IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Rachel Fraser	Art Unit	:	1791
Serial No.	:	15/398,479	Examiner	:	Elizabeth A. Gwartney
Filed	:	January 4, 2017	Conf. No.	:	2358
Title	:	METHODS AND COMPOSITIONS FOR AFFECTING THE FLAVOR AND AROMA PROFILE OF CONSUMABLES			

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

DECLARATION UNDER 35 U.S.C. § 1.132 OF DAVID J. LIPMAN M.D.

I, Dr. David J. Lipman, hereby declare as follows:

1. I am currently Chief Scientific Officer of Impossible Foods, the assignee of U.S. Application Serial No. 15/398,479 ("the '479 application"). I have held this position since May 2017, and am responsible for research and development and information technology. I am submitting this Declaration in support of the '479 application.

2. It is my understanding that the Office has rejected the pending claims in the '479 application as being anticipated and/or obvious over a number of references, including Tong et al. ("Blood Composition of Different Beef Breed Types", Can. J. Anim. Sci, 1986;66: 915-924); Shimbayashi et al. ("Free Amino Acids and Phosphorylethanolamine in Milk Whey of Cow", Agr. Biol. Chem, Vol. 29, No. 1, 1965;13-19); Koutsidis et al. ("Water-soluble precursors of beef flavor: I. Effect of diet and breed", Meat Science, 79, 2008;79:124-130); Field et al. ("Heme Pigment Content of Bovine Hemopoietic Marrow and Muscle", J. Food Sci., 1980;45:1109-1112); and Jamieson ("Iroquois Stew With Beef, Chicken and Pork", Bon Appetit, November 1995, http://w ww.epicurious.conilrecipesifoodiviewsiiroquois-stewwith-beef-chicken-and-pork-865). I have read the '479 application, the Office Action, and the cited art. I have also read the Declarations of Dr. Keith R. Cadwallader, Dr. Devin Grant Peterson, Dr. S. Christopher Davis and Dr. Celeste Holz-Schietinger, the cited art, and prior Office Action Responses that Impossible Foods has submitted with prior responses to Office Actions, including

those submitted in U.S. Application Serial No. 14/797,006, now U.S. Patent No. 9,700,067, which is the parent application of the '479 application.

3. I have a Bachelor of Science degree conferred on me by Brown University in 1976, and a M.D. degree conferred on me in 1980 by the State University of New York at Buffalo.

4. I am a nationally and internationally recognized expert in the field of bioinformatics. I was actively involved in the development of a series of sequence similarity algorithms, including the Wilbur-Lipman algorithm in 1983, FASTA search in 1985, BLAST in 1990, and Gapped BLAST and PSI-BLAST in 1997.

5. I am an elected member of the National Academy of Sciences and the National Academy of Medicine, and a fellow of the International Society of Computational Biology, the American Academy of Arts and Sciences, and the American College of Medical Informatics. I received the Jim Gray eScience Award from Microsoft in 2013.

6. I served as Director of the National Center for Biotechnology Information (NCBI) at the National Institutes of Health (NIH) from 1989 to 2017. NCBI is the home of GenBank, the U.S. node of the International Sequence Database Consortium, and PubMed, one of the most heavily used sites in the world for the search and retrieval of biomedical information.

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

8. Under my direction, a pairwise alignment was performed on 27 heme-containing protein sequences provided in the '479 application (i.e., SEQ ID NOs. 1-27 disclosed in the '479 application), representing a diverse range of organisms, to evaluate amino acid sequence similarity. All but three of the proteins are heme-containing globin proteins from a wide phylogenetic spread. The amino acid sequences of the three non-globin proteins are provided in SEQ ID Nos: 6-8.

