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 at Impossible Foods. 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. Gas Chromatography-Mass Spectroscopy (GCMS) and Gas Chromatography-Olfactory (GCO) methods were used to evaluate the production of volatile compounds during cooking of flavor reaction mixtures, and in particular, to evaluate the production of volatile compounds that are associated with meaty aroma. The first flavor reaction mixture set contained only Leghemoglobin (LegH-only) at different concentrations (0.01%, 0.2%, 1%, and 3%) (note 1% =10 mg/mL); the second set contained a mixture representing the estimated concentrations of amino acids, sugars, and other small molecules in beef based on their values reported in literature as shown below (Table 1, "the Precursor Mix"):

Table 1

Precursor 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
Thiamine	0.5
GMP	0.24
IMP	0.6
Lactic acid	1
Creatine	1
NaCl	10
KCl	10
Potassium phosphate pH 6.5	10

and the third contained both Leghemoglobin at different concentrations (0.01%, 0.05% 0.2%, 0.5%, 1%, and 3%) and the Precursor Mix. 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 mL 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.

5. 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.

6. Extracted samples were run on the GCMS by desorbing the SPME fiber in the Gerstel controled 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.

7. 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 threshold of 650. 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 seconds, and the peak must have been present in at least two samples.

8. The amount of each aroma compound was determined by averaging the signal intensities from the duplicate experiments. The amount of each aroma compound that can be attributed to leghemoglobin (LegH)[1] was determined by using the average signal intensity for each aroma compound produced in the LegH-only sample as a baseline to standardize the average signal intensity for the same aroma compound produced in the LegH + Precursor Mix sample. The fold-change in each aroma compound was determined by dividing the signal intensity produced in the presence of LegH (i.e., in the LegH + Precursor Mix sample) by the signal intensity produced in the absence of LegH (i.e., Precursor Mix sample). If the signal intensity in the absence of LegH (i.e., Precursor Mix) was not detectable, the aroma compound

[1] Aroma 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 Precursor Mix in the absence of LegH).

produced in the presence of LegH (i.e., in the LegH + Precursor Mix sample) was indicated as "novel," meaning that the aroma compound was newly produced (to the limits of detection of the technique) in the LegH + Precursor Mix sample.

9. The results of the above-described experiments are shown in **Exhibit B**. The first column shows the list of aroma compounds, grouped into eight (8) different classes of chemicals. The next columns show the amount of each aroma compound, calculated as described above, for each of the samples (LegH only, Precursor Mix only, and LegH + Precursor Mix) followed by a column indicating the fold-change, calculated as described above, or indicating that the aroma compound is novel as described above. The next column provides, if available, one or more peer-reviewed publications in which that particular aroma compound is associated with meaty aroma.

10. The presence of **0.01% LegH** with precursor mix resulted in the production of **twenty-two (22)** novel meat associated aroma compounds upon cooking that were not produced in the absence of the LegH. The 22 different novel aroma compounds can be divided into eight (8) different classes of compounds as follows. Representative aroma compounds in each class are shown below; the full list can be found in Exhibit B.

- *Lactones*: one (1) novel lactone was produced (i.e., dihydro-5-pentyl-2(3H)-furanone);
- *Oxygen (O)- and/or Nitrogen (N)-containing heterocyclic*: no (0) novel O- and/or N-containing heterocyclic compounds were produced;
- *Sulfur (S)-containing heterocyclic*: five (5) novel S-containing heterocyclic compounds were produced (i.e., 3-thiophenemethanol, 3-methyl-thiophene, 2-ethyl-thiophene, 2-methyl-5-propyl-thiophene, 4-(methylthio)thiophenol);
- *Carboxylic acids*: three (3) novel carboxylic acids were produced (i.e., hexanoic acid, octanoic acid, propanoic acid);
- *Aldehydes*: eight (8) novel aldehydes were produced (i.e., decanal, undecanal, butanal, heptanal, 2-methyl-propanal, propanal, 3-methyl-butanal, 3-methyl-2-butenal);
- *Sulfides*: two (2) novel sulfide compounds were produced (i.e., dimethyl sulfide, methanethiol);

- *Ketones*: three (3) novel ketones were produced (i.e., 2-nonanone, 2-heptanone, 2,3-pentanedione); and
- *Alcohols*: no (0) novel alcohols were produced.

