seafood Quality*. Nollet, L.M.L. (Ed.) and Boylsten, T., Chen, F. Coggins, P.C., Gloria, M.B., Hyldig, G., Kerth, C.R., McKee, L.H., Hui, Y.H. (Assoc. Eds.), Blackwell Publishing, Inc., Lancaster, PA, pp. 201-210.

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Other Publications (UIUC, 2000-present)

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Invited Talks

Cadwallader, K.R., Yin, Y., Hausch, B. and Chen, F. 2016. Assuring accuracy in the quantitation of the unstable odorant 2-acetyl-1-pyrroline in aromatic rice. ACS Symposium: *Flavor Stability: Chemical Changes in Flavor Molecules, Food-Flavor Matrix Interactions, Flavor Encapsulation.* 252nd Americal Chemical Society Meeting. Philadelphia, PA, August 21-25.

Cadwallader, K.R. 2016. Occurrence, formation and stabilization of the potent odorant 2-acetyl-1-pyrroline. Meeting of the Illinois Heartland Local Section of the American Chemical Society, Bloomington, IL, May 16, 2016.

Ickes, C. and Cadwallader, K.R. 2016. Characterizing aroma components of rum. ACS Symposium: *Flavor Chemistry of Alcoholic Beverages.* 251st American Chemical Society Meeting, San Diego, CA, March 13-17.

Cadwallader, K.R., Hausch, B. and Yin, Y. Quest for stability of 2-acetyl-1-pyrroline: The key odorant in aromatic rice and pandan. 17th Food Innovation Asia Conference 2015, Bangkok, Thailand, June 18-19.

Ickes, C. and Cadwallader, K.R. 2015. Understanding the effects of ethanol-flavor interactions on flavor perception in alcoholic beverages. ACS Symposium: *Challenges in Applied Flavor Sciences.* ACS National Meeting, Boston, MA, August 16-20, AGFD paper no. 297.

Pang, X., Wu, J. and Cadwallader, K.R. 2014. Identification of predominant contributors to off-odors formed in thermally processed melon juice. ACS Symposium: *Chemical Changes During Processing.* 248th American Chemical Society Meeting, San Francisco, CA, August 10-14. AGFD paper no. 177.

Cadwallader, K.R. and Lapsongphon, N. 2012. Identification of key odorants in fish sauce. US-Korea Conference (UKC) 2012, Los Angeles, August 8-11.

Fang, M. and Cadwallader, K.R. 2011. Convenient synthesis of stable deuterium labeled alkyl pyrazines for use in stable isotope dilution assays. ACS Symposium: *Recent Advances in Analysis of Food Flavors.* 242nd American Chemical Society Meeting, Denver, CO, August 28 – September 1, 2011, AGFD paper no. 11.

Hausch, B. and Cadwallader, K.R. 2010. Flavor chemistry of lemon-lime flavored carbonated beverages. ACS Symposium: *Symposium Honoring Prof. Terry E. Acree on the Occasion of His 70th Year.* 240th ACS National Meeting, Boston, MA, August 22-26, AGFD paper no. 40.

Lahne, J. and Cadwallader, K.R. 2010. Streamlined analysis of potent odorants in distilled alcoholic beverages. ACS Symposium: *Flavor Chemistry of Alcoholic Beverages*. 240th ACS National Meeting, Boston, MA, August 22-26, AGFD paper no. 106.

Cadwallader, K.R. and Kim, H. 2010. Flavor chemistry of fish sauce. ACS Symposium: *Fermented Foods.* 239th ACS National Meeting, San Francisco, CA, March 21-25, AGFD paper no. 112.

Cadwallader, K.R. 2010. Flavor chemistry of tree nuts. IFT Symposium: *Nuts: Phytochemicals, Health Effects and Flavor.* IFT Annual Meeting and Food Expo, Chicago, IL, July 17-20, paper no. 054-05.

Lozano, P.R. and Cadwallader, K.R. 2009. Effect of deamidation on binding of flavor compounds by dehydrated soy protein isolates. ACS Symposium: *Food Proteins and Food-based Protein Chemistry* 237th ACS National Meeting, Finley, J., organizer, Salt Lake City, UT, March 22-26.

Cadwallader, K.R. 2008. Sensory evaluation of aroma models for flavor characterization. *Symposium in Honor of Ronald Buttery G. Kenneth Recipient of the 2008 Spencer Award for Outstanding Achievement in Agricultural and Food Chemistry*, Kansas City Section, American Chemical Society, Kansas City, MO, Oct 27.

Cadwallader, K.R. 2008. AGFD and 100 years of chemical research on grains and legumes. ACS Symposium: *AGFD 100th Anniversary Symposium*, Brine, C. and Tunick, M.H., organizers, 236th ACS National Meeting, Philadelphia, PA, August 17-21.

Liu, Y and Cadwallader, K.R. 2008. Aroma components of "Carlos" muscadine (*Vitis rotundifolia* Michx) grape juice. ACS Symposium: Flavor and Health Benefits of Small Fruits, Rimando, A.M. and Qian, M.C., organizers, 236th ACS National Meeting, Philadelphia, PA, August 17-21.

Lozano, P.R. and Cadwallader, K.R. 2008. Flavor binding by soy proteins. ACS Symposium: Chemistry, Texture and Flavor of Soy, Cadwallader, K.R. and Chang, S. K. C. Chang, organizers, 236th ACS National Meeting, Philadelphia, PA, August 17-21.

Watcharananun, W., Cadwallader, K.R. and Huangrak, K. 2008. Predominant odorants in Thai desserts flavored by smoking with *tian op*, a traditional Thai scented candle. ACS Symposium: *Recent developments in flavor chemistry*, Cadwallader, K.R., organizer, 235th ACS National Meeting, New Orleans, LA, April 6-10.

Cadwallader, K.R. Lozano, P.R. and Kim, H. 2007. Challenges in the isolation and identification of key aroma components of complex functional foods. ACS Symposium: *Food Bioactives and Nutraceuticals: Production, Chemistry, Analysis and Health Effects: Symposium Honoring Fereidoon Shahidi*, Rimando, A.M., organizer. 234th ACS National Meeting, Boston, MA, August 19-23, AGFD Paper 92.

Cook, S., Cadwallader, K.R. and Kim, H. 2007. Identification of key thermally-derived odorants in cooked chicken liver. ACS Symposium: *Thermal Generation of Flavors and Off-flavors,* Ho, C.-T., Raghavan, S., McGorrin, R.J., organizers. 234th ACS National Meeting, August 19-23. AGFD Paper 206.

Cadwallader, K.R., Lorjaroenphon, Y., Kim, H. and Lee, S-Y. 2007. Evaluation of key odorants in chipotle pepper by quantitative analysis, calculation of odor-activity values and omission studies. 8th Wartburg Symposium on Flavor Chemistry & Biology, Eisenach, Germany, February 28 – March 2.

Cadwallader, K.R. 2007. Protein-based functional beverages and flavoring considerations. In IFT Symposium: *Functional Beverages and Human Health.* Annual Meeting of the Institute of Food Technologists, Chicago, IL, July 28 - August 1, Session-Paper 174-02.

Cadwallader, K.R. 2006. Chemical and sensory characterization of food aroma: An academic approach. Meeting of the Society of Flavor Chemists, Chicago, IL, October 19.

Cadwallader, K.R. 2006. Flavor chemistry of fish and shellfish. ACS Symposium: Molecular Cuisine. (Tunick, M.H., Risch, S.J. and Johnson, S.C., organizers). 232nd ACS National Meeting, San Francisco, CA, September 10-14, AGFD paper 61.

Cadwallader, K.R. and Zhou, Q. 2006. Characteristic aroma components of tree nuts. ACS Symposium: Nutrition and Sensory Quality of Nuts. (Pattee, H.E.; Shahidi, F.; Yu, L., organizers). 331st ACS National Meeting, Atlanta, GA, March 26 – March 30, AGFD paper 40.

Cadwallader, K.R. 2006. Modern techniques to characterize complex food flavor systems. International Symposium on *Functional Foods*, ATAM (Nutrition Society of Mexico) and Institute of Food Technologists, Mexico City, Mexico, August 17.

Rotsachakul, P., Cadwallader, K.R. and Chaiseri, S. 2005. Comparison of DHS and solvent extraction-SAFE for GCO-dilution analysis of key aroma components of Thai fried chili paste. ACS Symposium: *Aroma-active Compounds in Foods and Reaction Flavors: Formation, Measurement and Stability.* (Ho, C.-T., Weerasinghe, D.K. and Cadwallader, K.R., organizers). 330th ACS National Meeting, Washington, DC, August 28 – September 1, AGFD paper 9.

Cadwallader, K.R., Bordignon, J.R., Wang, P. and Sugiharto, R. 2005. Identification of odorants responsible for off-flavor of corn gluten meal. ACS Symposium: *Grain-based Products: Health and Flavor, and Safety Aspects* (Cadwallader, K.R., Schieberle, P. and Morello, M.J., organizers). 330th ACS National Meeting, Washington, DC, August 28 – September 1, AGFD paper 167.

Cadwallader, K.R., Drake, M.A., Carunchia-Whetstine, M.E. and Singh, T.J. 2005. Characterisation of Cheddar cheese flavour by sensory directed instrumental analysis and model studies. 11th Weurman Flavour Research Symposium, Roskilde, Denmark, June 21-24.

Gnadt, T.A., Jasso, L. and Cadwallader, K.R. 2005. Characteristic aroma components of chipotle peppers. ACS Symposium: *Chemistry and Flavor of Hispanic Foods* (de Mejia, E. and Tunick, M.H., organizers). 229th ACS National Meeting, San Diego, CA, March 13-17.

Lozano, P.R., Nicolalde, C.L. and Cadwallader, K.R. 2005. Identification of characteristic aroma components of mate (*Ilex paraguariensis*) tea. ACS Symposium: *Chemistry and Flavor of Hispanic Foods* (de Mejia, E. and Tunick, M.H., organizers). 229th ACS National Meeting, San Diego, CA, March 13-17.

Lecona-Uribe, S., Loarca-Piña, G., Arcila-Lozano, C.C. and Cadwallader, K.R. 2005. Chemical composition of essential oil from Mexican orégano. ACS Symposium: *Chemistry and Flavor of Hispanic Foods* (de Mejia, E. and Tunick, M.H., organizers). 229th ACS National Meeting, San Diego, CA, March 13-17.

Cadwallader, K.R. Importance of sample preparation technique on the composition of aroma extracts from fresh herbs. ACS Sympsosium: *Challenges in Herb Research – Chemical Analysis and Quality* (Ho, C.T. and Hwang, L.S., organizers). 227th ACS National Meeting, Anaheim, CA, March 28-April 1, 2004.

Cadwallader, K.R., Jarunrattanasri, A. and Theerakulkait, C. 2003. Aroma components of HVP made from rice bran protein. ACS Symposium: *Processed/Reaction Flavors, Ingredients and Intermediate Reactions.* (Weerasinghe, D.K. and Sucan, M.K., organizers). 226th ACS National Meeting, New York, NY, September 7-11.

Cadwallader, K.R. and Pojjanapimol, S. 2003. Characteristic aroma components of the cilantro mimics. ACS Symposium: *Natural Flavor and Fragrance Ingredients: Chemistry, Production and Application.* (Frey, C. and Rouseff, R.L., organizers). 226th ACS National Meeting, New York, NY, September 7-11.

Cadwallader, K.R. Relating Cheddar cheese flavor to chemical components. IFT Symposium: *Flavor Chemistry of Dairy Products.* (Drake, M.A., organizer). Annual Meeting of the Institute of Food Technologists, Chicago, IL, July 12-16, #52-5.

Cadwallader, K.R., Pojjanapimol, S. and Chaiseri, S. 2002. Heat-induced changes in holy basil aroma components. ACS Symposium: *Challenges in the Characterization of Flavor Compound.* (Deibler, K.D. and Delwiche, J., organizers). 224th ACS National Meeting, Boston, MA, August 18-22.

Cadwallader, K.R., Zhou, Q. and Huda, M.S. 2002. Measuring flavor release from microencapsulants under controlled environmental conditions. ACS Symposium: *Controlled Release Systems for Foods.* (Shefer, S. and Yildiz, M.E., organizers). 223rd ACS National Meeting, Orlando, FL, April 7-11.

Cadwallader, K.R., Brehart, D.M., Schmidt, S.J. and Huda, M.S. 2002. Volatile release from a sucrose encapsulated flavoring. IFT Symposium: *Use of Carbohydrates for Encapsulation and Sustained Release.* (Obanni, M. and Asquith, T.N., organizers). Annual Meeting of the Institute of Food Technologists, Anaheim, CA, June 15-19, #67-1.

Cadwallader, K.R., Lopez, J.R. and Menke, S.D. 2001. Aroma components of wines from Chardonel, a French-American hybrid grape variety. ACS Symposium: *Chemistry and Flavor of Nutritional Beverages.* (Shahidi, F. and Weerasinghe, D., organizers). 222nd ACS National Meeting, Chicago, IL, August 26-31.

Guthrie, B. and Cadwallader, K.R. 2001. Measuring flavor retention in dry food systems. IFT Symposium: *Developments in the Analysis of Complex Flavor Systems*. (Cadwallader, K.R. and McGorrin, R.J., organizers). Annual Meeting of the Institute of Food Technologies, New Orleans, LA, June 23-27, #76-2.

Cadwallader, K.R. 2001. Use of SPME Technology to Evaluate Antioxidant Effectiveness. Symposium: *Antioxidant Analysis*, (Engeseth, N.J., organizer). 92nd AOCS Annual Meeting and Expo, Minneapolis, MN, May 13-16.

Karagul-Yuceer,Y., Drake, M.A. and Cadwallader, K.R. 2001. Aroma characterization of fresh and stored nonfat dry milk. ACS Symposium: *Freshness and Shelf-life of Foods.* (Weenen, H. and Cadwallader, K.R., organizers). 222nd ACS National Meeting, Chicago, IL, August 26-30.

Cadwallader, K.R. 2000. Flavor Chemistry of Saffron. Symposium: *Carotenoid-Derived Aroma Compounds.* (Winterhalter, P. and Rouseff, R., organizers). 219th ACS National Meeting, San Francisco, CA, March 26-30, paper #46 AGFD.

Cadwallader, K.R. 1999. Sample dilution analysis for identification of aroma-impact components of edible oils. ACS Symposium: *Advances in Gas Chromatography/Olfactometry.* (Leland, J., Acree, T.E., organizers). 218th ACS National Meeting, New Orleans, LA, August 22-26.

Cadwallader, K.R. and Shahidi, F. 1999. Identification of potent odorants in seal blubber oil by direct thermal desorption-gas chromatography-olfactometry. ACS Symposium: *Chemistry and Nutrition of Highly Unsaturated Fatty Acids.* (Shahidi, F. and Finley, J.W., organizers). 217th ACS National Meeting, Anaheim, CA, March 21-25.

Cadwallader, K.R. and Howard, C.L. 1997. Analysis of aroma-active compounds in light-activated milk. ACS Symposium: *Challenges in the Isolation and Characterization of Flavor Compounds.* (Mussinan, C.J. and Morello, F., organizers). 214th National Meeting of the American Chemical Society, Las Vegas, NV, September 7-11.

Baek, H.-H. and Cadwallader, K.R. 1996. Character-impact aroma compounds of crustacea. ACS Symposium: *Flavor and Lipid Chemistry of Seafoods.* (Shahidi, F. and Cadwallader, K.R., organizers). 212th National Meeting of the American Chemical Society, Orlando, FL, August 25-29.

Cha, Y.J., Lee, G.H. and Cadwallader, K.R. 1996. Aroma-active compounds in salt-fermented anchovy. ACS Symposium: *Flavor and Lipid Chemistry of Seafoods.* (Shahidi, F. and Cadwallader, K.R., organizers). 212th National Meeting of the American Chemical Society, Orlando, FL, August 25-29.

Wang, H.-J., Cadwallader, K.R., Baek. H.H., D'Abramo, L.R. and Sullivan, J.A. 1996. Manipulating the flavor of crustacea using postharvest seawater acclimation. ACS Symposium: *Flavor and Lipid Chemistry of Seafoods.* (Shahidi, F. and Cadwallader, K.R., organizers). 212th National Meeting of the American Chemical Society, Orlando, FL, August 25-29.

