IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Patrick O'Reilly Brown M.D., Ph.D.	Art Unit	:	1792
			Examiner	:	Assaf Zilbering
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Title	:	METHODS AND COMPOSITIONS FOR CONSUMABLES			

Commissioner for Patents
P.O. Box 1450
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DECLARATION UNDER 37 CFR §1.132

I, S. Christopher Davis, D.Phil, hereby declare as follows:

1. I am currently a Senior Director at Impossible Foods in Redwood City, California. I have held this position since 2012, and I am responsible for long term research, intellectual property strategies, and research partnerships with global businesses. I have lead the product development, pilot and engineering team in their work to discover, develop and scale-up food products. I have also lead the research team responsible for examining the flavor profile of animal products and for developing similar flavor profiles in products derived from plant materials.

2. Prior to my position at Impossible Foods, I was a Director at Codexis, Inc. in Redwood City, California from 2002 to 2012. In that position, I was responsible for the analytical biochemistry group, which was focused on the quantitative analysis of chemical and biological compositions to support the creation of proteins and microbial biocatalysts for the production of pharmaceutical drugs, drug intermediates, chemicals, and biofuels. I was also responsible for creating the process chemistry, analytical chemistry and fermentation groups and for leading a series of product development programs. My work was recognized by two Presidential Green Chemistry Challenge Awards (2006 and 2012). Prior to my position at Codexis, I was a Scientist III at Maxygen, Inc. in Redwood City, California from 1998 to 2002. In that position, I developed new tools and methods for directed evolution.

3. I received Bachelors of Science (B.S.) degrees in Chemistry and Biochemistry in 1989 from Southampton University, and I received a Doctor of Philosophy (D.Phil) degree in Organic Chemistry in 1992 from Oxford University. I was a postdoctoral research associate at the University of California at San Francisco from 1993 to 1998, where I worked on the control

of catalysis by cytochrome P450 enzymes and on the development of novel gene therapy methods.

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

5. In the preparation of my declaration, I have reviewed U.S. Application Serial No. 14/152,499 ("the '499 application"), as well as the Office Action mailed March 10, 2017, in which the claims of the '499 application were rejected as being obvious over Van den Briel et al. (EP 1759593)("Van den Briel") in view of Proulx et al. (2006, *J. Agric. Food Chem.*, 54:1518-22)("Proulx"), Asgar et al. (2010, Comp. Rev. Food Sci. Food Safety, 9:513-29)("Asgar") and Brewer (2006, "The Chemistry of Beef Flavor – Executive Summary," National Cattlemen's Beef Association, #12814).

6. Proulx examined the bioavailability of heme iron derived from soy root nodule and purified soy leghemoglobin sources in a corn flour matrix (a tortilla made with corn flour and water) using a Caco-2 cell model system.

7. We performed a number of compositional analyses on corn flour. To perform these experiments, I purchased a 4.84 pound bag of Maseca masa instant corn flour ("the corn flour") from the Casa Lucas Market on 7/16/2016. Casa Lucas Market is located at 2934 24th St. in San Francisco, California. I brought the bag of the corn flour back to Impossible Foods.

8. On July 27, 2016, I weighed and then placed 500 grams of the corn flour into a clean Ziplock bag and shipped the bag to Silliker's Northern California Laboratory (referred to hereafter as "Silliker"). Silliker is located at 5262 Pirrone Court in Salida, California.

9. I instructed Silliker to analyze the elemental composition of the corn flour. Specifically, I instructed Silliker to perform compositional analysis of the corn flour for ash, carbohydrates, fats, total protein, sulfur, minerals (using ICP-MS to quantitate heavy metals), free amino acids, moisture, and monosaccharides and disaccharides including fructose, glucose, sucrose, maltose and lactose.

10. A copy of the Certificate of Analysis prepared by Silliker is provided in **Exhibit B**. Included in the Certificate of Analysis is a literature citation for each method that was used in the analysis determining the elemental composition of the corn flour. Silliker follows a validated sample preparation method appropriate for measurement of each compound identified in the

Certificate of Analysis and a validated method to determine the amount of each compound identified in the Certificate of Analysis. Silliker performs all of their analyses under good laboratory practice (GLP) conditions.

11. As shown in **Exhibit B**, the amount of each free amino acid, including methionine, was at or below the limit of quantitation for this assay. The limit of quantitation of 0.01% w/w corresponds to a concentration of 0.7 millimolar (mM) for methionine, which has a molecular weight of 149. Therefore, methionine is present at a concentration of less than 0.7 mM in the corn flour.

12. The methods utilized by Silliker cause cysteine and cystine (also known as disulfide-bonded cysteine) to degrade and so are inappropriate for quantitation of cysteine and cystine. To further evaluate the content of amino acids, including cysteine and cystine, in the corn flour, we asked Alphalyse Inc. (referred to hereafter as "Alphalyse") to determine the amounts of individual amino acids (i.e., free amino acids) in the corn flour.

13. On October 27, 2016, I placed 10 g grams of the corn flour into a clean Ziplock bag and shipped the bag to Alphalyse. Alphalyse is located at 200 Page Mill Road, Suite 100, in Palo Alto, California. I instructed Alphalyse to determine the amounts of the free amino acids in the corn flour.

