

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor :	Rachel Fraser	Art Unit :	1791
Serial No. :	14/797,006	Examiner :	Elizabeth A. Gwartney
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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, Devin Grant Peterson, PhD, hereby declare as follows:

1. I am currently a Professor in the Department of Food Science and Technology at the Ohio State University in Columbus, Ohio. I have held this position since early 2016. I have worked and taught in the field of Food Science since 2001. Prior to my current position, I held Professor/Associate Professor positions at the University of Minnesota in Saint Paul, Minnesota from 2009 to 2016; and was a faculty member in the Department of Food Science at the Pennsylvania State University in University Park, Pennsylvania from 2001 to 2009. In addition, I have been the Director of the Flavor Research and Education Center for the past seven years, at both the University of Minnesota and the Ohio State University.

2. I am a nationally recognized expert and scholar in the field of flavor science. In 2015, I received the Excellence in Flavor Science Award from the Flavor and Extract Manufacturers Association. In 2008, I received a Pan-American Nutrition, Food Science and Technology Award. In 2004, during my tenure at Pennsylvania State University, I was awarded the Presidential Early Career Award for Scientists and Engineers (PECASE), which was presented by the White House Office of Science and Technology Policy.

3. I received a Bachelor's of Science (B.S.) degree in Food Science in 1994 from the University of Manitoba in Winnipeg, Manitoba. I earned a Master's of Science (M.S.) degree in 1997 and a Doctor of Philosophy (Ph.D.) degree in 2001 in Flavor Chemistry from the Department of Food Science from the University of Minnesota in Saint Paul, Minnesota. I was named to the Dean's List during my academic career at both the University of Manitoba and at the University of Minnesota. While I was obtaining my Ph.D., I completed a six-month Internship as a Research Flavor Scientist at Givaudan Flavors in Cincinnati, Ohio.

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

5. My research focuses on food flavor and related chemistry, with an emphasis on identification of flavor stimuli (e.g., taste, aroma, chemesthetic, mouthfeel), pathways of formation and degradation of flavor stimuli, taste and aroma modulation, and mechanisms of delivery.

6. In preparing my Declaration, I have reviewed U.S. Patent Application No. 14/797,006 ("the '006 application"), as well as the Office Action mailed June 6, 2016, in which the claims of the '006 application were rejected as being anticipated by Proulx et al. (2006, J. Agric. Food Chem., 54:1518-22) ("Proulx") and as being obvious considering Hsieh et al. (US 2012/0093994) ("the '994 application") in view of Proulx. I also reviewed the Declaration under 37 CFR § 1.132 of Dr. Celeste Holz-Schietinger ("the Holz-Schietinger Declaration"). A list of the materials I reviewed in preparing this Declaration is provided in **Exhibit B**.

7. In forming my opinion, I reviewed and considered Applicants' experiments related to their discovery and demonstration that the addition of heme protein (in some cases as

leghemoglobin or LegH) surprisingly contributes to the development of meaty aroma in a "ground beef-like food product" comprising no animal products. These experiments are described in the '006 application and in the Holz-Schietinger Declaration.

8. Flavor is a complex sensation, consisting of different modalities such as olfaction (aroma), gustation (sweet, sour, salty, bitter, umami), and chemesthesis (heat, cooling, pain), as well as others. The aroma attributes are known to be a key component of flavor perception and have been the most widely studied by flavor chemists.

9. In my opinion, the scientific protocols in the '006 application and in the Holz-Schietinger Declaration for a) sample preparation and treatment; and b) chemical analysis of aqueous thermal reaction samples are reasonable and consistent with methods that would be used by skilled individuals in the art. Each of these aspects are reviewed in more detail below.

10. The Holz-Schietinger Declaration describes chemical analysis of aqueous thermal reaction samples using art recognized instrumental aroma analysis methods, specifically HS-SPME combined with GC-MS, to monitor the effect of LegH addition on the formation of volatile compounds, specifically those with meaty aroma characteristics, during heating (i.e., cooking). The objective was to demonstrate that LegH was capable of promoting or catalyzing the formation of meaty aroma compounds via aqueous thermal reactions between ribose and cysteine. Three flavor reaction systems were compared, consisting of: 1) 1% (10 mg/mL) LegH alone (**LegH control**), 2) 50 mM ribose + 50 mM cysteine alone (**Rib + Cyst**) and 3) 1% (10 mg/mL) LegH + 50 mM ribose + 50 mM cysteine (**LegH + Rib + Cyst**). The above reaction mixtures were diluted in a buffer (50 mM potassium phosphate and 100 mM sodium chloride)

and adjusted to pH 6.5, which is near the physiological pH of ground beef. A 2 mL aliquot of each reaction mixture was transferred to sample vials (sealed) which were subsequently heated (150°C for 3 min) and then analyzed by HS-SPME-GC-MS.

11. The concentrations (i.e. 0.1 to 50 mM) of flavor precursors (i.e., ribose and cysteine) of the aqueous thermal reaction samples described in the '006 application and the Hotz-Schietinger Declaration are consistent with peer-reviewed publications that have used similar aqueous reaction samples to investigate the thermal generation of volatile aroma compounds contributing to the flavor sensation of a food product. While aqueous thermal reaction samples are simplistic compared to food matrices, they provide relevant guidance and generalized outcomes for understanding the influence of flavor precursors on the formation of volatile compounds contributing to aroma, and to the flavor sensation generally in a number of different food products.

12. For example, a widely utilized and referenced publication for understanding the impact of flavor precursor composition on the formation of volatile aroma compounds generated therefrom is Lane and Nursten, "Variety of odors produced in Maillard model systems and how they are influenced by reaction conditions" in Maillard Reaction in Foods and Nutrition, Waller and Feather, eds., ACS Symposium, Series No. 215, pp 141-158. This publication describes aqueous combinations of sugar and amino acid mixtures heated under different time and temperature conditions. Lane and Nursten provides directional guidance for flavor development, and how flavor is impacted by flavor precursors, in various types of food matrices.