Cadwallader, K.R. and Howard, C.L. 1996. Characterization of saffron flavor by aroma extract dilution analysis. ACS Symposium: *Spices: Flavor Chemistry and Antioxidant Properties.* (Risch, S.J. and Ho, C.T., organizers). 211th National Meeting of the American Chemical Society, New Orleans, LA, March 24-28.

Cadwallader, K.R., Baek, H.H., Chung, H.Y. and Moody, M.W. 1993. Contribution of lipid-derived components to the flavor of alligator meat. ACS Symposium: *Lipids and Food Flavors.* (Ho, C.T. and Hoffman, T.G., organizers). 205th National Meeting of the American Chemical Society, Denver, CO.

Braddock, R.J. and Cadwallader, K.R. 1993. Bioconversion of citrus d-limonene. Symposium paper presented at the 206th National Meeting of the American Chemical Society, Chicago, IL.

Braddock, R.J. and Cadwallader, K.R. 1991. Citrus by-products manufacture for food use. Symposium paper presented at the 52nd Annual Meeting of the Institute of Food Technologists, Dallas, TX, June 1-5.

Volunteered Abstracts

Kelley, L. and Cadwallader, K.R. 2016. Identification and quantitation of potent odorants in spearmint oils. 11th Wartburg Syposium on Flavor Chemistry & Biology, Eisenach, Germany, June 21-24.

McKenna, S. and Cadwallader, K.R. 2016. Identification and characterization of mint lactones: Trace-level odor-important compounds in peppermint oil. IFT Annual Meeting and Food Expo, Chicago, IL, July 17-19, paper no. PO2-020

Ickes, C., Lee, S. and Cadwallader, K.R. Novel creation of rum flavor lexicon through the use of web-based material. IFT Annual Meeting and Food Expo, Chicago, IL, July 17-19, paper no. P03-111

Yu, L., Thomas, L., Yin, L, Jerrell, J., Cadwallader, K. and Schmidt, S. 2015. Impact on sucrose crystal composition and chemistry on its thermal behavior. IFT Annual Meeting and Food Expo, Chicago, IL, July 11-14, paper no. 046-093.

Yu, L., Thomas, L., Yin, L, Jerrell, J., Cadwallader, K. and Schmidt, S. 2015. Impact on sucrose crystal composition and chemistry on its thermal behavior. IFT Annual Meeting and Food Expo, Chicago, IL, July 11-14, paper 046-093.

Cadwallader, K.R. and Fang, M.-C. 2014. A novel and attractive method for the stabilization of the potent odorant 2-acetyl-1-pyrroline. 14th Weurman Flavor Research Symposium, Cambridge, UK, September 15-19.

Donovan, J.D., Cadwallader, K.R. and Lee, Y. 2014. Improvement of protein microcapsule structure and core retention through the use of inulin. IFT Annual Meeting and Food Expo, New Orleans, LA, June 20-25, paper 181-01.

Erten, E. and Cadwallader, K.R. 2014. Characterization of aroma components of raw, dry roasted, and oil roasted almonds using solvent assisted flavor evaporation (SAFE) and aroma extract dilution analysis (AEDA). IFT Annual Meeting and Food Expo, New Orleans, LA, June 20-25, paper 206-26.

Lu, Y., Lee, J., Thomas, L., Jerrell, J., Cadwallader, K.R. and Schmidt, S.J. 2014. Investigating the thermal decomposition differences between beet and cane sugars using HPLC. IFT Annual Meeting and Food Expo, New Orleans, LA, June 20-25, paper 050-07.

Rosales, E., Cadwallader, K.R. and Engeseth, N. 2014. Effect of ultrasonic application on fatty acid composition and aroma compounds of dark chocolate. IFT Annual Meeting and Food Expo, New Orleans, LA, June 20-25, paper 206-94.

Burnsides, E. and Cadwallader, K. 2012 Comparison of aroma compounds in rums and rum ethers. IFT Annual Meeting and Food Expo, Las Vegas, NV, paper no. 193-28.

Su, C., Padua, G. and Cadwallader, K. 2012. Zein applications in flavor encapsulation. IFT Annual Meeting and Food Expo, Las Vegas, NV, paper no. 228-23.

Cadwallader, K.R., Hausch, B. and Lorjaroenphon, Y. 2011. Identification, characterization and relative aroma impact of 2,3-dehydro-1,8-cineol in lemon-lime flavored carbonated beverages. 13th Weurman Flavor Research Symposium, Zaragoza, SPAIN, September 26-30.

Suppavorasatit, I., deMejia, E. and Cadwallader, K.R. 2011. Effect of enzymatic deamidation of soy protein isolate by protein-glutaminase on the functional properties of the protein. IFT Annual Meeting and Food Expo, New Orleans, LA, June 11-14, paper no. 236-57.

Lapsongphon, N., Yongsawatdigul, J. and Cadwallader, K.R. 2011. Aroma characterization of premium fish sauce. IFT Annual Meeting and Food Expo, New Orleans, LA, June 11-14, paper no. 236-61.

Tinchan, P., Chaiseri, S. and Cadwallader, K.R. 2011. Quantification of δ-lactones in canned coconut milk by stable isotope dilution assay-GC-MS. IFT Annual Meeting and Food Expo, New Orleans, LA, June 11-14, paper no. 236-64.

Lorjaroenphon, Y. and Cadwallader, K.R. 2011. Identification of potent odorants in cola flavored carbonated beverages. IFT Annual Meeting and Food Expo, New Orleans, LA, June 11-14, paper no. 236-73.

Gao, Y., Cadwallader, K.R. and Schmidt, S.J. 2011. Investigating the chemical and physical stability of glass encapsulated spray-dried flavors stored below their glass transition temperatures. IFT Annual Meeting and Food Expo, New Orleans, LA, June 11-14, paper no. 290-18

Lee, H., Kim, H., Cadwallader, K.R. and Feng, H. 2011. Analysis of aroma compounds of apple cider treated by thermal-pasteurization, manosonication, thermosonication, and manothermosolication. IFT Annual Meeting and Food Expo, New Orleans, LA, June 11-14, paper no. 293-14.

Cadwallader, K.R. 2010. Potent odorants in soymilk as affected by processing and storage. *Nutraceuticals, Ingredients, Challenges, Formulations, Flavours and Health Effects.* International Conference, Exhibition and Short Course on Nutraceuticals and Functional Foods, Denpasar, Bali, Indonesia, October 11 – 12, paper no. 92.

Genthner, E.R. and Cadwallader, K.R. 2010. Characterizing aroma components of fresh-cut watermelon. 9th Wartburg Symp. on Flavor Chemistry & Biology, Eisenach, Germany, April 13-16.

He, Y., Garces, M., Toshkov, S., Kim, H., Cadwallader, K.R., Engeseth, N., Parker, T. and Jackson, M. 2010. Impact of irradiation processing on papaya nectar flavor. IFT Annual Meeting and Food Expo, Chicago, IL, July 17-20, paper no. 188-11

Kim, H. and Cadwallader, K.R. 2010. Comparison of key odorants between commercial aseptic Korean and American soy milks. IFT Annual Meeting and Food Expo, Chicago, IL, July 17-20, 188-05

Tinchan, P., Chaiseri, S. and Cadwallader, K.R. 2010. Changes in the volatile profile of canned coconut milk during storage. IFT Annual Meeting and Food Expo, Chicago, IL, July 17-20, 188-14.

Cadwallader, K.R. 2009. Flavor challenges in functional and nutraceutical beverages. International Conference and Exhibition on Nutraceuticals and Functional Foods 2009, International Society for Nutraceuticals & Functional Foods, San Francisco, CA, October 31-November 4, paper 87.

LaFond, S., Cadwallader, K. and Artz, W. 2009. The formation of 4-HNE in frying oils: A controlled study using a French fry model. 110th AOCS Annual Meeting & Expo, Orlando, FL, May 3-6.

Choi, I.-S., Kim, H. and Cadwallader, K.R. 2009. Comparison of the aroma components between dairy and soy yogurt. Annual Meeting of the Institute of Food Technologists, Anaheim, CA, June 6-9, #024-02.

Lorjaroenphon, Y., Moy, M., Rosales, E. and Cadwallader, K.R. 2009. Identification of the key aroma components of fresh and baked Bartlett pear. Annual Meeting of the Institute of Food Technologists, Anaheim, CA, June 6-9, #057-15.

Lozano, P.R. and Cadwallader, K.R. 2009. Effect of nonenzymatic deamidation of soy protein isolate on the binding of selected flavor compounds. Annual Meeting of the Institute of Food Technologists, Anaheim, CA, June 6-9, #024-12

Lozano, P. and Cadwallader, K. 2008. Binding interactions of selected sulfur compounds with soy protein isolate under controlled relative humidity. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 28 – July 1, #006-12.

Carrasco Hernandez, G. and Cadwallader, K.R. 2008. Headspace reduction by different terpenoids of the main volatile sulfur compounds responsible for garlic breath. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 28 – July 1, #006-24.

Waimaleongora-Elk, P, Herrera Corredor, J., Prinyawiwatkul., W., Suvanich, V. and Cadwallader, K. Sensory attributes driving acceptance and purchase intent of watermelon juice containing no added sugar. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 28 – July 1, #138-12

Cadwallader, K.R. and Puangpraphant, S. 2008. Changes in the aroma components of pecans during roasting. 12th Weurman Flavour Research Symposium, Interlaken, Switzerland, July 1-4

Kim, H., Cadwallader, K.R. and Kido, H. 2008. Effect of commercial rosemary extract powders on formation of lipid-derived odorants in cooked pork sausages during cold storage. 235th ACS National Meeting, New Orleans, LA, April 6-10.

Carrasco, G. and Cadwallader, K.R. 2007. Mechanism of action of menthone in ameliorating oral cavity malodor caused by consumption of garlic. 234th ACS National Meeting, Boston, MA, August 19-23.

Lozano, P.R., Miracle, E.R., Krause, A.J. and Drake, M.A. 2007. Effect of cold storage and packaging material on the major aroma components of sweet cream butter. 234th ACS National Meeting, Boston, MA, August 19-23.

Gnadt, T.A., Lee, S.-Y. and Cadwallader, K.R. 2007. Effects of commercial masking agents and lactones on soymilk aroma and flavor. Annual Meeting of the Institute of Food Technologists, Chicago, IL, July 28 - August 2, #4-224.

Hong, L., Sajuti, S.C. and Cadwallader, K.R. 2007. Identification of characteristic aroma components of Pomegranate (Punica granatum). Annual Meeting of the Institute of Food Technologists, Chicago, IL, July 28 - August 2, #3-187

Mosca, C., Esgro, S. and Cadwallader, K.R. 2007. Flavor Characterization of unrefined shea butters. American Oil Chemists' Society's (AOCS) 98th Annual Meeting and Expo, Quebec City, QC, Canada, May 13-16.

Andrae, L.M., Cadwallader, K.R. and Engeseth, N.J. 2006. Flavor changes of stored dark chocolate by dynamic headspace sorption – gas chromatography/mass spectrometry. Annual Meeting of the Institute of Food Technologists, Orlando, FL, June 24-28, #39F-12.

Lozano, P. and Cadwallader, K.R and Drake, M.A. 2006. Instrumental and sensory characterization of heat-induced odorants in aseptic packaged soymilk. Annual Meeting of the Institute of Food Technologists, Orlando, FL, June 24-28, #39F-12.

Gnadt, T.A., Jasso, L. and Cadwallader, K.R. 2005. Comparison of direct solvent extraction and dynamic headspace sampling for the analysis of characteristic aroma components of chipotle pepper. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, July 16-20, #17D-34.

Klein, B.P., Khanna, P., Cadwallader, K.R. and Endres, J.M. 2005. Reducing fat in school lunches by using soy-enhanced entrees. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, July 16-20, #70E-27.

Nowak, M.C., Singh, T.K. and Cadwallader, K.R. 2005. Identification of potent odorants in commercial hickory and mesquite liquid smoke. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, July 16-20, #17D-36.

Song, H. and Cadwallader, K.R. 2005. Aroma components of American country-style ham. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, July 16-20, #17D-35

Yu, X., Song, Y.S. and Cadwallader, K.R. 2005. Identification of the characteristic aroma components of commercial dried California bay leaves (*Laurus nobilis* L.). Annual Meeting of the Institute of Food Technologists, New Orleans, LA, July 16-20, #17D-19.

Zhou, Q. and Cadwallader, K.R. 2005. Application of IGC for measurement of binding of volatile flavor compounds by soy proteins in low-moisture food systems. 229th ACS National Meeting, San Diego, CA, March 13-17.

Bohn, D.M. Cadwallader, K.R. and Schmidt, S.J. 2004. Kinetic analysis of benzaldehyde release in artificial cherry Durarome® upon humidification using dynamic vapor sorption-fast gas chromatography-flame ionization detection. Annual Meeting of the Institute of Food Technologists, Las Vegas, NV, July 12-16, #114B-27.

Lopez, J.R., Cadwallader K.R. and Menke, S.D. 2004. Menke Aroma-impact components of Illinois Frontenac wine. Annual Meeting of the Institute of Food Technologists, Las Vegas, NV, July 12-16, #83D-3.

Singh, T.K., Young, N.D., Drake, M.A., Cadwallader, K.R. 2004. Production of a bitter peptide from casein and its sensory characterization. Annual Meeting of the Institute of Food Technologists, Las Vegas, NV, July 12-16, #17B-17.

Ugarte-Romero, E.E. Feng, H, Martin, S.E. and Cadwallader, K.R. 2004. Power ultrasound as an alternative for apple cider pasteurization. Annual Meeting of the Institute of Food Technologists, Las Vegas, NV, July 12-16, #32-10.

Yoon, Y., Carunchia-Whetstine, M.E., Friedeck, K.G., Drake, M.A. and Cadwallader, K.R. 2004. Characterization of aroma-active compounds in fresh sweet cherry cultivars. Annual Meeting of the Institute of Food Technologists, Las Vegas, NV, July 12-16, #114B-3.

Zhou, Q. and Cadwallader, K.R. 2004. Investigation of flavor-soy protein interactions under controlled relative humidity by inverse gas chromatography (IGC). Annual Meeting of the Institute of Food Technologists, Las Vegas, NV, July 12-16, # 114B-2.

Brisske, L.K., Lee, S.Y., Klein, B.P. and Cadwallader, K.R. 2003 Consumer ratings of energy bars: Should the bar be raised? Annual Meeting of the Institute of Food Technologists, Chicago, IL, July 12-16, #12-2.

Bohn, D.M., Cadwallader, K.R. and Schmidt, S.J. 2003. Measurement of volatile release from an amorphous carbohydrate matrix as a function of relative humidity using dynamic vapor sorption technology. Annual Meeting of the Institute of Food Technologists, Chicago, IL, July 12-16, #12-2.

Singh, T.K., Drake, M.A. and Cadwallader, K.R. 2003. Characterization of aroma components of Muenster cheese by aroma extract dilution and headspace dilution analyses. Annual Meeting of the Institute of Food Technologists, Chicago, IL, July 12-16, #71-3.

Suriyaphan, O., Zhou, Q. and Cadwallader, K.R. 2003. Identification of key aroma components of *Piper betel* L. leaf. Annual Meeting of the Institute of Food Technologists, Chicago, IL, July 12-16, #45C-16.

Drake, M.A., Karagul-Yuceer, Y., Civille, G.V., Cadwallader, K.R. and Tong, P.S. 2002. Development of a descriptive sensory language for milk powders. Annual Meeting of the Institute of Food Technologists, Anaheim, CA, June 15-19, #100D-2.

Karagul-Yuceer, Y., Drake, M.A. and Cadwallader, K.R. 2002. Evaluation of the character-impact odorants in nonfat dried milk by sensory studies on model mixtures. Annual Meeting of the Institute of Food Technologists, Anaheim, CA, June 15-19, #12-10.

Karagul-Yuceer, Y., Vlahovich, K., Drake, M.A. and Cadwallader, K.R. 2002. Compounds responsible for musky off-odor of rennet casein. Annual Meeting of the Institute of Food Technologists, Anaheim, CA, June 15-19, #15B-12.