14. A copy of the Amino Acid Analysis Report prepared by Alphalyse is provided in **Exhibit C**. Included in **Exhibit C** is a description of the methods that were used to analyze the free amino acids in the corn flour. Briefly, Alphalyse extracted the free amino acids by treating the corn flour with dilute acid, followed by chromatographic separation of the compounds contained within the resulting extract. The specific chromatographic conditions and the resulting chromatograms provided by Alphalyse also are shown in **Exhibit C**.

15. As shown in **Exhibit C**, none of methionine, cysteine, or cystine were detected in the corn flour at a limit of quantitation of 50 ppm. 50 ppm of methionine (molecular weight of 149) corresponds to a concentration of 0.3 mM, 50 ppm of cysteine (molecular weight of 121) corresponds to a concentration of 0.4 mM, and 50 ppm of cystine (molecular weight of 240) corresponds to a concentration of 0.2 mM. Therefore, methionine, cysteine and cystine, as free

amino acids, are present in the corn flour at a concentration of less than 0.3 mM, 0.4 mM and 0.2 mM, respectively, or less than 0.9 mM in total.

16. In a letter dated November 23, 2016 (**Exhibit D**), Alphalyse confirmed their results in respect to cysteine/cystine, stating that their analysis "did not find any trace of free Amino Acids, including cysteine/cystine" in the corn flour.

17. The methods utilized by Silliker and Alphalyse did not specifically detect for the presence of thiamine. Thiamine, however, is known to be present in white masa flour (e.g., white corn flour) at a level of 0.22 mg/100 g flour (see Suri et al., 2016, Comprehensive Reviews in Food Science and Food Safety, 15:912-926, see pg. 915; **Exhibit E**), which corresponds to a concentration of 8 micromolar (μM) thiamine in corn flour.

18. To further examine the composition of the masa flour, and, particularly, the sulfur-containing free amino acid composition of the masa flour, I weighed and then placed about 100 grams of the corn flour into three different clean Ziplock bags and shipped the bags to the Proteomics & Mass Spectrometry Facility at the Donald Danforth Plant Science Center (referred to hereafter as "Danforth") on December 13, 2016. Danforth is located at 975 North Warson Road in St. Louis, Missouri.

19. I instructed Danforth to analyze the elemental composition of the corn flour. Specifically, I instructed Danforth to perform free amino acid analysis of the corn flour.

20. A copy of the Report prepared by Danforth is provided in **Exhibit F**. Included in the Report is a description of the method that was used to analyze the free amino acids in the corn flour. Danforth follows a validated sample preparation method appropriate for measurement of free fatty acids and a validated method to determine the amount of each free fatty acid. Danforth performs all of their analyses under good laboratory practice (GLP) conditions.

21. Briefly, Danforth extracted the free amino acids by treating the flour samples with several different extraction solvents as indicated, followed by treatment with a mild acid and chromatographic separation of the compounds contained within the resulting extract. The specific chromatographic conditions and the resulting chromatograms provided by Danforth also are shown in **Exhibit F**.

22. As shown in **Exhibit F**, the amount of cysteine in the three flour samples (i.e., 0.00000%, 0.00000% and 0.00000%) were all below the limit of quantitation for this assay (i.e., 0.00050%). For cysteine, which has a molecular weight of 121, the limit of quantitation corresponds to a concentration of 0.04 mM. Therefore, cysteine is present at a concentration of less than 0.04 mM in the corn flour.

23. As shown in **Exhibit F**, the amount of cysteic acid (Cya) in the three flour samples (i.e., 0.00000%, 0.00000% and 0.00000%) were all below the limit of quantitation for this assay (i.e., 0.00050%). For Cya, which has a molecular weight of 169, the limit of quantitation corresponds to a concentration of 0.03 mM. Therefore, Cya is present at a concentration of less than 0.03 mM in the corn flour.

24. As shown in **Exhibit F**, the amount of disulfide bonded cysteine (Cys-Cys), also known as cystine, in the three flour samples (i.e., 0.00000%, 0.00000% and 0.00000%) were all below the limit of quantitation for this assay (i.e., 0.00050%). For Cys-Cys, which has a molecular weight of 240, the limit of quantitation corresponds to a concentration of 0.02 mM. Therefore, Cys-Cys is present at a concentration of less than 0.02 mM in the corn flour.

25. As shown in **Exhibit F**, the amount of methionine in the three flour samples (i.e., 0.00041%, 0.00037% and 0.00040%) were all below the limit of quantitation for this assay (i.e., 0.00050%). For methionine, which has a molecular weight of 149, the limit of quantitation corresponds to a concentration of 0.03 millimolar (mM). Therefore, methionine is present at a concentration of less than 0.03 mM in the corn flour.

26. As shown in **Exhibit F**, the amount of methionine sulfoxide (MetS) in the three flour samples (i.e., 0.00000%, 0.00000% and 0.00000%) were all below the limit of quantitation for this assay (i.e., 0.00050%). For MetS, which has a molecular weight of 165, the limit of quantitation corresponds to a concentration of 0.03 mM. Therefore, MetS is present at a concentration of less than 0.03 mM in the corn flour.

27. In summary, based on the analyses described above and the published literature, it is my opinion that the corn flour tortilla of Proulx contains no more than 0.15 mM of free amino acids that contain sulfur (i.e., methionine, cysteine, disulfide bonded cysteine, cysteic acid, and methionine sulfoxide).

28. 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: 20th July 2017

S. Christopher Davis, Ph.D.