11. The presence of **0.2% LegH** with precursor mix resulted in the production of **twenty-seven (27)** novel meat associated aroma compounds upon cooking that were not produced in the absence of the LegH. The 27 different novel aroma compounds can be divided into eight (8) different classes of compounds as follows. Representative aroma compounds in each class are shown below; the full list can be found in Exhibit B.

- *Lactones*: no (0) novel lactones were produced;
- *Oxygen (O)- and/or Nitrogen (N)-containing heterocyclic*: two (2) novel O- and/or N-containing heterocyclic compound was produced (i.e., 2,5-dimethyl-pyrazine, indole);
- *Sulfur (S)-containing heterocyclic*: five (5) novel S-containing heterocyclic compounds were produced (i.e., 3-thiophenemethanol, 3-methyl-thiophene, 2-ethyl-thiophene, 2-methyl-5-propyl-thiophene, 4-(methylthio)thiophenol);
- *Carboxylic acids*: four (4) novel carboxylic acids were produced (i.e., hexanoic acid, hexadecanoic acid, octanoic acid, propanoic acid);
- *Aldehydes*: nine (9) novel aldehydes were produced (i.e., decanal, undecanal, butanal, heptanal, 2-methyl-propanal, propanal, hexanal, 3-methyl-butanal, 3-methyl-2-butenal);
- *Sulfides*: four (4) novel sulfide compounds were produced (i.e., dimethyl sulfide, dimethyl trisulfide, dimethyl disulfide, methanethiol);
- *Ketones*: three (3) novel ketones were produced (i.e., 2-nonanone, 2-heptanone, 2,3-pentanedione); and
- *Alcohols*: no (0) novel alcohols were produced.

12. The presence of **1% LegH** with precursor mix resulted in the production of **thirty two (32)** novel meat associated aroma compounds upon cooking that were not produced in the absence of the LegH. The 32 different novel aroma compounds can be divided into eight (8) different classes of compounds as follows. Representative aroma compounds in each class are shown below; the full list can be found in Exhibit B.

- *Lactones*: no (0) novel lactones were produced;

- *Oxygen (O)- and/or Nitrogen (N)-containing heterocyclic*: four (4) novel O- and/or N-containing heterocyclic compounds were produced (i.e., 2-pentyl-pyridine, 2-ethyl-5-methyl- pyrazine, 2,5-dimethyl-pyrazine, indole);
- *Sulfur (S)-containing heterocyclic*: seven (7) novel S-containing heterocyclic compounds were produced (i.e., 3-thiophenemethanol, 1-(4,5-dihydro-2-thiazolyl)-ethanone, 3-methyl-thiophene, 2-ethyl-thiophene, 2-methyl-5-propyl-thiophene, 4-(methylthio)thiophenol, 2,4-dimethyl-thiazole);
- *Carboxylic acids*: four (4) novel carboxylic acids were produced (i.e., hexanoic acid, hexadecanoic acid, octanoic acid, propanoic acid);
- *Aldehydes*: ten (10) novel aldehydes were produced (i.e., decanal, undecanal, butanal, heptanal, 2-methyl-propanal, propanal, hexanal, 3-methyl-butanal, pentanal, 3-methyl-2-butenal);
- *Sulfides*: three (3) novel sulfide compounds were produced (i.e., dimethyl sulfide, dimethyl trisulfide, dimethyl disulfide);
- *Ketones*: three (3) novel ketones were produced (i.e., 2-nonanone, 2-heptanone, 2,3-pentanedione); and
- *Alcohols*: one (1) novel alcohol was produced (i.e., furaneol).

13. The presence of **3% LegH** with precursor mix resulted in the production of **thirty-three (33)** novel meat associated aroma compounds upon cooking that were not produced in the absence of the LegH. The 33 different novel aroma compounds can be divided into eight (8) different classes of compounds as follows. Representative aroma compounds in each class are shown below; the full list can be found in Exhibit B.