Karagul-Yuceer, Y, Cadwallader, K.R. and Drake, M.A. 2002. Odor-active compounds of liquid whey. Annual Meeting of the Institute of Food Technologists, Anaheim, CA, June 15-19, #61B-4.

Pojjanapimol, S., Chaiseri, S. and Cadwallader, K.R. 2002. Aroma components of distilled essential oil of Holy basil. Annual Meeting of the Institute of Food Technologists, Anaheim, CA, June 15-19, #61B-12.

Zhou, Q., Cadwallader, K.R. and Guthrie, B.D. 2002. Simultaneous measurement of permeability of aroma compound mixtures through polymer packaging films. Annual Meeting of the Institute of Food Technologists, Anaheim, CA, June 15-19, #100B-8.

Benitez, D. and Cadwallader K.R. 2001. Aroma-active components of Vietnamese coriander (*Polygonum odoratum*). Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 23-27, #88A-14.

Benitez, D., Kappes, S. and Cadwallader, K.R. 2001. Aroma of dried peaches. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 23-27, #44C-34.

Chen, D., Andrae, L., Suriyaphan, O., Weingartner, K. and Cadwallader, K. 2001. Characteristic aroma components of vegetable soybean (edamame). Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 23-27, #88A-17.

Drake, M.A., Gerard, P.D., Wright, S., Cadwallader, K.R. and Civille, G.V. 2001. Cross validation of a sensory language for Cheddar cheese. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 23-27, #73G-19.

Grady, J., Klein, B.P. and Cadwallader, K.R. 2001. Bitterness and astringency thresholds for soy isoflavones. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 23-27, #98-2.

Karagul-Yuceer, Y, Cadwallader, K.R. and Drake, M.A. 2001. Identification of volatile off-flavor compounds of nonfat dried milks. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 23-27, #88A-3.

Suvanich, V., Maciel-Pedrote, I., Cadwallader, K.R., Moser, E.B., Thongthai, C. and Prinyawiwatkul, W. 2001. Aroma indices of Southeast Asian fish sauces. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 23-27, #73F-16.

Suvanich, V., Maciel-Pedrote, I., Cadwallader, K.R., Moser, E.B., Thongthai, C. and Prinyawiwatkul, W. 2001. Developnebt if fish sauces quantitative descriptive sensory aroma profile. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 23-27, #73G-34.

Zhou, X., Winterstein, C. and Cadwallader, K.R. 2001. Aroma of buckwheat honey. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 23-27, #88A-12.

Zhu, L., Robbins, K. and Cadwallader, K.R. 2001. Effects of soy protein isolate on headspace aroma components of cooked ground beef patties. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 23-27, #88A-18.

Drake, M.A., McIngvale, S.C., Cadwallader, K.R. and Civille, G.V. 2000. Development of a descriptive language for Cheddar cheese. Book of Abstracts, Annual Meeting of the Institute of Food Technologists, Dallas, TX, June 10-14, #11-3.

Suriyaphan, O., Cadwallader, K.R. and Drake, M.A. 2000. Lipid oxidation of deoiled soy lecithin in fermented milk systems. Book of Abstracts, Annual Meeting of the Institute of Food Technologists, Dallas, TX, June 10-14, #14B-32.

Cadwallader, K.R. and Thongthai, C. 2000. Comparison of aroma-active headspace components of three types of fish sauces. Book of Abstracts, Annual Meeting of the Institute of Food Technologists, Dallas, TX, June 10-14, #36-3.

Chen, X.Q., Drake, M.A. and Cadwallader, K.R. 2000. Characteristic aroma components of English Farmhouse Cheddar Cheeses. Book of Abstracts, Annual Meeting of the Institute of Food Technologists, Dallas, TX, June 10-14, #78C-4.

Karagul, Y., Drake, M.A. and Cadwallader, K.R. 2000. Aroma-active components of nonfat dry milk powders. Book of Abstracts, Annual Meeting of the Institute of Food Technologists, Dallas, TX, June 10-14, #78C-6.

Cadwallader, K.R. and Jung, J. 1999. Evaluation of warmed-over flavor in chicken wings by sensory and instrumental methods. 60th Annual Meeting of the Institute of Food Technologists, Chicago, IL, #50A-13, p. 122.

Wu, Y.F., Baek, H.H. and Cadwallader, K.R. 1999. Development of a meat-like process flavoring from soybean-based hydrolyzed vegetable protein. 60th Annual Meeting of the Institute of Food Technologists, Chicago, IL, #50A-9, p.121.

Suriyaphan, O., Cadwallader, K.R. and Drake, M.A. 1999. Lecithin associated off-flavors in fermented dairy products—A model study. 60th Annual Meeting of the Institute of Food Technologists, Chicago, IL, #17-2, p. 31.

Cadwallader, K.R. and Suriyaphan, O. 1998. Aroma-impact components of loroco. 59th Annual Meeting of the Institute of Food Technologists, Atlanta, GA, #45-2, p. 97.

Surakarnkul, R. and Cadwallader, K.R. 1998. Release of glycosidically-bound aroma compounds during fermentation of muscadine juice. 59th Annual Meeting of the Institute of Food Technologists, Atlanta, GA, #72-18, p. 180.

Suriyaphan, O., Drake, M.A., and Cadwallader, K.R. 1998. Identification of volatile off-flavors in reduced fat cheeses containing lecithin. 59th Annual Meeting of the Institute of Food Technologists, Atlanta, GA, #72-31, p. 183.

Baek, H.H. and Cadwallader, K.R. 1997. Free and glycosidically bound volatile aroma compounds in muscadine grape juice. 58th Annual Meeting of the Institute of Food Technologists, Orlando, FL, #23D-6, p.63.

Cai, M. and Cadwallader, K.R. 1997. (Z)-3-Nonenal and (Z,Z)-3,6-nonadienal: Character-impact aroma compounds in watermelon. 58th Annual Meeting of the Institute of Food Technologists, Orlando, FL, #23D-9, p.64.

Cha, Y.J., Kim, H., and Cadwallader, K.R. 1997. Aroma-active compounds in Kimchi during aging. 58th Annual Meeting of the Institute of Food Technologists, Orlando, FL, #23D-13, p.65.

Baek, H.H., Kim, C.J., Ahn, H.S., Nam, H.S., and Cadwallader, K.R. 1997. Aroma extract dilution analysis of beef-like process flavor from extruded enzymatically hydrolyzed soybean protein. 58th Annual Meeting of the Institute of Food Technologists, Orlando, FL, #23D-24, p.67.

Fu, S.G., Baek, H.H., and Cadwallader, K.R. 1997. Comparison of aroma-active compounds extracted from blue crab meat with processing by-products hydrolysate using aroma extract dilution analysis. 58th Annual Meeting of the Institute of Food Technologists, Orlando, FL, #23D-25, p.67.

Aryana, K.J., Cadwallader, K.R., and Haque, Z.U. 1996. Isolation of flavor volatiles in lowfat Cheddar cheese by vacuum simultaneous distillation-solvent extraction. 73rd Annual Meeting of the Southern Branch, American Dairy Science Association, February 3-7.

Baek, H.H. and Cadwallader, K.R. 1996. Evaluation of the enzymatic hydrolysis of defatted soybean meal by response surface methodology. 57th Annual Meeting of the Institute of Food Technologists, New Orleans, LA, #53A-16, p.120.

Baek, H.H., Cadwallader, K.R., Marroquin, E., and Silva, J.L. 1996. Gas chromatography/olfactometric analysis of muscadine grape aroma. 57th Annual Meeting of the Institute of Food Technologists, New Orleans, LA, #34-7, p.74.

Cadwallader, K.R. 1996. Potent odorants in hickory and mesquite smokes and liquid smoke extracts. 57th Annual Meeting of the Institute of Food Technologists, New Orleans, LA, #34-6, p. 74.

Cadwallader, K.R., Baek, H.H., Chang-Lee, G.-H. 1996. Aroma extract dilution analysis of cooked freshwater prawn. 57th Annual Meeting of the Institute of Food Technologists, New Orleans, LA, #80D-20, p.197.

Cha, Y.J., Kim, E.J., Baek, H.H., and Cadwallader, K.R. 1996. Development of a flavoring agent from sea mussel. 57th Annual Meeting of the Institute of Food Technologists, New Orleans, LA, #80D-22, p.197.

Kim, H.R., Ahn, C.B., Jung, K.J., Baek, H.H., Cadwallader, K.R. 1996. Characterization and utilization of waste water from oyster processing plants as flavoring agents. 57th Annual Meeting of the Institute of Food Technologists, New Orleans, LA, #80D-21, p.197.

Aryana, K.J., Cadwallader, K.R., and Haque, Z.U. 1996. Development of methodology for measuring+ flavor volatiles in Cheddar cheese made with and without fat replacers. International Conference on Food Science and Technology of The Fine Particle Society, Chicago, IL, August 24-26.

Cha, Y.J., Baek, H.H., and Cadwallader, K.R. 1995. Volatile flavor components in oyster hydrolysate produced with protease. 56th Annual Meeting of the Institute of Food Technologists, Anaheim, CA, #54E-16, p.166.

Conner, T.M., McGregor, J.U., and Cadwallader, K.R. 1995. Identifying flavor components in light-activated milk. J. Dairy Sci. 48(supplement 1): 317.

Baek, H.H. and Cadwallader, K.R. 1994. Comparison of volatile compounds in flavor concentrates produced from crawfish processing by-products with and without protease treatment. 55th Annual Meeting of the Institute of Food Technologists, June 25-29, Atlanta, GA, #79C-5, p.244.

Cadwallader, K.R., Tan, Q., Chen, F., and Meyers, S.P. 1994. Identification of character-impact aroma compounds in spiny lobster (*Panulirus argus*) tail meat. 55th Annual Meeting of the Institute of Food Technologists, June 25-29, Atlanta, GA, #7-7, p.13.

Chung, H.Y. and Cadwallader, K.R. 1994. Comparison of the aroma of three different extracts prepared from blue crab (*Callinetes sapidus*) processing by-product. 55th Annual Meeting of the Institute of Food Technologists, June 25-29, Atlanta, GA, #79C-7, p.244.

Chung, H.Y., Chen, F., and Cadwallader, K.R. 1994. Evaluation of aroma of blue crab (*Callinetes sapidus*) claw and lump meats. 55th Annual Meeting of the Institute of Food Technologists, June 25-29, Atlanta, GA, #79C-6, p.244.

Tan, Q. and Cadwallader, K.R. 1994. Biotransformation of ß-pinene by *Aspergillus cellulosae*. 55th Annual Meeting of the Institute of Food Technologists, June 25-29, Atlanta, GA, #24C-7, p.67.

Andrews, L.S. and Cadwallader, K.R. 1993. Microbial and sensory quality of ground ginger as affected by gamma irradiation. 54th Annual Meeting of the Institute of Food Technologists, July 10-14, Chicago, IL, Technical Program: Book of Abstracts, #817, p.206.

Baek, H.H. and Cadwallader, K.R. 1993. Identification of characteristic volatile flavor components of roasted chicory by aroma extract dilution analysis. 54th Annual Meeting of the Institute of Food Technologists, July 10-14, Chicago, IL, Technical Program: Book of Abstracts, #506, p.129.

Cha, Y.J. and Cadwallader, K.R. 1993. Volatile flavor components in Korean fermented fish paste. 54th Annual Meeting of the Institute of Food Technologists, July 10-14, Chicago, IL, Technical Program: Book of Abstracts, #510, p.130.

Cha, Y.J., Baek, H.H., and Cadwallader, K.R. 1993. Comparison of DHS and SDE for the analysis of volatile compounds in fermented shrimp paste. 54th Annual Meeting of the Institute of Food Technologists, July 10-14, Chicago, IL, Technical Program: Book of Abstracts, #511, p.131.

Chung, H.Y. and Cadwallader, K.R. 1993. Aroma extract dilution analysis of freshly picked cooked blue crab meat. 54th Annual Meeting of the Institute of Food Technologists, July 10-14, Chicago, IL, Technical Program: Book of Abstracts, #563, p.144.

Chung, H.Y., Chou, C.-C., and Cadwallader, K.R. 1993. Changes in the volatile flavor constituents of coconut during roasting. 54th Annual Meeting of the Institute of Food Technologists, July 10-14, Chicago, IL, Technical Program: Book of Abstracts, #504, p.129.

Yoo, S.K. and Cadwallader, K.R. 1993. Bioconversion of α- and ß-pinene. 54th Annual Meeting of the Institute of Food Technologists, July 10-14, Chicago, IL, Technical Program: Book of Abstracts, #516, p.132.

Baek, H.H. and Cadwallader, K.R. 1992. Enzymatic hydrolysis of crawfish waste for bioflavor production. Ninth International Biotechnology Symposium and Exposition, August 16-21, Crystal City, VA, Book of Abstracts, #472.

Baek, H.H., Pike, M.M., Bidner, T.D., Moody, M.W., and Cadwallader, K.R. 1992. Evaluation of the off-odor associated with wild alligator. 53rd Annual Meeting of the Institute of Food Technologists, June 20-24, New Orleans, LA. Book of Programs and Abstracts, #359, p.93.

Cha, Y.J., Baek, H.H., and Cadwallader, K.R. 1992. Volatile flavor components in concentrated snow crab extracts. 53rd Annual Meeting of the Institute of Food Technologists, June 20-24, New Orleans, LA, Book of Abstracts, #357, p.92.

Chung, H.Y. and Cadwallader, K.R. 1992. Comparison of volatile compounds in freshly picked crabmeat and crab processing waste. 53rd Annual Meeting of the Institute of Food Technologists, June 20-24, New Orleans, LA, Book of Abstracts, #358, p.93.

Kim, H.R., Baek, H.H., Cadwallader, K.R., Meyers, S.P., and Godber, J.S. 1992. Improved flavor extractibility from crab waste using the hepatopancreatic enzyme of crawfish, *Procambarus clarkii*. 53rd Annual Meeting of the Institute of Food Technologists, June 20-24, New Orleans, LA, Book of Abstracts, #360, p.93.

Cadwallader, K.R. and Braddock, R.J. 1990. Enzymatic hydration of limonene to α-terpineol. 51st Annual Meeting of the Institute of Food Technologists, June 16-20, Anaheim, CA, Book of Programs and Abstracts, #354, p.170.

Cadwallader, K.R. 1988. Bioconversion of (+)-limonene. 49th Annual Meeting of the Institute of Food Technologists, June 19-22, New Orleans, LA, Book of Programs and Abstracts, #293.

