IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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

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

DECLARATION UNDER 37 CFR §1.132

I, Celeste Holz-Schietinger, Ph.D., hereby declare as follows:

1. I am currently a Principal Scientist at Impossible Foods in Redwood City, California. I have held this position since 2015, and I am responsible for leading the Flavor Department. In this role, I direct flavor technology research as well as flavor product integration. I began working for Impossible Foods in 2012 as a Scientist, and I was elevated to a Senior Scientist in 2013. While at Impossible Foods, I have been the lead chemist on the identification, characterization and manipulation of biological flavor catalysis and potentiation, including through the use of heme-containing proteins.

2. I received my Bachelors of Science (B.S.) degree in Molecular Biology and Evolution, with a minor in Chemistry, in 2005 from The Evergreen State College in Olympia, Washington, and I received my Doctor of Philosophy (Ph.D.) degree in Biochemistry from the University of California at Santa Barbara in 2012. I was an Enzymology Research Associate at the University of California at Santa Barbara from 2007 to 2012, and I also was a Biochemistry Visiting Associate at Stanford University in 2009.

3. A copy of my curriculum vitae is attached as **Exhibit A**.

4. I am a named inventor on the above-referenced patent application.

5. I have read U.S. Application No. 14/797,006 ("the '006 application") as well as the Office Action mailed June 6, 2016, in which the claims were rejected as being anticipated by Proulx et al. (2006, *J. Agric. Food Chem.*, 54:1518-22) and obvious over Hseih et al. (US 2012/0093994) in view of Proulx et al.

6. Gas Chromatography-Mass Spectroscopy (GCMS) and Gas Chromatography-Olfactory (GCO) methods were used to evaluate the production of volatile compounds during cooking of three flavor reaction mixtures, and in particular, to evaluate the production of volatile compounds that are associated with meaty aroma. The first flavor reaction mixture contained 1% (10 mg/ml) Leghemoglobin (LegH-only); the second contained 50 mM Ribose with 50 mM Cysteine (Ribose + Cysteine); and the third contained 1% Leghemoglobin with 50 mM Ribose and 50 mM Cysteine (LegH + Ribose + Cysteine). All flavor reaction mixtures were adjusted to pH 6.5 using 50 mM of potassium phosphate buffer and 100 mM of sodium chloride buffer. Two mls of each flavor reaction mixture were prepared in 10 ml GCMS glass vials (referred to as "flavor reaction mixture samples" or "samples" hereinafter). Each sample was analyzed in duplicate.

7. An Agilent 7890A GC system coupled with a Leco TruTOF HT system was used along with a Gerstel MultiPurpose Sampler for auto-sampling. The Gerstel auto-sample was used to cook, cool, and perform HS-SPME on each of the samples. Each sample was heated to 150°C for 3 minutes with 750 rpm of agitation to "cook" the material. This was followed by a two-phase active cooling step that decreased the agitator temperature from 150°C to 50°C. The sample was extracted for 20 minutes at 50°C with 500 rpm of agitation using a 50/30 μm Divinylbenzene / Carboxen / Polydimethylsiloxane (DVB/CAR/PDMS), Stable flex, 23 Ga, Autosampler (Supleco, Cat# 57298-U) fiber.

8. Extracted samples were run on the GCMS by desorbing the SPME fiber in the Gerstel controlled CIS inlet for 6 minutes at 240°C. The desorbed sample was separated on a 60-meter wax column (Agilent, VF-Waxms 60 m x 0.25 mm x 0.25 mm, Part# CP9207) using a 35 minutes GCMS method (35°C for 2 minutes, ramp of 5C°/min until 172°C, ramp of 60C°/min until 255°C, 255°C for 4 minutes) and 1.75 mL/min Helium flow rate and splitless mode. Separated compounds were analyzed by the mass spectrometer, and all data was collected over a 14-500 μ mass range, with an acquisition rate of 10 spectra/sec, and a detector voltage off-set of 100.

9. Samples were then analyzed using Leco ChromaTOF optimized for TruTOF HT (Version 4.51.6.0) coupled with the NIST MS Search 2.2. The identity of each peak in each sample was identified in a two-step process. First, the mass spectrum of each peak with a signal

to noise greater than 50 was matched to a mass spectrum in the NIST library using a match similarity score of 650, which in my experience is a conservative match similarity score. Additionally, an internally developed calibration method was applied to the data set to confirm the identity of the compounds of interest. In the second step, the Statistical Compare function of ChromaTOF was used to align the named analytes across all samples in a set. The criteria for aligning a single peak across all samples were a similarity score (similarity between the peak spectra across all samples) of 700, a maximum retention time difference of 3 secs, and the peak must have been present in at least two samples.