9. The pairwise alignments were performed using the Needleman & Wunsch alignment algorithm [Needleman SB, Wunsch CD. "A general method applicable to the search for similarities in the amino acid sequence of two proteins", J Mol Biol. 1970 Mar;48(3):443-53, attached as **Exhibit B**] as implemented by the National Center for Biotechnology Information [see https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&PROG_DEF=blastn&BLA

ST_PROG_DEF=blastn&BLAST_SPEC=GlobalAln&LINK_LOC=BlastHomeLink, attached as **Exhibit C**)] and run using the default parameters.

10. The results of the pairwise alignments are shown in **Exhibit D**. The table provided in Exhibit D reports the percentage of identical amino acids shared among each pair of aligned sequences. This global alignment approach was used in order to maximize the number of shared amino acids across the entire amino acid sequence, and thus it is a highly conservative approach in this context. Despite this conservative approach, this set of proteins is so diverse that many pairs exhibit very little sequence similarity.

11. Example 9 of the '479 application describes an experiment employing Gas Chromatography-Mass Spectroscopy (GCMS) and Gas Chromatography-Olfactory (GCO) methods to evaluate the production of volatile compounds, including those associated with a meaty aroma, during thermal treatment (cooking) of four aqueous flavor reaction mixtures containing four distinct globin heme-containing proteins (SEQ ID NOs: 3-5 and 18).

12. A summary of the pairwise alignment shared among each pair of aligned sequences for SEQ ID NOs: 3-5 and 18 shown in Exhibit B is provided in Table 1 below. As one can see from Exhibit B and Table 1, these 4 globin heme-containing proteins demonstrate less than 36% pairwise identity between each pair of aligned sequences. These four globin proteins represent heme-containing proteins obtained from animals, plants, and bacteria, which have been separated by over a billion years of evolution; (see, for example, Proc Natl Acad Sci U S A. 2001 Feb 27;98(5):2497-502, attached as **Exhibit E**; and Mol Biol Evol. 2015 Apr;32(4):835-45, attached as **Exhibit F**).

Table 1

		Pairwise Identity			
		SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:5	SEQ ID NO:18
Aquifex aeolicus	SEQ ID NO:3	100%	25%	19%	21%
Leghemoglobin	SEQ ID NO:4	25%	100%	36%	19%
Barley (*Hordeum vulgare*)	SEQ ID NO:5	19%	36%	100%	20%
Bovin Myoglobin	SEQ ID NO:18	21%	18%	20%	100%

13. The first flavor reaction mixture in Example 9 contained 1% (10 mg/ml) Leghemoglobin (SEQ ID NO: 4) mixed with 10 mM Cysteine and 10 mM Ribose; the second

flavor reaction mixture contained 1% (10 mg/ml) Barley hemoglobin (*H. vulgare*, (SEQ ID NO: 5) mixed with 10 mM Cysteine and 10 mM Ribose; the third flavor reaction mixture contained 1% (10 mg/ml) *B. myoglobin* hemoglobin (SEQ ID NO: 18) mixed with 10 mM Cysteine and 10 mM Ribose; and the fourth flavor reaction mixture contained 1% (10 mg/ml) an *A. aeolicus* heme-containing protein (SEQ ID NO: 3) mixed with 10 mM Cysteine and 10 mM Ribose. All flavor reaction mixtures were adjusted to pH 6.0, heated to 150°C for 3 minutes and evaluated by GCMS to identify volatile aroma compounds produced that are associated with meaty aroma.

14. The results of the above-identified experiments are set forth in Table 9 of the '479 application, which is reproduced below. The results show that that inclusion of <u>any</u> of the four members of this widely diverse set of heme containing proteins, proteins that in my opinion are from perhaps the farthest branches of the tree of life, in an aqueous thermal flavor reaction with Cysteine and Ribose, produced multiple aroma compounds associated with a meaty aroma upon heating.