Students/Postdoctoral/Visiting Scholar Researchers Supervised (1991-1999)

Visiting Scientists
Yong-Jun Cha (LSU, MSU) – Chang Won National University
Hetty Voermans (LSU) – University of Wegeningen, The Netherlands

Postdoctoral Researchers
Hyung-Hee Baek (MSU)

Graduate Students Completed (LSU/MSU)

MS Students
Sun-Kyun Yoo (LSU)
Min Cai (MSU)
Hsin-Jen (Connie) Weng (MSU)
Shi-Guei (Scott) Fu (MSU)
Shin-Pei (Peggy) Yang (MSU)
Yi-Fang (Grace) Wu (MSU)
Leyla Yuksel (MSU)
JiHyun Jung (MSU)
Min-Sheng Su (MSU)
Gwo-Yuh Li (MSU)
Jeong Heo (MSU)

Ph.D. Students
Hyung-Hee Baek (LSU)
Hau Yin Chung (LSU)
Gyu-Hee (Chang) Lee (MSU)
Ranee Surakarnkul (MSU)
Yonca Karagul-Yuceer (MSU)

Students/Postdoctoral/Visiting Scholar Researchers Supervised (UIUC, 2000-present)

Visiting Scholars
Byung Hak Ahn (2006-2007) - Korea Food Research Institute, South Korea
Supeeraya Arsa (2013-2014) – Kasetsart University, Bangkok, Thailand
Mantana Buttara (2011-present) – Suranaree Univeristy of Technology, Thailand
Dong Chen (2015-present) – China Agricultural University, Beijing, China

Rongrong Chen (2016-present) - Ever Maple Food Science and Technology Co., Ltd., China
Arporn Jarunrattanasri (2003-2004) – Naresuan University, Thailand
Young-Suk Kim (2015-present) – Ewha Womans University
Patthamawadi Kiatbenjaku. (2009-2011, 2012) – Suranaree University of Technology, Thailand
Fareeya Kulapichitr (2016-present) – Chulalongkorn University, Thailand
Sasinee Kunyaboon (2016-present) - Suranaree University of Technology, Thailand
Nawaporn Lapsongphon (2010-2011) - Suranaree University of Technology, Thailand
Yuping Liu (2014-2015) – Beijing Technology and Business University, China
Yaowapa Lorjaroenphone (2006-2007) – Kasetsart University, Thailand
Xueli Pang (2013-2014) – China Agricultural University, Beijing, China
Sompoche Pojjanapimol (2003-2004) - Kasetsart University, Thailand
Primseri Rotsachakul (2004-2005) – Kasetsart University, Thailand
Huanlu Song (2005-2006) – Beijing Technology and Business University, Beijing, China
Patcharaporn Tinchan (2010-2012) – Kasetsart University, Thailand
Jihong Wu (2011-2012) – China Agricultural University, Beijing, China

Postdoctoral Researchers (UIUC)
Masud Huda (2002-2003)
Orasa Suriyaphan (2001, 2003)
Tanoj Singh (2001-2005)
Hun Kim (2005-2012)

Graduate Students Completed (UIUC)
Daniel Benitez (MS 2003)
Laura Brisske (MS 2003)
Erin Burnside (MS 2012)
Gabriela Carrasco (MS 2007)
Shondra Cook (MS 2007)
Edibe Erten (Ph.D. 2016)
Ming Chih Fang (Ph.D.2013)
Samantha Gardiner (MS 2015)
Elizabeth Genthner (MS 2010; Ph.D. 2014)
Theresa Gnadt (MS 2007)
Julia Gregg (Nonthesis MS, CHEFS Program, 2009)
Bethany Hausch (MS 2010)
Lauren Kelley (MS)
Sean LaFond (MS 2009)
Jacob Lahne (MS 2010)
Yaowapa Lorjaroenphone (Ph.D. 2012)
Patricio Lozano (MS 2005 & Ph.D. 2009)
Samantha McKenna (MS, 2016)
Joshua Moore (MS 2006)
Linda Moran (MS 2013)
Ana Carolina Mosca (MS 2007)
Jason Niermann (Nonthesis MS, CHEFS Program 2001)
Sirima Puangpraphant (MS 2007)
Bethany Richardson (MS 2013)
Inthawoot Suppavorasatit (Ph.D. 2012)
Chenxing Sun (3+2 program, Nonthesis MS)
Qiaoxuan Zhou (MS 2001 & Ph.D. 2005)

Current Graduate Students (2016)
Courtney Beckett (MS)
Chelsea Ickes (Ph.D.)
Fengjia Chen (MS, 3+1)
Bethany Hausch (Ph.D.)
Nagiat Hwasa (Ph.D.)
Jing (Jenny) Jin (MS, 3+1)
Tiandan Wu (MS)
Wenqi Zhu (Ph.D.)
Yun Yin (Ph.D.)

Undergraduate Student Researchers
Kelsey Choquette (2012-2013)
Diniene Chreaton, Rap II (2001)
Ashley Davis, Rap II (ACES) (2001) & UIUC Ronald E. McNair Summer Research Program (2004)
Yaozheng (Tess) Liu (2013-present)
Rosemary Nash (2011-2012)
Mary Nowak (2004-2005)
Jieun Oh (2013)
Abigail Pishevar (2014-2015)
David Potts (2008-2010)
Stephanie Sajuti (2005-2007)
Dylan Troth (2015-present)
Katrina Vlahovich (2001-2002)

Involvement in Professional Organizations (2000-present)

Leadership

Chair and Program Chair for the Division of Agricultural and Food Chemistry (AGFD) of the American Chemical Society, 2003-2005. Responsibilities include organizing AGFD technical programs for two ACS National Meetings (fall 2004 and spring 2005 meetings).

Editorial Board

Flavour and Frangrance Journal

Associate Editor

Journal of Food Science – *Food Chemistry*

Symposia Organized

Cadwallader, K.R. and Shahidi, F. 2017. ACS Symposium: *Chemistry of Clean Ingredients.* Washington, DC, August 20-24.

Cadwallader, K.R. and Chang, S.K.C. 2008. ACS Symposium: *Chemistry, Texture and Favor of Soy,* Philadelphia, PA, August 17-21.

Cadwallader, K.R. 2008. ACS Symposium: *Recent Developments in Flavor Chemistry*. 235th ACS National Meeting, New Orleans, LA, April 6-10.

Ho, C.-T., Weerasinghe, D.K. and Cadwallader, K.R. 2005. ACS Symposium: *Aroma-active Compounds in Foods and Reaction Favors: formation, measurement and stability.* 330th ACS National Meeting, Washington, DC, August 28 – September 1.

Cadwallader, K.R., P. Schieberle and M.J. Morello. 2005. ACS Symposium: *Grain-based Products: Health and Flavor, and Safety Aspects.* 330th ACS National Meeting, Washington, DC, August 28 – September 1.

Cadwallader, K.R. *Obesity Management: Role of Soy Foods.* University of Illinois, Champaign, IL, May 25-26, 2005.

Cadwallader, K.R., Drake, M.A. and McGorrin, R.J. 2004. ACS Symposium: *Chemistry and Flavor of Dairy Products.* 228th ACS National Meeting, Philadelphia, PA, August 22-26.

Weenen, H. and Cadwallader, K.R. (organizers). 2001. ACS Symposium: *Freshness and Shelf-life of Foods*. 222nd ACS National Meeting, Chicago, IL, August 26-30.

Cadwallader, K.R. and McGorrin, R.J. (organizers). 2001. IFT Symposium: *Developments in the Analysis of Complex Flavor Systems*. Annual Meeting of the Institute of Food Technologies, New Orleans, LA, June 23-27.

Rouseff, R.L. and Cadwallader, K.R. 1998. ACS Symposium: *Headspace Analysis of Foods and Flavors.* 214th ACS National Meeting, Boston, MA, August 23-27.

Shahidi, F. and Cadwallader, K.R. 1996. ACS Symposium: *Flavor and Lipid Chemistry of Seafoods.* 212th National Meeting of the American Chemical Society, Orlando, FL, August 25-29.

Workshops/Short courses (organized)

A Place for Soy in a Better School Lunch Program. University of Illinois, Urbana, IL, March 12, 2003.

Traditional and Emerging Methods for the Manufacture and Analysis of Food Flavorings. Faculty of Agriculture Industry, King Mongkut's Institute of Technology Ladkraband (KMITL), Bangkok, Thailand, July 29, 2002

Soy Flavor Workshop. ICSF Workshop (Klein, B.P. et al. coorganizers). Illinois Center for Soy Foods, University of Illinois, Urbana, IL, April, 2002/2003.

Workshops/Short courses (lecturer)

Nutraceuticals, Ingredients, Challenges, Formulations, Flavours and Health Effects. International Society for Nutraceutical and Functional Foods. Denpasar, Bali, Indonesia, October 11 – 12.

Flavor Chemistry. (course co-taught with Drs. Chi-Tang Ho and Soo-Yeun Lee), Taiwan National University, May 10-19, 2008.

Flavor Research Workshop. ACS Short Course (Acree, T.E., course director), American Chemical Society, Boston, MA, August 17-18, 2007.

Flavor Research Workshop. ACS Short Course (Acree, T.E. and Deibler, K., organizers), American Chemical Society, August 25-26, 2005.

Cadwallader, K.R. Flavoring soy products: masking and creating. Preconference AOAC Short Course. *Soyfoods: Ingredients, Preparation, and Utilization*. Koseoglu, S. and Shahidi, F. (organizers), Cincinnati, OH, May 9, 2005.

Cadwallader, K.R. 2004. Aroma and flavor aspects of soy beverages. Preconference Shortcourse: *Functional Beverages*, Koseoglu, S and Shahidi, F. (organizers), Worldnutra: International Conference and Exhibition on Nutraceutacals and Functional Foods, San Francisco, CA, Nov 8, 2004

Flavor Research Workshop. ACS Short Course (Acree, T.E., organizer), American Chemical Society, Boston, MA, August 16-17, 2002; New York, NY, September 7-11, 2003.

Cheese Flavor and Texture: Sensory and Instrumental Analysis. SDFRC Short Course (Drake, M.A., course director), Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, NC, August 7-9, 2002.

Proposal Reviewer/Panel Participation (2010-present)

Panelist/Reviewer (2015) - Office of Scientific Quality Review (OSQR)-USDA-ARS, NP 306 Panel 14: Functional Foods (2015)

Panelist/Reviewer (2015) - Office of Scientific Quality Review (OSQR)-USDA-ARS, NP 107 Panel 14: Grain Engineering (2014)

Panelist/Reviewer (2014) - Office of Scientific Quality Review (OSQR)-USDA-ARS, NP 107 Panel 5: Food Chemistry Analysis (2014)

Panelist/Reviewer (2013) – 2012/2013 King Abdulaziz City for Science and Technology (KACST) Research Proposal Competition

EXHIBIT B

MATERIALS CONSIDERED

- U.S. Application Serial No. 14/797,006, Fraser, R. Brown, P.O., Karr, J. and Holz-Schietinger, C., 2014, Publication No. US 2015/0351435
- Hsieh, F-h and Huff, H.E. 2012. Meat analog compositions and process. U.S. Patent Application No. US 2012/0093994 ("the '994 application")
- Office Action Mailed June 6, 2016 and draft Response to same
- Declaration Under 37 CFR §1.132 of Celeste Holz-Schietinger, Ph.D.
- Calkins, C.R. and Hodgen, J.M. A fresh look at meat flavor. Meat Sci. 2007, 63-80.
- Proulx, A.K. and Reddy, M.B. Iron bioavailability of hemoglobin from soy root nodules using caco-2 cell culture model. J. Agric. Food Chem. 2006, 54, 1518-1522.
- Belitz, H.-D., Grosch, W., and Schieberle, P. Food Chemistry, 3rd revised edition. Springer-Verlag, Berlin (2006), p. 368.
- Tang, W., Jiang, D., Yuan, P. and Ho, C.-T. (2013) Flavor chemistry of 2-methyl-3-furanthiol, an intense meaty aroma compound. J. Sulfur Chem. 34: 38-47.
- Chaudhari, N. and Roper, S.D. (2010). The cell biology of taste. J. Cell Biol. 190(3): 285-296.
- Shahidi, F., Rubin, L.J., D'Souza, L.A., Teranishi, R. and Buttery, R.G. 1986. Meat flavor volatiles: A review of the composition, techniques of analysis, and sensory evaluation. CRC Crit. Rev. Food Sci. Nutr. 24: 142-243.
- Cadwallader, K.R. and MacLeod, A.J. 1998. Instrumental methods for analyzing the flavor of muscle foods. Ch. 16. In *Flavor of Meat, Meat Products, and Seafoods, 2nd Ed.* Shahidi, F. (Ed.). Blackie Academic & Professional, New York, pp.
- Jublot, L., Rosing, E.A.E, Blom, L. v.d., Desclaux, G., Martins, S.I.F.S., Batenburg, A.M.

and Smit, G. 2010. Quantitation of sulphur aroma compounds in Maillard model reaction systems of different composition. In *Expression of Multidisciplinary Flavour Science.* Proceedings of the 12th Weurman Symposium. Blank, I., Wüst, M., and Yeretzian, C. (Eds.). Winterthur: Zurich University of Applied Sciences.

- Spence, C. 2015. Multisensory flavor perception. Cell 161: 24-35.

EXHIBIT A

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Devin G Peterson

Experience:

Aug/16 **Professor** **Food Science & Technology** **Director of the Flavor Research & Education Center**	The Ohio State University	Columbus, OH
June/12 – Aug/16 **Professor** **Food Science & Nutrition** **Director of the Flavor Research & Education Center**	The University of Minnesota	St. Paul, MN
Aug/09 – June/12 **Associate Professor** **Food Science & Nutrition** **Director of the Flavor Research & Education Center**	The University of Minnesota	St. Paul, MN
July/07 – Aug/09 **Associate Professor/Food Science**	The Pennsylvania State University	University Park, PA
March/01 – June/07 **Assistant Professor/Food Science**	The Pennsylvania State University	University Park, PA
Dec/99 – Feb./01 **Research Assistant**	University of Minnesota	St. Paul, MN
July/99 - Dec./99 **Research Flavor Scientist (Internship)**	Givaudan Flavors	Cincinnati, OH
Sept./94 - July/99 **Research Assistant**	University of Minnesota	St. Paul, MN
June/94 –Aug./94 **Information Technician**	The Canadian Wheat Board	Winnipeg, MB
May – Aug./92&93 **QC Technician**	Coca-Cola Bottling Company	Winnipeg, MB

Education:	1997 - 2001 **PhD/Food Science** (Dean's List)	University of Minnesota	St. Paul, MN
	1994 - 1997 **MS/Food Science** (Dean's List)	University of Minnesota	St. Paul, MN
	1990 – 1994 **BS/Food Science** (Dean's List)	University of Manitoba	Winnipeg, MB

Awards:	Flavor and Extract Manufacturers Association - Excellence in Flavor Science Award	2015
	Waters Centers of Innovation Award	2012
	Pan-American Nutrition, Food Science and Technology Award	2008
	Presidential Early Career Award for Scientist and Engineers (PECASE)	2004
	John Brandt Memorial Foundation Scholarship; National	2000
	M. E. Franks Scholarship; National	1998, 1999 & 2000
	IFT Grad. Fellowship–Soc. of Flavor Chemists Memorial; National	1998/99 & 2000/01
	AACC Graduate Fellowship; National	1997/98
	IFT Scholarship; Minnesota	1997 & 2000
	Triticus Award; Departmental	1996
	The Canadian Wheat Board Scholarship; National	1994

Peer Reviewed Publications (*italics* is corresponding author):

1. Raithore, S. and ***Peterson, D.G.*** 2016. Delivery of taste and aroma components in sugar-free chewing gum: mass balance analysis. Chemosensory Perception. *Accepted.*
2. Paravisini, L. and ***Peterson, D.G.*** 2016. Characterization of Browning Formation in Orange Juice during Storage. ACS Symposium Proceedings, Browned Flavors: Analysis, Formation, & Physiology. *Accepted.*
3. Kokkinidou, S. ***Peterson, D.G.*** 2016. Identification of compounds that contribute to trigeminal burn in aqueous ethanol solutions. Food Chem. 211: 757-762 http://www.sciencedirect.com/science/article/pii/S0308814616308068
4. Bin, Q. and ***Peterson, D.G.*** 2016. Identification of Bitter Compounds in Whole Wheat Bread Crumb. Food Chem. 203: 8–15, http://www.sciencedirect.com/science/article/pii/S0308814616301182
5. Yeo, L., Thompson, D. and ***Peterson, D.G.*** 2016. Inclusion Complexation of Flavor Compounds by Dispersed High-Amylose Maize Starch (HAMS) in an Aqueous Model System. Food Chem. *199*: 393–400. http://www.sciencedirect.com/science/article/pii/S0308814615303320
6. Yucel, U. and ***Peterson, D.G.*** 2015. Effect of Protein–Lipid–Salt Interactions on Sodium Availability in the Mouth and Consequent Perception of Saltiness: In Solutions. J. Agric. Food Chem., 63: 7487–7493.
   Impact Factor: 3.1 http://pubs.acs.org/doi/abs/10.1021/acs.jafc.5b02311
7. Yucel, U. and ***Peterson, D.G.*** 2015. Effect of Protein–Lipid–Salt Interactions on Sodium Availability in the Mouth and Consequent Perception of Saltiness: As Affected by Hydration in Powders. J. Agric. Food Chem., 63: 7494–7498. http://pubs.acs.org/doi/abs/10.1021/acs.jafc.5b02312