- *Lactones*: one (1) novel lactone was produced (i.e., 5-ethyldihydro-2(3H)-furanone);
- *Oxygen (O)- and/or Nitrogen (N)-containing heterocyclic*: four (4) novel O- and/or N-containing heterocyclic compounds were produced (i.e., 2-pentyl-pyridine, 2-ethyl-5-methyl- pyrazine, 2,5-dimethyl-pyrazine, indole);
- *Sulfur (S)-containing heterocyclic*: seven (7) novel S-containing heterocyclic compounds were produced (i.e., 3-thiophenemethanol, 1-(4,5-dihydro-2-thiazolyl)-ethanone, 3-methyl-thiophene, 2-ethyl-thiophene, 2-methyl-5-propyl-thiophene, 4-(methylthio)thiophenol, 2,4-dimethyl-thiazole);

- *Carboxylic acids*: four (4) novel carboxylic acids were produced (i.e., hexanoic acid, hexadecanoic acid, octanoic acid, propanoic acid);
- *Aldehydes*: ten (10) novel aldehydes were produced (i.e., decanal, undecanal, butanal, heptanal, 2-methyl-propanal, propanal, hexanal, 3-methyl-butanal, pentanal, 3-methyl-2-butenal);
- *Sulfides*: three (4) novel sulfide compound was produced (i.e., dimethyl sulfide, dimethyl trisulfide, dimethyl disulfide);
- *Ketones*: three (3) novel ketones were produced (i.e., 2-nonanone, 2-heptanone, 2,3-pentanedione); and
- *Alcohols*: one (1) novel alcohol was produced (i.e., furaneol).

14. Across the four concentration ranges of LegH, **35** different novel aroma compounds were identified, each of which have been associated, directly or indirectly, with meaty aroma; see, for example, the references provided in **Exhibit B**. Specifically,

- twenty (20) of those novel compounds were produced in all four LegH reactions (0.01%, 0.2%, 1% and 3%) (i.e., 3-thiophenemethanol, 3-methyl-thiophene, 2-ethyl-thiophene, 2-methyl-5-propyl-thiophene, 4-(methylthio)thiophenol, hexanoic acid, octanoic acid, propanoic acid, decanal, undecanal, butanal, heptanal, 2-methyl-propanal, propanal, 3-methyl-butanal, 3-methyl-2-butenal, dimethyl sulfide, 2-nonanone, 2-heptanone, and 2,3-pentanedione);
- six (6) of those novel compounds were produced in all LegH reactions except at the lowest concentration (i.e., 0.2%, 1% and 3% LegH, but not 0.01% LegH) (i.e., 2,5-dimethyl-pyrazine, hexadecanoic acid, hexanal, dimethyl trisulfide, dimethyl disulfide, and indole); and
- four (4) of those novel compounds were produced in the presence of the higher concentrations of LegH (i.e., 1% and 3% LegH, but not 0.01% or 0.2% LegH) (i.e., 1-(4,5-dihydro-2-thiazolyl)-ethanone, 2,4-dimethyl-thiazole, pentanal, and furaneol).

15. The presence of LegH in the flavor reaction mixture with the Precursor Mix also resulted in a synergistic and dramatic increase in the amount of numerous meat associated aroma compounds produced upon heating compared to the amount of the same aroma compounds

produced upon heating in the absence of LegH (i.e., in the Precursor Mix sample) or in the LegH sample. For example, in the presence of LegH, the amount of:

- 3-thiophenecarboxaldehyde was increased almost 10-fold in the presence of 0.01% LegH, more than 250-fold in the presence of 0.2% LegH, about 3600-fold in the presence of 1% LegH, and over 4300-fold in the presence of 3% LegH;
- methyl-2-thiophenecarboxaldehyde was increased over 350-fold in the presence of 1% LegH and over 1460-fold in the presence of 3% LegH.;
- methyl pyrazine was increased over 10-fold in the presence of 0.2% LegH, over 120-fold in the presence of 1% LegH, and almost 150-fold in the presence of 3% LegH;
- 2-methyl-3-furanthiol was increased over 120-fold in the presence of 0.01% LegH, and about 320-fold in the presence of 0.2% LegH;
- dihydro-3-(2H)-thiophenone was increased almost 5-fold in the presence of 0.2% LegH, over 15-fold in the presence of 1% LegH, and over 30-fold in the presence of 3% LegH; and
- 2,3-butanedione was increased over 8-fold in the presence of 0.2% LegH, almost 650-fold in the presence of 1% LegH, and almost 620-fold in the presence of 3% LegH.
- 2-butanone was increased over 15-fold in the presence of 0.2% LegH, over 125-fold in the presence of 1% LegH, and over 165-fold in the presence of 3% LegH.
- 2-acetylthiazole was increased over 8-fold in the presence of 0.2% LegH, almost 215-fold in the presence of 1% LegH, and almost 275-fold in the presence of 3% LegH.
- furfural was increased almost 40-fold in the presence of 1% LegH and over 30-fold in the presence of 3% LegH.