Table 9 of the '479 Application

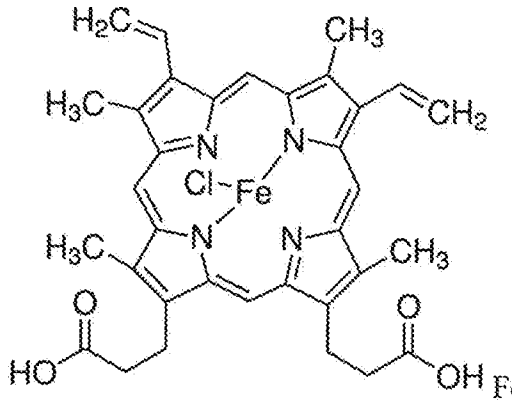
Name	LegH	Barley	*B. myoglobin*	*A. aeolicus*
Furan	x	x	x	x
Thiazole	x	x	x	x
benzaldehyde	x	x	x	x
2-acetylthiazole	x	x	x	x
2-methyl-propanal	x	x	x	x
furfural	x	x	x	x
2,3-butanedione	x	x	x	x
2-pentyl-furan	x	x	x	x
2-pentanone	x	x		
pyrazine	x	x	x	x
dimethyl trisulfide	x	x	x	x
3-methyl-butanal	x	x		x
2-methyl-thiazole	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

15. Example 13 of the '479 application describes an experiment employing GCMS methods to evaluate the production of volatile aroma compounds associated with a meaty aroma during cooking of reaction mixtures containing either myoglobin free in solution or hemin (heme unbound to protein) coupled to a solid support. A first reaction mixture included heme protein (equine myoglobin -Sigma) at 0.35% in a phosphate buffer (20 mM) at pH 6.0 with 100 mM NaCl; this was then mixed with Magic Mix (see Table 13 of the '479 application). A second reaction mixture was created with immobilized hemin (bovine hemin (Sigma Aldrich)) 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 of the '479). The reaction mixtures were heated to 150°C for 3 minutes and evaluated by GCMS to identify volatile aroma compounds that are associated with meaty aroma.

16. Hemin is an example of a conjugated heterocyclic ring complexed to an iron ion, as described in the '479 application. More specifically, hemin is an iron-containing porphyrin

(protoporphyrin IX) containing a ferric iron ion (heme B) with a chloride ligand, having the structure of Formula I below.



Formula I

17. The results of the above-identified experiments are set forth in Table 17 of the '479 application, which is reproduced below. The results provided in Table 17 show that reaction mixtures containing the immobilized hemin and the heme-protein both produce multiple aroma compounds associated with a meaty aroma upon heating. Notably, the profiles of volatile compounds, measured by GCMS, produced by the reaction mixtures containing the immobilized hemin and the heme-protein, respectively, were very similar.

Table 17 of the '479 Application

Flavor compound	myoglobin	hemin-linker-resin
2-methyl-5-(methylthio)-thiophene	Low	
dihydro-5-propyl-2(3H)-furanone	Low	
octane	Low	
pyrrole	Low	Low
methanethiol	Low	Low
2-thiophenecarboxaldehyde	Low	Low
methyl-pyrazine	Low	Low
1-hydroxy-2-propanone	Low	Low
propanal	Low	Low
thiophene	Low	medium
pyridine	Low	Low
2-methyl-furan	Low	medium
oxalic acid, butyl propyl ester	Low	Low
pyrazine	medium	Low
oxalic acid, diallyl ester	medium	medium
2-butenal	medium	large
furfural	medium	medium

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

18. In my opinion, the experimental data provided in Example 9 of the '479 application relating to the surprising ability of four very distinct members of the set of heme containing proteins to generate meat-associated volatile aroma compounds upon heating, when coupled with the experimental data provided in Example 13 of the '479 application, demonstrating that even heme unbound to protein (i.e., hemin), also shared such meat aroma and flavor production properties when heated in a reaction mixture, make it reasonable to conclude that these functional properties are not based on protein sequence similarity, but are instead based on the commonality among the proteins and the hemin experiments, namely, the inclusion of the heme co-factor.

19. 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: July 11, 2017

David J. Lipman, M.D.