8. Forero, D.P, Orrego, C.E. **Peterson, D.G.** and *Osoria, C.* 2015. Chemical and sensory comparison of fresh and dried lulo (*Solanum quitoense* Lam.) fruit aroma. Food Chemistry, 169: 85–91. http://www.sciencedirect.com/science/article/pii/S0308814614011595
9. Kokkinidou, S. and ***Peterson, D.G.*** 2015. Characterization of compounds that influence alcohol trigeminal burn in spirits. Weurman Symposium Proceedings Flavour Science Proceedings of the XIV Weurman Flavour Research Symposium, Andrew Taylor and Donald Mottram (Eds), pg 31-37.
10. Ronningen, I. and ***Peterson, D.G.*** 2015. Application of untargeted LC/MS techniques (Flavoromics) to identify changes related to freshness of food, *in* The Chemical Sensory Informatics of Food: Measurement, Analysis, Integration. Eds. Guthrie, et al.; ACS Symposium Series; American Chemical Society: Washington, DC, Vol. 1191, pg 269-277. http://pubs.acs.org/doi/pdf/10.1021/bk-2015-1191.ch019
11. Zhang, L., Xia, Y. and ***Peterson, D.G.*** 2014. Identification of bitter modulating Maillard-catechin reaction products. J. Agric. Food Chem. 62, 10092−10100. http://pubs.acs.org/doi/abs/10.1021/jf502040e
12. Karametsi, K., Kokkinidou, S., Ronningen, I. and ***Peterson, D.G.*** 2014. Identification of Bitter Peptides in Aged Cheddar Cheese. J. Agric. Food Chem. 62, 8034−8041. http://pubs.acs.org/doi/abs/10.1021/jf5020654
13. Troise, A. D. Fiore, A. Colantuono, A. Kokkinidou, S., **Peterson, D.G.** and *Fogliano, V.* 2014. Effect of olive mill wastewater phenol compounds on reactive carbonyl species and Maillard reaction end-products in ultrahigh-temperature-treated milk. J. Agric. Food Chem. 62, 10092−10100. http://pubs.acs.org/doi/abs/10.1021/jf501919y
14. Kokkinidou, S. and ***Peterson, D.G.*** 2014. Control of Maillard-type Off-Flavor Development in Ultra High Temperature Processed Bovine Milk By Phenolic Chemistry. J. Agric. Food Chem. 62, 8023−8033. http://pubs.acs.org/doi/abs/10.1021/jf501919y
15. Lui, X., Jiang, D. and ***Peterson, D.G.*** 2014. Identification of Bitter Peptides in Whey Protein Hydrolysate. J. Agric. Food Chem. 62, 5719−5725. http://pubs.acs.org/doi/abs/10.1021/jf4019728
16. Kokkinidou, S. and ***Peterson, D.G.*** 2013. Response Surface Methodology as Optimization Strategy for Reduction of Reactive Carbonyl Species in Heat-processed Foods by Means of Phenolic Chemistry. Food and Function. 4(7): 1093-1104. http://pubs.rsc.org/en/content/articlelanding/2013/fo/c3fo60032g
17. Jiang, D. and ***Peterson, D.G.*** 2013. Identification of Bitter Compounds in Whole Wheat Bread. Food Chem. 141; 1345-1353. http://www.sciencedirect.com/science/article/pii/S0308814613003233
18. Moskowitz, M., Bing, Q., Elias, R. and ***Peterson, D.G.*** 2012. Influence of endogenous ferulic acid in whole wheat flour on bread crust aroma. J. Agric. Food Chem. 60: 11245-11252. http://pubs.acs.org/doi/abs/10.1021/jf303750y
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20. Bin, Q., Jiang, D. Cho, I.H., ***Peterson, D. G.*** 2012. Chemical markers for bitterness in wheat bread. 2012. Flavour Fragr. J. 27, 454–458. http://onlinelibrary.wiley.com/doi/10.1002/ffj.3124/abstract

21. **Peterson, D.G**. 2012. Refining Maillard-type flavor chemistry: Phenolic reaction pathways, *in* Advances and Challenges in Flavor Chemistry & Biology. Plenary Lecture, 9th Wartburg Symposium Proceedings on Flavor Chemistry & Biology, Eisenach, Germany, pp 155-160
22. Bin, Q. **Peterson, D.G.** and *Elias, R.J.* 2012. Influence of phenolic compounds on the mechanisms of pyrazinium radical generation in the Maillard reaction. J. Agric. Food Chem. *60* (21), pp 5482–5490. http://pubs.acs.org/doi/full/10.1021/jf3010838
23. Jiang, D. Huang, Y. and **Peterson, D.G**. 2012. Application of Two-Dimensional Orthogonal Reversed-Phase Liquid Chromatography for Taste Compound Analysis. In Recent Advances in the Analysis of Food and Flavors; Toth, S., et al.; ACS Symposium Series; American Chemical Society: Washington, DC. http://pubs.acs.org/doi/abs/10.1021/bk-2012-1098.ch010
24. Moskowitz, M. and **Peterson, D. G.** 2011. Hydroxycinnamic acid-Maillard reactions in simple aqueous model systems. *in* Controlling Maillard Pathways. Washington, D.C.: American Chemical Society Symposium Proceedings 1042, pp 53-62. http://pubs.acs.org/doi/abs/10.1021/bk-2010-1042.ch006
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26. Cho, I. H. and ***Peterson, D.G.*** Chemistry of bread aroma: A review. 2010. Food Sci. Biotechnol. 19, 575-582. http://link.springer.com/article/10.1007/s10068-010-0081-3
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35. Vunta H, Davis F, Palempalli UD, Bhat D, Arner RJ, Thompson JT, **Peterson DG**, Reddy CC, *Prabhu KS*. 2007. The Anti-inflammatory Effects of Selenium Are Mediated through 15-Deoxy-Δ12,14-prostaglandin J2 in Macrophages. J. Biol. Chem. V. 282, No. 25, pp. 17964–17973. Impact Factor 4.651. http://www.jbc.org/content/282/25/17964
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41. Ghosh, G., **Peterson, D.G.** and *Coupland, J.N.* 2006. Effects of droplet crystallization and melting on the aroma release properties of a model oil-in-water emulsion. J. Ag. Fd. Chem. 54; 1829-1837. http://pubs.acs.org/doi/abs/10.1021/jf052262w
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http://www.sciencedirect.com/science/article/pii/S0022030205726552
47. Morawicki, R.O., *Beelman, R.B.*, **Peterson, D.G**. 2005. Recovery and purification of 10-oxo-*trans*-8-decenoic acid enzymatically produced using a crude homogenate of *Agaricus bisporus*. J. Food Sci. 70(8): C490-494.
Impact factor: 1.775. http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.2005.tb11506.x/abstract
48. Morawicki, R.O., *Beelman, R.B.*, **Peterson, D.G.** and Demirci, A. 2005. Biosynthesis of 1-octen-3-ol and 10-oxo-trans-8-decenoic acid using a crude homogenate of Agaricus bisporus: Reaction Scale Up. J. Food Sci. 70(5):367-371.
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http://www.sciencedirect.com/science/article/pii/S095869460400216X
50. Morawicki, R.O., *Beelman, R.B.*, **Peterson, D.G**. and Ziegler, G. 2005. Biosynthesis of 1-octen-3-ol and 10-oxo-trans-8-decenoic acid using a mushroom homogenate. Optimization of the reaction, part I: kinetic factors. Process Biochemistry. 40:131-137.
http://www.sciencedirect.com/science/article/pii/S0032959203004618
51. **Peterson, D.G.** and V.A. Totlani. 2005. Influence of flavonoids on the thermal generation of aroma compounds. in Phenolics in Foods and Natural Health Products, New York, ACS Symposium Series #909, September 7-11, 2003, pg 143-160.
http://pubs.acs.org/doi/abs/10.1021/bk-2005-0909.ch013
52. Schober, A.L. and ***Peterson, D.G.*** 2004. Flavor release and perception in hard candy: Influence of flavor compound-compound interactions. J. Ag. Fd. Chem. 52: 2623-2627.
http://pubs.acs.org/doi/full/10.1021/jf0353133
53. Schober, A.L. and ***Peterson, D.G.*** 2004. Flavor release and perception in hard candy: Influence of flavor compound-flavor solvent interactions. J. Ag. Fd. Chem. 52: 2628-2631.
http://pubs.acs.org/doi/full/10.1021/jf0354287
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http://onlinelibrary.wiley.com/doi/10.1002/ffj.1228/abstract
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http://www.sciencedirect.com/science/article/pii/S0963996900001757
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Patents (inventor):
International Application (PCT). April 21st, 2015. Peterson, D. and Kokkinidou, S. Methods of Making Modified Alcohol Containing Products.

USA application. An umami-enhanced food product and method of enhancing the umami taste of food products.

Courses Taught:
Flavor Chemistry (FScN 8337), Flavor Technology (FScN 5521); Current Issues in Food Science (FScN 8318); Food Chemistry and Functional Foods (FScN 4112)

Professional Memberships:
American Chemical Society
Institute of Food Technologists

EXHIBIT B

MATERIALS CONSIDERED

- U.S. Patent Application No. 14/797,006 ("the '006 application")
- Office Action mailed June 6, 2016
- Proulx et al. (2006, J. Agric. Food Chem., 54:1518-22) ("Proulx")
- Hsieh et al. (US 2012/0093994) ("the '994 application")
- Declaration under 37 CFR § 1.132 of Dr. Celeste Holz-Schietinger ("the Holz-Schietinger Declaration")
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Electronic Patent Application Fee Transmittal

Application Number:	14796970
Filing Date:	10-Jul-2015
Title of Invention:	METHODS AND COMPOSITIONS FOR CONSUMABLES
First Named Inventor/Applicant Name:	Marija Vrljic
Filer:	M. Angela Parsons/Judith Witzel
Attorney Docket Number:	38767-0010001

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 3 months with $0 paid	1253	1	1400	1400
Miscellaneous:				
RCE- 2ND AND SUBSEQUENT REQUEST	1820	1	1700	1700
		Total in USD ($)		**3100**

Electronic Acknowledgement Receipt

EFS ID:	30681721
Application Number:	14796970
International Application Number:	
Confirmation Number:	8501
Title of Invention:	METHODS AND COMPOSITIONS FOR CONSUMABLES
First Named Inventor/Applicant Name:	Marija Vrljic
Customer Number:	26191
Filer:	M. Angela Parsons/Judith Witzel
Filer Authorized By:	M. Angela Parsons
Attorney Docket Number:	38767-0010001
Receipt Date:	17-OCT-2017
Filing Date:	10-JUL-2015
Time Stamp:	16:17:33
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$3100
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Continued Examination (RCE)	RCE.pdf	114746 1e8dd607058d2f799e829357118f08a08fcbfd16	no	1
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Information:					
2		Response.pdf	211367 e51de06caa5468cf341cf04c02ecdaeee970e9b2	yes	21
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Amendment Submitted/Entered with Filing of CPA/RCE		1	1	
	Claims		2	8	
	Applicant Arguments/Remarks Made in an Amendment		9	21	
Warnings:					
Information:					
3	Examination support document	Exhibit1_Holz_SchietingerDeclaration.pdf	5205922 5724ca190d98546751aa10f3f0ed13b2437e9639	no	12
Warnings:					
Information:					
4	Examination support document	Exhibit2_CadwalladerDeclaration.pdf	576745 35fce1c755f88743438a4b462c7ecbd47d7096ad	no	45
Warnings:					
Information:					

5	Examination support document	Exhibit3_PetersonDeclaration.pdf	2443149 cb448c5054d6fe4e422770bf7a8d5a2d8ac24edc	no	20
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Warnings:					
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8	Examination support document	EXHIBIT_B_hemegradient8_18_17.pdf	80412 988b585c31779eb22092bb698d90d0597ba477b7	no	2
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Warnings:					
Information:					
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Information:					
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19	Examination support document	Exhibit7_Lipman_HemeAlignmentDeclaration.pdf	3516702 be45db7bc3c2e0216a1504ea76420a109d6fd02b	no	8
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26	Examination support document	EXH_A_Holz_Schietinger_CV.pdf	174592 13f7721a42d281ff8217f7851172650ef7860a3f	no	4
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Warnings:					
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National Stage of an International Application under 35 U.S.C. 371
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New International Application Filed with the USPTO as a Receiving Office
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PTO/SB/06 (09-11)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 14/796,970	Filing Date 07/10/2015	☐ To be Mailed

ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	10/17/2017	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 33	Minus	** 33	= 0	X $80 =	0
	Independent (37 CFR 1.16(h))	* 3	Minus	***4	= 0	X $420 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
KAREN WASHINGTON

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**
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Attorney Docket No.: 38767-0010001 / M2CON-Track 1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Marija Vrljic	Art Unit	:	1791
Serial No.	:	14/796,970	Examiner	:	Elizabeth A. Gwartney
Filed	:	July 10, 2015			
			Conf. No.	:	8501
Title	:	METHODS AND COMPOSITIONS FOR CONSUMABLES			

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Please consider the references listed on the enclosed PTO-1449 Form. Foreign patent documents and non-patent literature are enclosed; U.S. patents will be provided upon request. Copies of a Communication from a foreign Patent Office in a counterpart application also is enclosed.

This statement is being filed after a Request for Continued Examination (RCE) and before the mailing of a first Office Action. Therefore, no fees are believed due. If this is incorrect, please apply any charges, or credits, to Deposit Account No. 06-1050, referencing the above-indicated Attorney Docket Number.

Please contact the undersigned if there are any questions regarding this statement.

Respectfully submitted,

Date: /November 17, 2017/

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M. Angela Parsons, Ph.D.
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Sheet 1 of 1

Substitute Disclosure Form	U.S. Department of Commerce Patent and Trademark Office	Attorney Docket No. 38767-0010001	Application No. 14/796,970
Information Disclosure Statement by Applicant (Use several sheets if necessary)		Applicant Impossible Foods Inc.	
(37 CFR §1.98(b))		Filing Date July 10, 2015	Group Art Unit 1791

U.S. Patent Documents

Examiner Initial	Desig. ID	Document Number	Publication Date	Patentee	Class	Subclass	Filing Date If Appropriate
	1.	2,934,437	04/26/1960	Morton et al.			
	2.	3,394,017	07/23/1968	Giacino et al.			
	3.	5,039,543	08/13/1991	Lee et al.			

Foreign Patent Documents or Published Foreign Patent Applications

Examiner Initial	Desig. ID	Document Number	Publication Date	Country or Patent Office	Class	Subclass	Translation Yes	Translation No
	4.	EP 0136428	04/10/1985	EPO				
	5.	EP 2943072	11/18/2015	EPO				
	6.	JP S42-22194	10/31/1942	JAPAN				
	7.	JP S49-39824	10/29/1974	JAPAN				
	8.	JP S51-63971	10/24/1975	JAPAN			Engl. Transl.	
	9.	WO 2014/110532	07/17/2014	WIPO				

Other Documents (include Author, Title, Date, and Place of Publication)

Examiner Initial	Desig. ID	Document
	10.	Supplementary European Search Report in European Application No. 15774164, dated October 27, 2017, 11 pages

Examiner Signature	Date Considered
EXAMINER: Initials citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Substitute Disclosure Form

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 136 428**
A2

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: 84108340.5

(22) Date of filing: 16.07.84

(51) Int. Cl.[4]: A 23 L 1/226
A 23 L 1/231

(30) Priority: 01.09.83 US 528561

(43) Date of publication of application:
10.04.85 Bulletin 85/15

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

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(54) **Flavoring agents.**

(57) A process for the production of meat or fish flavoring agents comprises reacting a sulfur-containing compound with a lipid which is oxidized prior to or during the reaction, desirably to a peroxide value equal or close to zero. The reaction mixture may include an amino acid source.

EP 0 136 428 A2

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BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention pertains to the field of flavorants and particularly pertains to flavorants which impart cooked meat flavor to foodstuffs. More specifically, the present invention is concerned with the preparation of meat flavorants having increased flavor and aromatic intensity and which more closely resemble the natural flavor and aroma of cooked meat.

2. Description of Related Art

The desirability of providing flavoring agents which closely simulate the flavor and aromatic characteristics of cooked meat has long been recognized. Such flavoring agents may, for example, be employed with non-meat sources of protein so as to make them more palatable and as meat-like as possible. So, too, they may also be employed with meat-containing or meat-based foods or vegetable-type foodstuffs such as condensed soups, dried meats, packaged gravies, casseroles, etc., in order to supplement or enhance these foods whose organoleptic properties may have been affected by their processing.