Significantly, each of these compounds have been associated, either directly or indirectly, with meaty or beefy aroma. See, for example, the references cited in **Exhibit B**. Specifically, 2-

methyl-3-furanthiol[2,3] and 2-acetylthiazole[4], for example, are considered important components in the aroma of meat or beef.

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[2] See paragraph 15 in the Declaration under 37 CFR § 1.132 by Dr. Keith R. Cadwallader ("the Cadwallader Declaration"), submitted in U.S. Application No. 14/797,006, now U.S. Patent No. 9,700,067, on December 5, 2016, which states:

> Among the 28 compounds identified in [the accompanying Holz-Schietinger Declaration] experiments, two in particular are well-known to possess meaty sensory characteristics and to be important and potent odorants in cooked meat. Specifically, 2-methyl-3-furanthiol (MFT) and its dimerized form bis(2-methyl-3-furyl)disulfide (MFT-MFT) are important aroma compounds in cooked meat (Tang et al., 2013). Both compounds have extremely low odor detection thresholds of 0.7 parts-per-trillion (pptr) and 0.02 pptr, respectively (Belitz et al. 2006), and are usually only present in low abundance (pptr range) in cooked meat (Tang et al., 2013). Despite their low abundance in meat they are still among the most impactful aroma compounds because of their extremely low odor detection thresholds.

[3] See paragraph 21 in the Declaration under 37 CFR § 1.132 by Dr. Devin Grant Peterson ("the Peterson Declaration"), submitted in U.S. Application No. 14/797,006, now U.S. Patent No. 9,700,067, on December 5, 2016, which states:

> Additionally, the [accompanying Holz-Schietinger Declaration] demonstrates that 2-methyl-3-furanthiol (MFT) and the major degradation product of MFT, the dimer, bis(2-methyl-3-furyl) disulfide, were detected only in the aqueous thermal reaction samples that contained Leghemoglobin. MFT is a very potent meaty odorant, with an extremely low odor threshold at 0.007 μg/kg in water, and is known to have an important contribution to meat aroma. See, for example, Kerscher and Grosch (1998, J. Agric. Food Chem., 46:1954-58); Tang et al. (2013, J. Sulfur Chem., 34:38-47); and Withcombe and Mussinan (1988, J. Food Sci., 53:658-60). It is surprising to me that an intense meaty aroma compound such as MFT was uniquely identified in the reaction mixture upon the addition of LegH.

[4] See paragraph 19 of the Peterson Declaration, submitted in U.S. Application No. 14/797,006, now U.S. Patent No. 9,700,067, on December 5, 2016, which states:

> 2-acetylthiazole is particularly interesting as it has been associated in the literature with a meaty/roasty aroma. See, for example, Chemistry and Technology of Flavours and Fragrances, 2005, Ed Rowe, Blackwell Publishing Ltd; Flavor of Meat and Meat Products, 1994, Ed Shahidi, Springer-Science+Business Media, BV; Handbook of Meat and Meat Processing, 2012, 2nd Ed, Ed Hui, CRC Press; Mottram and Mottram (2002, "An Overview of the Contribution of Sulfur-Containing Compounds to the Aroma in Heated Foods," in Heteroatomic Aroma Compounds, ACS Symposium Series, Vol 826); and Chen and Ho (2002, J. Agric. Food Chem., 50(8):2372–76).

16. 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: 8/17/17

Celeste Holz-Schietinger