10. The amount of each volatile compound was determined by averaging the signal intensities from the duplicate experiments. The amount of each volatile compound that can be attributed to Leghemoglobin (LegH)[1] was determined by using the average signal intensity for each volatile compound produced in the LegH-only sample as a baseline to standardize the average signal intensity for the same volatile compound produced in the LegH + Ribose + Cysteine sample. The fold-change in each volatile compound was determined by dividing the signal intensity produced in the presence of LegH (i.e., in the LegH + Ribose + Cysteine sample) by the signal intensity produced in the absence of LegH (i.e., the Ribose + Cysteine sample). If the signal intensity in the absence of LegH (i.e., the Ribose + Cysteine sample) was not detectable, the volatile compound produced in the presence of LegH (i.e., in the LegH + Ribose + Cysteine sample) was indicated as "novel," meaning that the volatile compound was newly produced (within the limits of detection of the technique) in the LegH + Ribose + Cysteine sample.

11. The results of the above-described experiments are shown in **Exhibit B**. The first column shows a list of volatile compounds, grouped into eight (8) different classes of chemicals. The next three columns show the amount of each volatile compound, calculated as described above, for each of the samples (LegH only, Ribose + Cysteine, and LegH + Ribose + Cysteine) followed by a column indicating the fold-change, calculated as described above, or indicating that the volatile compound is novel as described above. The next column provides, where

[1] Volatile compounds that can be attributed to LegH refer to compounds that are, independently, either not detectably produced or produced in a detectably smaller amount upon cooking compared with the control samples (e.g., LegH-alone or Ribose + Cysteine in the absence of LegH).

available, one or more peer-reviewed publications in which that particular volatile compound is associated with meaty aroma.

12. The presence of LegH resulted in the production of **twenty-eight (28)** novel volatile compounds that were not produced in the absence of LegH. The 28 novel volatile compounds can be divided into eight (8) different classes of compounds as follows. Representative volatile compounds in each class are shown below; the full list can be found in **Exhibit B**.

- *Oxygen (O)- and/or Nitrogen (N)-containing heterocyclic*: six (6) novel O- and/or N-containing heterocyclic compounds were produced (i.e., 2-methyl furan; 2-pentyl furan; bis(2-methyl-3-furyl) disulfide; 2,5-dimethyl-pyrazine; methyl-pyrazine; and 2-ethyl-5-methyl-pyrazine);
- *Sulfur (S)-containing heterocyclic*: nine (9) novel S-containing heterocyclic compounds were produced (e.g., 2-methyl-3-furanthiol; 3-thiophenemethanol; and 2-methyl-thiazole);
- *sulfides*: one (1) novel sulfide compound was produced (i.e., dimethyl sulfide);
- *aldehydes*: four (4) novel aldehydes were produced (e.g., (E,E)-2,4-hexadienal; and decanal);
- *lactones*: two (2) novel lactones were produced (i.e., 5-ethyldihydro-2(3H)-furanone; and dihydro-5-pentyl-2(3H)-furanone);
- *ketones*: three (3) novel ketones were produced (e.g., 2-octanone; and 3-nonanone);
- *alcohols*: one (1) novel alcohol was produced (i.e., p-cresol); and
- *carboxylic acids*: two (2) novel carboxylic acids were produced (i.e., hexanoic acid; and hexadecanoic acid).

Significantly, each of the 28 different novel volatile compounds have been associated, directly or indirectly, with a meaty aroma; see, for example, the references provided in **Exhibit B**. Of further significance, the presence of LegH in the flavor reaction mixture with Cysteine and Ribose resulted in the generation of two (2) novel volatile compounds upon heating: **2-methyl-3-furanthiol** and **bis(2-methyl-3-furyl)disulfide**. As noted in the Cadwallader

Declaration, which I have reviewed, both compounds are important aroma compounds in cooked meat and have extremely low odor detection thresholds of 0.7 parts-per-trillion (pptr) and 0.02 pptr, respectively (Belitz et al., 2006, Food Chemistry, Springer-Verlag, Berlin, p. 368). Both compounds usually are only present in low abundance (pptr range) in cooked meat (Tang et al., 2013, J. Sulfur Chem., 34:38-47). Despite their low abundance in meat, they are still among the most impactful aroma compounds because of their extremely low odor detection thresholds.

13. The presence of LegH in the flavor reaction mixture with Cysteine and Ribose also resulted in a synergistic and dramatic increase in the amount of **two (2)** volatile compounds produced upon heating compared to the amount of the same volatile compounds produced upon heating in the absence of LegH (i.e., in the Ribose + Cysteine sample) or in the LegH-only sample. In the presence of LegH, the amount of:

- 2-acetylthiazole was increased **2,851-fold**; and
- thiophene was increased **89-fold**.

Significantly, 2-acetylthiazole and thiophene have been associated with a meaty aroma. See, for example, the references cited in **Exhibit B**.

14. In sum, the presence of LegH with Cysteine and Ribose dramatically increased the number and amount of aroma compounds associated with meat upon cooking.

15. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully submitted,

Date: Dec 2nd 2016

Celeste Holz-Schietinger, Ph.D.

EXHIBIT A

Celeste Holz-Schietinger, PhD

Principal Scientist, Impossible Foods

Redwood City, CA
celeste.holz@impossiblefoods.com

Innovative R&D Scientist and Product Developer responsible for leading the Flavor Department at Impossible Foods. Technical skills include enzymology, flavor chemistry, sensory science, lipid and protein biochemistry, protein purification, analytical chemistry, metabolic pathways and small molecule identification.

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.

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.