Various expedients have been suggested in the prior art in attempts to provide flavoring agents having the organoleptic profile of cooked meat. Thus, for example, U.S. Patent No. 2,934,437 describes the preparation of a meat-like flavor by the

reaction of a mixture of monosaccharide and a source of amino acid. U.S. Patent No. 3,394,015 describes the preparation of a meat-like flavorant from the reaction of a proteinaceous substance with a sulfur-containing compound in the absence of a monosaccharide. U.S. Patent No. 3,532,514 describes the preparation of a meat-like flavorant from a mixture of an amino acid source, a mono-, di-, tri-, or polysaccharide and an animal or vegetable fat. U.S. Patent No. 3,394,017 describes the preparation of a meat-like flavorant by reacting thiamine with a sulfur-containing polypeptide or an amino acid mixture derived therefrom and thereafter adding aldehydes and ketones to the product.

These and similar techniques may suffer from a number of disadvantages. Firstly, these meat flavorants may possess only a small fraction of the total flavor and aromatic notes possessed by natural cooked meat. This is attributable, in part, to the particular reactive ingredients employed to produce the flavorants which can provide only a limited number of the required chemical precursors -- such as various aldehydes and ketones -- which are necessary to obtain the full organoleptic profile of cooked meat.

Moreover, the addition of separately prepared aldehydes and/or ketones to the flavorants in an effort to enhance the organoleptic profile of the flavorant produced is generally uneconomical.

Still further, the intensity or strength of these flavorants may be relatively weak so that a considerable amount of such flavorants would be required to obtain a perceptible flavoring effect upon a foodstuff. This, of course, may be economically undesirable. It would be far more advantageous to employ as little flavorant as possible and still obtain the desired flavoring effect.

SUMMARY OF THE INVENTION

Applicants have discovered a process for preparing flavorants for imparting a cooked meat flavor to foodstuffs which avoids substantially all of the disadvantages that may be associated with the prior art flavorants discussed above.

The process of the present invention economically and desirably utilizes a lipid material, and more specifically an oxidized lipid material, to provide a wide range of chemical precursors which facilitate the preparation of flavorants having the characteristic species which more closely resemble that of natural cooked meat. Still further, the flavorants produced by this process are enhanced and intensified so that a relatively small amount of these flavorants is needed to obtain a desired flavoring effect upon a foodstuff.

More particularly, applicants have found that superior meat flavorants may be obtained by reacting at elevated temperatures a sulfur-containing compound and an oxidized lipid material, such as oxidized triglycerides and/or oxidized unsaturated fatty acid components thereof. The lipid material may be separately oxidized prior to being combined with the sulfur-containing compound or, alternatively, may be oxidized during the reaction step itself after having been combined with the sulfur-containing compound.

The oxidation of the lipid material leads to the formation of a wide range of various hydroperoxides which in turn form a broad range of fatty unsaturated aldehydes and ketones. These carbonyl compounds then react with the sulfur-containing compound(s) to form heterocyclic ring structures which greatly enhance and intensify the characteristic meat flavor and aroma in the flavorants. Moreover, by virtue of employing an oxidized lipid material in the process of the present invention, the unsaturated aldehydes and ketones which are formed are endogenous to the starting lipid material and therefore need not be separately added from some other source.

In a preferred embodiment of the present invention, a source of amino acid is additionally reacted with the sulfur-containing compound(s) and the oxidized lipid material to even further enhance the flavorants produced. In this embodiment, the fatty aldehydes and ketones derived from the oxidized lipid

material undergo non-enzymatic browning reactions with the amino acid source to provide an even broader organoleptic profile to the flavorant. As a source of amino acid, enzymatically treated meat hydrolysates are especially preferred. It is noted that in the context of the present invention, the term "meat" also includes fish.

The ability to produce an acceptable flavorant at all, much less, a flavorant which has an even broader organoleptic profile and increased intensity when employing an oxidized lipid material is quite unexpected and contrary to what would be predicted by those skilled in the art. Thus, it has long been recognized that lipid oxidation is a major cause of deterioration in meat, particularly of its organoleptic qualities, resulting in off-flavors and rancidity. Generally, steps are taken to inhibit such undesirable lipid oxidation through the use of metal chelators (e.g., polyphosphates, citric acid, ascorbic acid and EDTA), phenolic antioxidants (propyl gallate, BHA and BHT), tocopherols, vacuum packaging, etc. Still, some oxidation nevertheless takes place during the cooking of the meat.

Yet, in the process of the present invention, rather than avoiding lipid oxidation, steps are actually taken to ensure that oxidized lipids are indeed present in order to form the novel flavorants discovered by applicants. These oxidized lipid materials, typically thought of as being the cause of rancidity, are actually utilized in the process of the present invention to

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

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

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

DETAILED DESCRIPTION OF THE INVENTION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

EXAMPLE 1

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

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

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

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

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

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

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

EXAMPLE 2

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

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

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

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

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

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

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

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

EXAMPLE 3
(COMPARISON EXAMPLE)

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

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

EXAMPLE 4
(COMPARISON EXAMPLE)

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

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

EXAMPLE 5

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

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

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

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

EXAMPLE 6

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

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

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

EXAMPLE 7

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

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

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

EXAMPLE 8

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

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

Chicken fat	97.0 parts
L-Cysteine HCl	3.0

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

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

EXAMPLE 9

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

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

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

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

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

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

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

Claims

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

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

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

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

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

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

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

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

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

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

Espacenet

Description: EP2943072 (A2) — 2015-11-18

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

Description not available for EP2943072 (A2)
Description of corresponding document: WO2014110532 (A2)

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AU2014205114 (A1) CA2897600 (A1) CN105050414 (A) JP2016502867 (A) WO2014110532 (A2) AU2014205121 (A1) CA2897602 (A1) CN105050426 (A) JP2016508716 (A) WO2014110539 (A1)

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

CROSS-REFERENCE TO RELATED APPLICATIONS

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

TECHNICAL FIELD

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

BACKGROUND

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

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

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

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

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

SUMMARY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DESCRIPTION OF THE DRAWINGS

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

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

DETAILED DESCRIPTION

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

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

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

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

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

TABLE 1

Flavor Precursor Molecules

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

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

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

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

guanosine, guanoside monophosphate (GMP), adenosine, adenosine

monophosophate (AMP)

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

Misc: phospholipid, lecithin, pyrazine, creatine, pyrophosphate

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

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

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

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

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

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

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

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

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

Any of the heme-containing proteins described herein that can be used for producing food products can have at least 70% (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%), 98%o, 99%), or 100%) sequence identity to the amino acid sequence of the corresponding wild-type heme-containing protein or fragments thereof that contain a heme-binding motif. For example, a heme-containing protein can have at least 70% sequence identity to an amino acid sequence set forth in FIG. 1, including a non-symbiotic hemoglobin such as that from Vigna radiata (SEQ ID NO: l), Hordeum vulgare (SEQ ID NO:5), Zea mays (SEQ ID NO: 13), Oryza sativa subsp. japonica (rice) (SEQ ID NO: 14), or Arabidopsis thaliana (SEQ ID NO: 15), a Hell's gate globin I such as that from Methylacidiphilum infernorum (SEQ ID NO:2), a flavohemoprotein such as that from Aquifex aeolicus (SEQ ID NO:3), a leghemoglobin such as that from Glycine max (SEQ ID NO:4), Pisum sativum (SEQ ID NO: 16), or Vigna unguiculata (SEQ ID

NO: 17), a heme-dependent peroxidase such as from Magnaporthe oryzae, (SEQ ID

NO:6) or Fusarium oxysporum (SEQ ID NO:7), a cytochrome c peroxidase from

Fusarium graminearum (SEQ ID NO: 8), a truncated hemoglobin from Chlamydomonas moewusii (SEQ ID NO:9), Tetrahymena pyriformis (SEQ ID NO: 10, group I truncated), Paramecium caudatum (SEQ ID NO: I 1, group I truncated), a hemoglobin from

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

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

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

Biotechnology Information web site (www.ncbi.nlm.nih.gov). Instructions explaining how to use the B12seq program can be found in the readme file accompanying BLASTZ. B12seq performs a comparison between two amino acid sequences using the BLASTP algorithm. To compare two amino acid sequences, the options of B12seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seqI .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:\seqI .txt -j c:\seq2.txt -p blastp -o c:\output.txt. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology, then the designated output file will not present aligned sequences.

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

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

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

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

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

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

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

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

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

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

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

Modulating Flavor and/or Aroma Profiles

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

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

TABLE 2

Image available on "Original document"
yeasty goaty

vegetable broth

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

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

TABLE 3

Compounds Produced

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

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

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

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

1-heptanol octanoic acid heptane

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

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

2-nonanone pyrazine acetophenone

2-pentanone 1 ,3,5-cycloheptatriene tridecane

thiophosphoramide, s-

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

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

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

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

3-ethyl-2,2-dimethyl-

2-octanone thiophene pentane

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

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

3 -ethylcyclopentanone butanal 2,6-dimethyl-nonane

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

4-methyl-5-

3-octen-2-one carbon disulfide thiazoleethanol

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

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

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

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

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

2-

3 -methyl-2-butenal ethanol thiophenemethanamine

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

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

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

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

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

4-amino- 1,2,5-

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

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

1 ,2-benzisothiazol-

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Food Products

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

EXAMPLES

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

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

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

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

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

TABLE 4

Replica Burger Ingredients

Repl ica biiruer " .) precooked w w

Pea vicilin 3.86

Soy protein concentrate (SPC) 2.52

Bamboo fiber 0.34
Image available on "Original document"

TABLE 5

Sensory evaluation of replica burger with Heme

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

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

TABLE 6

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

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

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

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

Image available on "Original document"

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

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

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

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

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

Image available on "Original document"
trichloromethane X

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

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

2-pentanone X furfural X X

2-nonanone X X

(Z)-2-heptenal X X

(E)-2-heptenal X X

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

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

3-pentyl-furan X X

2-propenal X

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

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

4-penten-2-one X X

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

3-ethylcyclopentanone X pyrrole X X

2-thiophenecarboxaldehyde X

3 -thiophenecarboxaldehy de X

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Example 9 - Flavor compound production by different Heme-proteins

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

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

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

TABLE 9

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

Name LegH Barley B. myoglobin A. aeolicus

Furan X X X X

Thiazole X X X X

benzaldehyde X X X X

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

2,3-butanedione X X X X

2-pentyl-furan X X X X

2-pentanone X X

pyrazine X X X X dimethyl trisulfide X X X X

3 -methy 1-butanal X X X

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

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

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

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

1-octene X X X butanal X X X X

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

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

2-propenal X X X

2-decenal X X X

2-nonanone X X

2-octanone X X X

2-tridecen-l-ol, X X

2-octanone X

2-octenal X X

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

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

3-methylheptyl acetate X x

Example 10 - Generation of Meat flavors from different lipids

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

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

Table 10

Legend showing components of each sample

Sample Name Solution Additives

MRM None MaiUard Reaction Mix None

MRM Linoelic Acid MaiUard Reaction Mix 1% linoleic acid

MRM Oleic Acid MaiUard Reaction Mix 1% oleic acid

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

MRM C18 MaiUard Reaction Mix 1% CI 8:0 free fatty acid

MRM Canola MaiUard Reaction Mix 3% Canola Oil

MRM Coconut MaiUard Reaction Mix 3% Coconut Oil

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

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

KPhos6 Linoelic Acid PPB, pH 6 1% linoelic acid

KPhos6_01eic Acid PPB, pH 6 1% oleic acid

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

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

KPhos6 Canola PPB pH 6 3% Canola Oil

KPhos6_Coconut PPB, pH 6 3% Coconut Oil

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

KPhos6_Biolipon95 PPB, pH 6 1% Biolipon95 (emulsifier)

KPhos6_NatCholinePC40 PPB, pH 6 1% NatCholinePC40 (emulsifier)

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

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

compounds made.

TABLE 11

Aroma descriptions of each sample after it was cooked.

Image available on "Original document"
KPhos6_NatCholinePC4

0 light fatty, fresh

MRM_NatCholinePC40 fatty, beef tallow, brothy

K-Phos6_C14 light/faint plastic/waxy

MRM_C14 brothy, beefy, minty, fresh

K-Phos6_C18 light/faint plastic/waxy

MRM_C18 beefy with cucumber &/or pepper aroma

K-Phos6_Canola fresh, cucumber

MRM_Canola fatty, brothy, oil, roasted nuts

K-Phos6_Coconut nothing

MRM_Coconut brothy. beefy, slight fatty, crackers

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

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

K-Phos6_I_inoelic Acid light plastic

MRM_Linoelic Acid fatty, light waxy, brothy. herbal

Table 12 fMRMBH teeea r

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

C 40P ei MRMLno <ec>

i da c

Compounds in Beef

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

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

1-heptene N Y Y Y

2-methyl- 1 -heptene N N N N

1-hexanol N Y Y Y

2-ethyl- 1 -hexanol N N N N

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

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

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

1-octene N N N N

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

1-propanol N N N N

8-methyl- 1 -undecene N Y Y Y

1,3-hexadiene N N N Y

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

1,3-octadiene Y N N Y

1 ,3,5-cycloheptatriene N N N N

2.3- dihydro-5,6-dimethyl-

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

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

2-acetyl-2-thiazoline 1111 N N N

2-acetylthiazole Y Y Y Y

2-butanone N Y Y Y

2-butenal N Y Y Y

2-ethyl-2-butenal N N N Y

3 -methyl-2-butenal N N Y Y

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

2-decanone Y Y Y N

(E)-2-decenal N N N N

(Z)-2-decenal Y Y Y Y

2-furanmethanol N N N N

2-heptanone Y Y Y Y

6-methyl-2-heptanone N N Y N

(E)-2-heptenal N Y Y Y

(Z)-2-heptenal N N N Y

(E)-2-hexenal N Y Y Y

2-ethyl-2-hexenal N N N N

2-methyl-2-heptene llll N N N

2-n-heptylfuran llll N N N

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

(E)-2-nonenal Y Y Y Y

(Z)-2-nonenal N N N Y

2-octanone v Y Y Y

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

(E)-2-octenal N Y Y Y

2-pentanone N Y Y N

1 -propoxy-2-propanol N N N N

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

1 -hydroxy-2-propanone Y N N N

2-propenal N N N Y

2-thiophenecarboxaldehyde Y Y Y Y

2-undecenal N Y Y Y

2,3-butanedione N N N Y

2,3-pentanedione N N N N

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

2,4-decadienal N N N Y

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

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

2,6-dimethylpyrazine N N N N

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

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

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

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

3 -ethylcyclopentanone N Y Y Y

3-hexanone N N N N

3-methyl-2- thiophenecarboxaldehyde N N N N

3-oetanone Y Y N Y

3-octen-2-one N Y Y

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

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

2-ethyl-furan iiiii N N N

2-hexyl-furan 1111 N N Y

2-methyl-furan N N N Y

2-pentyl-furan N Y Y Y

2-propyl-furan N N Y Y

3-methyl-furan Y N N N

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

MRM_NatCholinePC40, KPhos6_Linoleic acid, and MRM Oleic Acid samples.

Example 11 - Creation of beefy flavors using complex precursor mixtures

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

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

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

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

Table 13

Chemical entities added to the Magic Mix

Image available on "Original document"
Valine 0.9

glucose 5.6

Ribose 6.7

Maltodextrin 5.0

Thiamine 0.5

GMP 0.24

IMP 0.6

Lactic acid 1.0

creatine 1.0

NaCl 10

KC1 10

Kphos pH 6.0 10

TABLE 14

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

LegH alone (average of five samples)

MM

with LegH

R.T.(s) Name LegH alone

248 acetaldehyde L S

256.3 carbon disulfide L S

264.3 dimethyl sulfide S 0

265 oxalic acid, isobutyl pentyl ester M 0

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

269.2 methanethiol S 0

283.4 propanal M 0

285.4 octane M 0

287.1 furan M 0

295.3 2-methyl-propanal L s

297.6 acetone L s

319.3 2-propenal M s

338.1 2-methyl-furan M s

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

346.3 methacrolein M 0

357.4 methyl-thiirane L 0

360.2 3-methyl-furan S 0

363.7 butanone L s

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

376.4 2-methyl-butanal L M

381.1 3 -methy 1-butanal L M

390.6 isopropyl alcohol 0 s

399.6 ethanol L M

406.2 2-propenoic acid, methyl ester M 0

408.2 benzene S 0

414.4 methyl vinyl ketone M 0

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

422.6 2-ethyl-furan S 0

438.4 2-ethylacrolein M 0

449.9 2-pentanone S 0

453.2 pentanal/2,3-butanedione L 0

453.8 2,3-butanedione L M

472.8 4,7-dimethyl-undecane M s

485.9 2-methyl-pentanal M 0

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

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

508.6 l-penten-3-one M 0

510.6 trichloromethane M M

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

525.5 3 -methyl-pentanal M 0

535.1 (E)-5-decene M 0

536.5 toluene M s

537.9 2-butenal M s

543.8 4-penten-2-one M 0

550.8 methyl thiolacetate M 0

683.7 p-xylene S 0

727.4 dimethyl selenone M 0

738.3 methyl isopropyl disulphide M 0

755 2-heptanone M 0

758.7 heptanal L 0

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

789.4 3 -methy 1-2 -butenal M 0

793.4 4-methyl-2-heptanone M 0

810.4 pyrazine M 0

818.8 isothiazole S 0

827.1 acetyl valeryl M 0

831.8 2-pentyl-furan L 0

851 2-methyl-thiazole S 0

853.3 isothiocyanato-methane S 0

870.9 thiazole L 0

879.2 styrene M 0

890.7 1 -(methylthio)-propane M 0

895.6 methyl-pyrazine M 0

910.5 thiocyanic acid, methyl ester S 0

918.6 4-methylthiazole M 0

921.4 2-octanone M 0

923.9 2-methyl-cyclopentanone M 0

927.9 octanal L s

934.3 tridecane M 0

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

961.9 1 -hydroxy-2-propanone M 0

974.5 (E)-2-heptenal M 0

987.4 5 -methyl- 1 -undecene M 0

993.8 2-hexyl-furan M 0

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

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

1058.6 2-butyl-l-decene M 0

1079.3 dimethyl trisulfide L s

1085.3 2-nonanone M 0

1093.2 nonanal L M

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

1149.6 (E)-2-octenal M 0

1164.5 1-heptanol M 0

1193.5 methional L 0

1198.8 acetic acid M s

1207.2 furfural M 0

1242.1 2-decanone M 0

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

1283.3 pyrrole M 0

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

1294.3 benzaldehyde L M

1303.7 2-n-octylfuran M 0

1305.6 (E)-2-nonenal M 0

1341.4 1-octanol M 0

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

1391.7 2-undecanone M 0

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

1448 butyrolactone S s

1456.3 (E)-2-decenal M 0

1462.4 phenylacetaldehyde L s

1466.3 2-acetylthiazole L 0

1471.3 acetophenone M s

1475.4 1-nonanol M 0

1487 methyl (methylthio)methyl disulfide M 0

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

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

1512 3 -thiophenecarboxaldehyde M 0

1518.8 2-nonen-4-one M 0

1531.7 2-thiophenecarboxaldehyde S 0

1543.9 dodecanal M 0

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

1558.2 3 -(methy lthio)-propanenitrile S 0

1561.2 3-decen-2-one M 0

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

1615.6 1,10-undecadiene M 0

1619.5 2-undecenal S 0

1668.9 2-phenylpropenal M 0

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

1733.1 3-phenyl-furan S 0

4-nitrophenyl 2-thiophenecarboxylic acid

1739.7 ester S 0

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

1749.7 isobutyl ester M 0

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

1774.2 isobutyl ester S 0

1796.9 dodecanal M 0

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

1825.6 1-undecanol M s

1827.9 2-methyl-3 -furanthiol M 0

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

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

1844.1 phenol S s

1845.3 [(methylsulfonyl)methyl]-benzene s 0

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

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

1859.9 naphthalene s 0

1863.2 2,4-decadienal s 0

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

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

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

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

1944.5 9-octadecenal M 0

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

1968.4 4-methyl-5 -thiazoleethanol M s

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

2019.8 benzoic acid s s

2026.4 4-quinolinecarboxaldehyde s 0

2027.8 m-aminophenylacetylene M 0

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

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

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

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

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

TABLE 15

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

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Preparation of Flavor Reaction Containing Ferrous Chlorin or Leghemoglobin

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

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

TABLE 16

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

1-heptanol acetone

1-hexanol acetonitrile

1-octanol benzaldehyde

l-octen-3-ol butanal

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

1-pentanol dimethyl trisulfide

2-acetylthiazole ethyl acetate

2-butenal furan

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

(Z)-2-decenal 2-hexyl furan

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

(E)-2-heptenal furfural

(E)-2-hexenal heptanal

2-methyl-3 -furanthiol aminophenylacetylene

(E)-2-nonenal methacrolein

(E)-2-octenal methional

2-pentanone octanal

1 -hydroxy-2-propanone octane

2-thiophenecarboxaldehyde oxalic acid, diallyl ester

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

3 -thiophenecarboxaldehyde pyrazine

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

Example 13 - Flavor creation by immobilized Hemin

Preparation of hemin linked CM Sepharose.

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

inky black in color.

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

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

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

TABLE 17

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

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

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

octane Low

pyrrole Low Low

methanethiol Low Low

2-thiophenecarboxaldehyde Low Low

methyl-pyrazine Low Low

1 -hydroxy-2-propanone Low Low

propanal Low Low thiophene Low medium pyridine Low Low

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

2-butenal medium large furfural medium medium nonanal medium medium

2-ethyl-furan medium Low ethanol medium very large

L i L-U LallOl meui m

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

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

2-acetylthiazole medium medium cyclohexane medium

ethyl tert-butyl ether medium

carbon disulfide medium medium thiazole medium medium acetonitrile medium large

2-pentyl-furan medium Low

3 -thiophenecarboxaldehyde medium medium

2-methyl-butanal medium medium thiazole medium large

2-methyl-3 -furanthiol larege large

2-propenal large large

3 -methy 1-2 -butenal large medium

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

2-butanone large Low

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

methyl thiolacetate large medium

acetaldehyde very large very large

2-methyl-propanal very large very large

dimethyl trisulfide very large very large

3 -methy 1-butanal very large very large

propyl-cyclopropane medium

(E)-2-octenal medium

2-n-propylazirdine medium

thiirane medium

ethyl formate medium

methyl vinyl ketone medium

2-propenoic acid, ethyl ester medium

1-nonanol large

1-octene large

1-heptanol large

1-dodecene large

phorone very large

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

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

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

propanal medium

pyrazine medium

(Z)-2-heptenal medium

2-methyl- 1 -heptene medium

2-ethyl-furan medium

octanal medium

(E)-4-octene low

(E)-2-octenal low

2-methyl-thiazole low

2-propenal low

l-octen-3-one low

1-octene low

2-octanone low

dimethyl sulfide low

3-pentyl-furan low

2-n-octylfuran low

2-pentyl-thiophene low

OTHER EMBODIMENTS

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

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 July 2014 (17.07.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/110532 A2

(51) International Patent Classification:
A23J 1/00 (2006.01) *A23J 1/14* (2006.01)
A23J 1/06 (2006.01) *A23J 1/12* (2006.01)

(21) International Application Number:
PCT/US2014/011347

(22) International Filing Date:
13 January 2014 (13.01.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/751,816	11 January 2013 (11.01.2013)	US
13/941,211	12 July 2013 (12.07.2013)	US
61/908,634	25 November 2013 (25.11.2013)	US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

WO 2014/110532 A2

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

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

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

CROSS-REFERENCE TO RELATED APPLICATIONS

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

TECHNICAL FIELD

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

BACKGROUND

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

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

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

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

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

SUMMARY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DESCRIPTION OF THE DRAWINGS

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

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

DETAILED DESCRIPTION

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

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

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

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

TABLE 1

Flavor Precursor Molecules

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Modulating Flavor and/or Aroma Profiles

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

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

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

TABLE 2

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

yeasty	goaty
vegetable broth	

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

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

TABLE 3

Compounds Produced

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Food Products

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

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

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

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

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

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

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

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

Assessment of Food Products

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

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

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

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

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

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

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

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

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

EXAMPLES

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

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

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

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

TABLE 4

Replica Burger Ingredients

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

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

TABLE 5

Sensory evaluation of replica burger with Heme

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

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

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

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

TABLE 6

Improvement of overall taste with precursors added to LegH burgers

	with precursors	without precursors
Average	3.5	1.8
STDV	0.6	0.5

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

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

TABLE 7

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

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

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

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

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

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

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

TABLE 8

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

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

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

pyrrole	X	X
2-thiophenecarboxaldehyde		X
3-thiophenecarboxaldehyde		X

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Example 9 - Flavor compound production by different Heme-proteins

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

TABLE 9

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

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

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

2-pentyl-thiophene				x

Example 10 - Generation of Meat flavors from different lipids

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

Table 10

Legend showing components of each sample

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

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

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

TABLE 11

Aroma descriptions of each sample after it was cooked.

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

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

Table 12

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Example 11 - Creation of beefy flavors using complex precursor mixtures

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

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

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

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

Table 13

Chemical entities added to the Magic Mix

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

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

TABLE 14

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

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

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

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

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

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

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

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

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

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

TABLE 15

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

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

Preparation of Flavor Reaction Containing Ferrous Chlorin or Leghemoglobin

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

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

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

TABLE 16

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

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

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

Example 13 - Flavor creation by immobilized Hemin

Preparation of hemin linked CM Sepharose.

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

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

Flavor reaction

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

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

TABLE 17

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

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

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

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

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

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

Table 18

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

Compound	Precursor mix	LegH	Precursor mix + Leg H
carbon disulfide	medium	medium	high
isopropyl alcohol	medium	medium	low
2-methyl-furan	low		low
butanal	low		medium
thiophene	low		low
2,3-butanedione	low	low	high
furan	low		medium
2,4-dimethyl-1-heptene		high	high
acetone		high	high
dimethyl trisulfide		medium	medium
2-methyl-heptane		medium	medium
2-pentanone		medium	
pentanal		medium	medium
2-pentyl-furan		medium	medium
2-methyl-propanal		low	high
2-acetatyl-1-propene		low	low
2-methyl-butanal		low	medium
1,3-dimethyl-benzene		low	low
octane		low	low
benzene		low	low
benzaldehyde			very high
2-butanone			very high
furfural			very high
thiazole			high
nonanal			high
thiazole			high
2-acetylthiazole			medium
3-methyl-butanal			medium
(Z)-2-heptenal			medium
heptanal			medium
methyl-thiirane			medium
3-ethyl-pentane			medium
phenylacetaldehyde			medium
2-hexyl-furan			medium

2-nonanone			medium
propanal			medium
pyrazine			medium
(Z)-2-heptenal			medium
2-methyl-1-heptene			medium
2-ethyl-furan			medium
octanal			medium
(E)-4-octene			low
(E)-2-octenal			low
2-methyl-thiazole			low
2-propenal			low
1-octen-3-one			low
1-octene			low
2-octanone			low
dimethyl sulfide			low
3-pentyl-furan			low
2-n-octylfuran			low
2-pentyl-thiophene			low

OTHER EMBODIMENTS

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

WHAT IS CLAIMED IS:

1. A food product comprising:

a) a highly conjugated heterocyclic ring complexed to iron ion; and

b) one or more flavor precursor molecules selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, corn oil, soybean oil, palm fruit oil, palm kernel oil, safflower oil, flaxseed oil, rice bran oil, cottonseed oil, olive oil, sunflower oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone.

2. The food product of claim 1, wherein said highly conjugated heterocyclic ring complexed to an iron ion is selected from the group consisting of a heme moiety, and a porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochorophyll, a corphin, a chlorophyllin, a bacteriochlorin, and an isobacteriochlorin moiety complexed to iron ion.

3. The food product of claim 2, wherein said heme moiety is a heme-containing protein, a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid support.

4. The food product of claim 2, wherein said highly conjugated heterocyclic ring complexed to iron is ferrous chlorin.

5. A food product comprising:

a) a heme-containing protein; and

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

6. The food product of any one of claims 1-5, wherein said food product comprises two to fifty flavor precursors.

7. The food product of any one of claims 1-5, wherein said food product comprises two to forty flavor precursors.

8. The food product of any one of claims 1-5, wherein said food product comprises two to thirty-five flavor precursors.

9. The food product of any one of claims 1-5, wherein said food product comprises two to ten flavor precursors.

10. The food product of any one of claims 1-5, wherein said food product comprises two to six flavor precursors.

11. The food product of any one of claims 1-10, wherein said one or more flavor precursor molecules are selected from the group consisting of glucose, ribose, sucrose, maltose, cysteine, a cysteine derivative, thiamine, lysine, a lysine derivative, glutamic acid, a glutamic acid derivative, IMP, GMP, lactic acid, maltodextrin, creatine, alanine, arginine, asparagine, aspartate, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, linoleic acid, lecithin, and mixtures thereof.

12. The food product of any one of claims 3-11, wherein the heme-containing protein is a plant heme-containing protein.

13. The food product of any one of claims 3-11, wherein the heme-containing protein is a mammalian heme-containing protein.

14. The food product of any one of claims 3-11, wherein the heme-containing protein is a yeast or filamentous fungus heme-containing protein.

15. The food product of any one of claims 3-11, wherein the heme-containing protein is a bacterial or archeal heme-containing protein.

16. The food product of any one of claims 3-12, wherein the heme-containing protein is a non-symbiotic hemoglobin.

17. The food product of any one of claims 3-12, wherein the heme-containing protein is a leghemoglobin.

18. The food product of claim 17, wherein the leghemoglobin is a from soybean, alfalfa, lupin, fava bean, pea, cow pea, or lupine.

85 19. The food product of any one of claims 12-18, wherein the heme-containing
86 protein is recombinantly produced, chemically synthesized, or transcribed and translated
87 *in vitro*.
88
89 20. The food product of any one of claims 3-19, wherein the heme-containing protein
90 comprises an amino acid sequence having at least 80% sequence identity to a polypeptide
91 set forth in SEQ ID NOs:1-26.
92
93 21. The food product of any one of claims 1-20, wherein said heme-containing
94 protein is isolated and purified.
95
96 22. The food product of any one of claim 1-21, further comprising a food-grade oil, a
97 seasoning agent, a flavoring agent, a protein, a protein concentrate, an emulsifier, a
98 gelling agent, or a fiber.
99
100 23. The food product of any one of claims 1-18, wherein said food product is a meat
101 substitute.
102
103 24. The food product of any one of claim 1-19, wherein said food product is a soup
104 base, stew base, snack food, bouillon powder, bouillon cube, or a flavor packet.
105
106 25. The food product of any one of claims 1-20, wherein said food product is free of
107 animal products.
108
109 26. The food product of any one of claims 1-21, wherein said food product is a frozen
110 food product.
111
112 27. The food product of any one of claims 1-22, wherein said food product is sealed
113 within a packet or shaker.
114

115 28. A method for producing a flavor compound, said method comprising:
116 a) combining a highly conjugated heterocyclic ring complexed to iron ion with
117 one or more flavor precursor molecules to form a mixture, said one or more flavor
118 precursor molecules selected from the group consisting of glucose, ribose, fructose,
119 arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol,
120 maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a
121 phospholipid, a lecithin, inosine, inosine monophosphate (IMP), guanosine
122 monophosphate (GMP), pyrazine, adenosine monophosphate (AMP), lactic acid, succinic
123 acid, glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower
124 oil, cottonseed oil, soybean oil, corn oil, palm fruit oil, palm seed oil, rice bran oil,
125 safflower oil, olive oil, canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid,
126 cysteine, methionine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan,
127 valine, arginine, histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine,
128 proline, serine, tyrosine, glutathione, an amino acid derivative, a protein hydrolysate, a
129 malt extract, a yeast extract, and a peptone; and
130 b) heating the mixture to form one or more flavor compounds selected from the
131 group consisting of phenylacetaldehyde, 1-octen-3-one, 2-n-heptylfuran, 2-
132 thiophenecarboxaldehyde, 3-thiophenecarboxaldehyde, butyrolactone, 2-undecenal,
133 methyl-pyrazine, furfural, 2-decanone, pyrrole, 1-octen-3-ol, 2-acetylthiazole, 2-(E)-
134 octenal, decanal, benzaldehyde, (E)-2-nonenal, pyrazine, 1-hexanol, 1-heptanol, dimethyl
135 trisulfide, 2-nonanone, 2-pentanone, 2-heptanone, 2,3-butanedione, heptanal, nonanal, 2-
136 octanone, 1-octanol, 3-ethylcyclopentanone, 3-octen-2-one, (E,E)-2,4-heptadienal, (Z)-2-
137 heptenal, 6-methyl-2-heptanone, (Z)-4-heptenal, (E,Z)-2,6-nonadienal, 3-methyl-2-
138 butenal, 2-pentyl-furan, thiazole, (E,E)-2,4-decadienal, hexanoic acid, 1-ethyl-5-
139 methylcyclopentene, (E,E)-2,4-nonadienal, (Z)-2-decenal, dihydro-5-pentyl-2(3H)-
140 furanone, trans-3-nonen-2-one, (E,E)-3,5-octadien-2-one, (Z)-2-octen-1-ol, 5-
141 ethyldihydro-2(3H)-furanone, 2-butenal, 1-penten-3-ol, (E)-2-hexenal, formic acid,
142 heptyl ester, 2-pentyl-thiophene, (Z)-2-nonenal, 2-hexyl-thiophene, (E)-2-decenal, 2-
143 ethyl-5-methyl-pyrazine, 3-ethyl-2,5-dimethyl-pyrazine, 2-ethyl-1-hexanol, thiophene, 2-
144 methyl-furan, pyridine, butanal, 2-ethyl-furan, 3-methyl-, butanal, 2-methyl-, butanal,

145 methacrolein, 2-methyl-propanal, propanal, acetaldehyde, 2-propyl-furan, dihydro-5-
146 propyl-2(3H)-furanone, 1,3-hexadiene, 4-decyne, pentanal, 1-propanol, heptanoic acid,
147 ethanethiol, 1-butanol, 1-penten-3-one, dimethyl sulfide, 2-ethyl furan, 2-pentyl-
148 thiophene, 2-Propenal, 2-Tridecen-1-ol, 4-Octene, 2-methyl thiazole,, methyl-pyrazine, 2-
149 butanone, 2-pentyl-furan, 2-methyl-propanal, butyrolactone,, 3-methyl-butanal, methyl-
150 thiirane, 2-hexyl-furan, butanal,, 2-methyl-butanal, trichloromethane, 2-methyl-furan,
151 furan, octanal, 2-Heptenal, 1-Octene, heptyl ester formic acid, 3-pentyl-furan, and 4-
152 penten-2-one.
153
154 29. The method of claim 28, wherein said highly conjugated heterocyclic ring
155 complexed to an iron ion is selected from the group consisting of a heme moiety, and a
156 porphyrin, a porphyrinogen, a corrin, a corrinoid, a chlorin, a bacteriochorophyll, a
157 corphin, a chlorophyllin, a bacteriochlorin, or an isobacteriochlorin moiety complexed to
158 iron ion.
159
160 30. The method of claim 29, wherein said heme moiety is a heme-containing protein,
161 a heme moiety bound to a non-peptidic polymer; or a heme moiety bound to a solid
162 support.
163
164 31. The method of claim 30, wherein said heme moiety is a heme-containing protein.
165
166 32. The method of claim 29, wherein said highly conjugated heterocyclic ring complexed
167 to an iron ion is ferrous chlorin.
168
169 33. The method of any one of claims 28-32, said method comprising combining
170 cysteine, ribose, lactic acid, lysine, and/or thiamine with said highly conjugated
171 heterocyclic ring complexed to iron.
172
173 34. A method for producing a flavor compound, said method comprising:

174 a) combining a heme-containing protein, cysteine, and a sugar, and one or more
175 optional flavor precursor molecules to form a mixture, said one or more optional flavor
176 precursor molecules selected from the group consisting of glucose, fructose, ribose,
177 arabinose, glucose-6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol,
178 maltose, sucrose, maltodextrin, glycogen, nucleotide-bound sugars, molasses, a
179 phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid,
180 glycolic acid, thiamine, creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil,
181 cottonseed oil, soybean oil, corn oil, palm fruit oil, palm seed oil, rice bran oil, safflower
182 oil, olive oil, canola oil, coconut oil, mango oil, a free fatty acid, methionine, isoleucine,
183 leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine,
184 asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione,
185 an amino acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a
186 peptone; and
187 b) heating the mixture to form one or more flavor compounds set forth in Table 3,
188 8, 9, 12, 14, 16, or 17.
189
190 35. A method for imparting a meat-like flavor to a food product comprising
191 contacting said food product with a flavoring composition, said flavoring composition
192 comprising i) a heme-containing protein; and ii) one or more flavor precursor molecules
193 selected from the group consisting of glucose, fructose, ribose, arabinose, glucose-6-
194 phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose,
195 maltodextrin, glycogen, nucleotide-bound sugars, molasses, a phospholipid, a lecithin,
196 inosine, IMP, GMP, pyrazine, AMP, lactic acid, succinic acid, glycolic acid, thiamine,
197 creatine, pyrophosphate, vegetable oil, algal oil, sunflower oil, cottonseed oil, soybean
198 oil, corn oil, palm fruit oil, palm seed oil, rice bran oil, safflower oil, olive oil, sunflower
199 oil, canola oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine, isoleucine,
200 leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine,
201 asparagine, aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione,
202 an amino acid derivative, a hydrolyzed protein, a malt extract, a yeast extract, and a

203 peptone; wherein after heating said food product and said flavoring composition together,
204 a meat-like flavor is imparted to said food product.
205
206 36. The method of claim 35, wherein said meat-like flavor is a beef-like, chicken-like,
207 pork-like, lamb-like flavor, or turkey-like.
208
209 37. The method of claim 35 or claim 36, wherein said flavoring composition further
210 comprises a seasoning agent, a flavoring agent, a protein, a protein concentrate, or an
211 emulsifier.
212
213 38. The method of any one of claims 35-37, wherein said flavoring composition is
214 sealed within a packet or shaker.
215
216 39. A method of making a food product, said method comprising combining an
217 isolated heme-containing protein and one or more flavor precursor molecules to form a
218 mixture, said one or more flavor precursor molecules selected from the group consisting
219 of glucose, fructose, ribose, arabinose, glucose-6-phosphate, fructose 6-phosphate,
220 fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen, nucleotide-
221 bound sugars, molasses, a phospholipid, a lecithin, inosine, IMP, GMP, pyrazine, AMP,
222 lactic acid, succinic acid, glycolic acid, thiamine, creatine, pyrophosphate, sunflower oil,
223 canola oil, flaxseed oil, coconut oil, mango oil, a free fatty acid, cysteine, methionine,
224 isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, valine, arginine,
225 histidine, alanine, asparagine, aspartate, glutamate, glutamine, glycine, proline, serine,
226 tyrosine, glutathione, an amino acid derivative, a hydrolyzed protein, a malt extract, a
227 yeast extract, and a peptone; and heating the mixture.
228
229 40. A method for producing a flavor compound, said method comprising:
230 a) incubating a heme-moiety bound to a solid support with one or more flavor
231 precursor molecules, said one or more flavor precursor molecules selected from the group
232 consisting of cysteine, ribose, glucose, fructose, arabinose, glucose-6-phosphate, fructose

233 6-phosphate, fructose 1,6-diphosphate, inositol, maltose, sucrose, maltodextrin, glycogen,
234 nucleotide-bound sugars, molasses, a phospholipid, a lecithin, inosine, inosine
235 monophosphate (IMP), guanosine monophosphate (GMP), pyrazine, adenosine
236 monophosphate (AMP), lactic acid, succinic acid, glycolic acid, thiamine, creatine,
237 pyrophosphate, vegetable oil, algal oil, sunflower oil, cottonseed oil, soybean oil, corn
238 oil, palm fruit oil, palm seed oil, rice bran oil, safflower oil, olive oil, canola oil, flaxseed
239 oil, coconut oil, mango oil, a free fatty acid, methionine, isoleucine, leucine, lysine,
240 phenylalanine, threonine, tryptophan, valine, arginine, histidine, alanine, asparagine,
241 aspartate, glutamate, glutamine, glycine, proline, serine, tyrosine, glutathione, an amino
242 acid derivative, a protein hydrolysate, a malt extract, a yeast extract, and a peptone; and
243 b) heating the heme-moiety bound to said solid support and one or more flavor
244 precursors to form one or more flavor compounds selected from the group consisting of
245 phenylacetaldehyde, 1-octen-3-one, 2-n-heptylfuran, 2-thiophenecarboxaldehyde, 3-
246 thiophenecarboxaldehyde, butyrolactone, 2-undecenal, methyl-pyrazine, 2-furfural,
247 decanone, pyrrole, 1-octen-3-ol, 2-acetylthiazole, (E)-2-octenal, decanal, benzaldehyde,
248 (E)-2-nonenal, pyrazine, 1-hexanol, 1-heptanol, dimethyl trisulfide, 2-nonanone, 2-
249 pentanone, 2-heptanone, 2,3-butanedione, heptanal, nonanal, 2-octanone, 1-octanol, 3-
250 ethylcyclopentanone, 3-octen-2-one, (E,E)-2,4-heptadienal, (Z)-, 2-heptenal, 6-methyl-2-
251 heptanone, (Z)-4-heptenal, (E,Z)-2,6-nonadienal, 3-methyl-2-butenal, 2-pentyl-furan,
252 thiazole, (E,E)-2,4-decadienal, hexanoic acid, 1-ethyl-5-methylcyclopentene, (E,E)-2,4-
253 nonadienal, (Z)-2-decenal, dihydro-5-pentyl-2(3H)-furanone, trans-3-nonen-2-one, (E,E)-
254 3,5-octadien-2-one, (Z)-2-octen-1-ol, 5-ethyldihydro-2(3H)-furanone, 2-butenal, 1-
255 penten-3-ol, (E)-2-hexenal, formic acid, heptyl ester, 2-pentyl-thiophene, (Z)-2-nonenal,
256 2-hexyl-thiophene, (E)-2-decenal, 2-ethyl-5-methyl-pyrazine, 3-ethyl-2,5-dimethyl-
257 pyrazine, 2-ethyl-1-hexanol, thiophene, 2-methyl-furan, pyridine, butanal, 2-ethyl-furan,
258 3-methyl-butanal, 2-methyl-butanal, methacrolein, 2-methyl-propanal, propanal,
259 acetaldehyde, 2-propyl-furan, dihydro-5-propyl-2(3H)-furanone, 1,3-hexadiene, 4-
260 decyne, pentanal, 1-propanol, heptanoic acid, ethanethiol, 1-butanol, 1-penten-3-one,
261 dimethyl sulfide, 2-ethyl furan, 2-pentyl-thiophene, 2-propenal, 2-tridecen-1-ol, 4-octene,
262 2-methyl thiazole, methyl-pyrazine, 2-butanone, 2-pentyl-furan, 2-methyl-propanal,

263 butyrolactone, 3-methyl-butanal, methyl-thiirane, 2-hexyl-furan, butanal,, 2-methyl-
264 butanal, trichloromethane, 2-methyl-furan, furan, octanal, 2-heptenal, 1-octene, heptyl
265 ester formic acid, 3-pentyl-furan, and 4-penten-2-one.

FIGURE 1

SEQ ID NO:1 *Vigna radiata*

MTTTLERGFTEEQEALVVKSWNVMKKNSGELGLKFFLKIFEIAPSAQKLFSFLRDSTVP
LEQNPKLKPHAVSVFVMTCDSAVQLRKAGKVTVRESNLKKLGATHFRTGVANEHFEVTK
FALLETIKEAVPEMWSPAMKNAWGEAYDQLVDAIKYEMKPPSS

SEQ ID NO:2 *Methylacidiphilum infernorum*

MIDQKEKELIKESWKRIEPNKNEIGLLFYANLFKEEPTVSVLFQNPISSQSRKLMQVLG
ILVQGIDNLEGLIPTLQDLGRRHKQYGVVDSHYPLVGDCLLKSIQEYLGQGFTEEAKAA
WTKVYGIAAQVMTAE

SEQ ID NO:3 *Aquifex aeolicus*

MLSEETIRVIKSTVPLLKEHGTEITARMYELLFSKYPKTKELFAGASEEQPKKLANAII
AYATYIDRLEELDNAISTIARSHVRRNVKPEHYPLVKECLLQAIEEVLNPGEEVLKAWE
EAYDFLAKTLITLEKKLYSQP

SEQ ID NO:4 *Glycine max*

MGAFTEKQEALVSSSFEAFKANIPQYSVVFYTSILEKAPAAKDLFSFLSNGVDPSNPKL
TGHAEKLFGLVRDSAGQLKANGTVVADAALGSIHAQKAITDPQFVVVKEALLKTIKEAV
GDKWSDELSSAWEVAYDELAAAIKKAF

SEQ ID NO:5 *Hordeum vulgare*

MSAAEGAVVFSEEKEALVLKSWAIMKKDSANLGLRFFLKIFEIAPSARQMFPFLRDSDV
PLETNPKLKTHAVSVFVMTCEAAAQLRKAGKITVRETTLKRLGGTHLKYGVADGHFEVT
RFALLETIKEALPADMWGPEMRNAWGEAYDQLVAAIKQEMKPAE

Magnaporthe oryzae, (SEQ ID NO:6)

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  1 mdgavrldwt gldltgheih dgvpiasrvq vmvsfplfkd qhiimsskes psrksstigq
 61 strngscqad tqkgqlppvg ekpkpvkenp mkklkemsqr plptqhgdgt yptekkltgi
121 gedlkhirgy dvktllamvk sklkgeklkd dktmlmervm qlvarlptes kkraeltdsl
181 inelwesldh pplnylgpeh syrtpdgsyn hpfnpqlgaa gsryarsvip tvtppgalpd
241 pglifdsimg rtpnsyrkhp nnvssilwyw atiiihdifw tdprdintnk sssyldlapl
301 ygnsqemqds irtfkdgrmk pdcyadkrla gmppgvsvll imfnrfhnhv aenlalineg
361 grfnkpsdll egeareaawk kydndlfqva rlvtsglyin itlvdyvrni vnlnrvdttw
421 tldprqdaga hvgtadgaer gtgnavsaef nlcyrwhsci sekdskfvea qfqnifgkpa
481 sevrpdemwk gfakmeqntp adpgqrtfgg fkrgpdgkfd dddlvrcise avedvagafg
541 arnvpqamkv vetmgiiqgr kwnvaglnef rkhfhlkpys tfedinsdpg vaealrrlyd
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 601 hpdnvelypg lvaeedkqpm vpgvgiapty tisrvvlsda vclvrgdrfy ttdftprnlt
 661 nwgykevdyd lsvnhgcvfy klfirafpnh fkqnsvyahy pmvvpsenkr ilealgradl
 721 fdfeapkyip prvnitsygg aeyiletqek ykvtwheglg flmgegglkf mlsgddplha
 781 qqrkcmaaql ykdgwteavk afyagmmeel lvsksyflgn nkhrhvdiir dvgnmvhvhf
 841 asqvfglplk taknptgvft eqemygilaa ifttiffdld psksfplrtk trevcqklak
 901 lveanvklin kipwsrgmfv gkpakdepls iygktmikgl kahglsdydi awshvvptsg
 961 amvpnqaqvf aqavdyylsp agmhyipeih mvalqpstpe tdalllgyam egirlagtfg
1021 syreaavddv vkedngrqvp vkagdrvfvs fvdaardpkh fpdpevvnpr rpakkyihyg
1081 vgphaclgrd asqiaitemf rclfrrrnvr rvpgpqgelk kvprpggfyv ymredwgglf
1141 pfpvtmrvmw dde
```

Fusarium oxysporum (SEQ ID NO:7)

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

Fusarium graminearum (SEQ ID NO:8)

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

SEQ ID NO: 9 *Chlamydomonas eugametos*

MSLFAKLGGREAVEAAVDKFYNKIVADPTVSTYFSNTDMKVQRSKQFAFLAYALGGASE
WKGKDMRTAHKDLVPHLSDVHFQAVARHLSDTLTELGVPPEDITDAMAVVASTRTEVLN
MPQQ

SEQ ID NO:10 *Tetrahymena pyriformis*

MNKPQTIYEKLGGENAMKAAVPLFYKKVLADERVKHFFKNTDMDHQTKQQTDFLTMLLG
GPNHYKGKNMTEAHKGMNLQNLHFDAIIENLAATLKELGVTDAVINEAAKVIEHTRKDM
LGK