13. It is my opinion that the experimental approach and subsequent data analysis techniques described in the Holz-Schietinger declaration are appropriate and acceptable for the purpose of evaluating and comparing aroma compound formation in the three aqueous thermal reaction systems under heating conditions. The aqueous thermal reaction samples described in the Holz-Schietinger Declaration are comparable with those described by Lane and Nursten. It is also my opinion that these conditions would simulate the cooking of a "ground beef-like food product" containing no animal products as described in the '006 application, and that the data obtained from the experimental approach would be reasonably predictive of the expected aroma compound formation from a "ground beef-like food product" containing no animal products and including the flavor reaction system components LegH, ribose, and cysteine.

14. It is well known that thermal generation of volatile aroma compounds in an aqueous reaction system is influenced by reaction temperature and pH. When utilizing aqueous reaction samples, the reaction systems often are sealed so they can be pressurized, which increases the temperature of the sample to simulate temperatures in excess of 100°C, which would be experienced with conventional food heating methods such as baking, grilling or pan-frying. The aqueous thermal reaction samples described in the '006 application and in the Holz-Schietinger Declaration were heated in sealed vials at 150°C, which would be consistent with temperatures experienced by common meat preparation (heating) techniques.

15. My research program has similarly utilized the benchtop autosampler apparatus utilized in the '006 application and the Holz-Schietinger Declaration to rapidly heat sealed vials of aqueous samples to between 100-150°C for the purpose of evaluating and comparing aroma

compound formation. See, for example, Kokkinidou and Peterson, 2013, *Food Funct.*, 4:1093-104.

16. The slightly acidic pH value of 6 - 6.5 for the aqueous thermal reaction samples used in the '006 application and the Holz-Schietinger Declaration also are consistent with the typical intrinsic pH values of meat products, as well as with peer-reviewed publications evaluating flavor development, and how it is impacted by flavor precursors, in various types of food matrices, including in meat food products.

17. It is my opinion that the experimental approach and analytical methods used in the '006 application and the Holz-Schietinger Declaration for evaluating and comparing aroma compound formation in aqueous thermal reaction samples were consistent with those used by flavor scientists of ordinary skill in the art. For example, the headspace solid phase micro-extraction (SPME) sampling technique coupled with GCMS utilized in the '006 application and the Holz-Schietinger Declaration has been widely utilized for characterizing the aroma of foodstuffs, such as meat food products. See, for example, Elmore et al., 2001, "A comparison of headspace entrainment on Tenax with solid phase micro-extraction for the analysis of the aroma volatiles of cooked beef," in *Headspace Analysis of Foods and Flavors*; Rouseff and Cadwallader, eds, Springer. Indeed, more than 100 articles are reported in SciFinder when the key words "meat, aroma, SPME" are set as filters to screen the existing scientific literature.

18. It is well known that the aroma profiles of meat food products are impacted by sulfur-containing compounds (typically, reaction products of sulfur-containing amino acids) and lipid degradation/oxidation products that are generated during heating. The different chemical

classes of volatile compounds formed in the aqueous thermal reaction samples of the '006 application and the Holz-Schietinger Declaration are consistent with volatile aroma compounds reported in the literature evaluating volatile compound formation contributing to meat aroma and flavor sensation. See, for example, Elmore et al. (2002, *J. Agric. Food Chem.*, 50:1126-32); Chen et al. (2003, "Influence of DNA on Volatile Generation from Maillard Reaction of Cysteine and Ribose," *in* *Nutraceutical Beverages*, ACS Symposium Series Vol 871); Mottram and Nobrega (2002, *J. Agric. Food Chem.*, 50(14):4080-86); Schieberle and Hofmann ("Characterization of Key Odorants in Dry-Heated Cysteine—Carbohydrate Mixtures: Comparison with Aqueous Reaction Systems," *in* *Flavor Analysis*, ACS Symposium Series, Vol 705); 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 Shu-C and Ho (1989, "Parameter Effects of the thermal reaction of cysteine and 2,5-dimethyl-4-hydroxy-3(2H)-furanone," *in* *Thermal Generation of Aromas*, ACS Symposium Series, Vol 409).

19. In my opinion, Applicant's experiments in the '006 application and the Holz-Schietinger Declaration demonstrate that the addition of LegH to an aqueous thermal reaction mixture comprising ribose and cysteine has the surprising result of enhancing the formation of volatile compounds associate with a meaty aroma. Significantly, the 2,851-fold increase in concentration of 2-acetylthiazole when LegH was combined with Ribose and Cysteine, which is shown in the Holz-Schietinger Declaration, in my opinion signifies that novel and unexpected chemical reactions occur in the presence of LegH. 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).

20. Yield changes of 0.5- to 10-fold would be consistent with optimizing a reaction pathway, however, changes of greater than 1000-fold, as is seen with 2-acetylthiazole, indicate that new pathways of formation for meaty aromas result from the inclusion of LegH in the aqueous thermal reaction mixture.

21. Additionally, the 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, Kerschler 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.

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Track 1

22. 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 2, 2016



Devin Grant Peterson, Ph.D.

EXHIBIT A

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, Brownd 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>
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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/jf501919v>
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/jf501919v>
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>
19. Ferruzzi, M.G., **Peterson, D.G.**, Singh, R.P. Schwartz, S.J. and *Freedman, M.* 2012. Nutritional Translation Blended With Food Science: 21st Century Applications. Adv. Nutr. 3: 813–819. <http://advances.nutrition.org/content/3/6/813>
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.
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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>
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Patents (inventor):

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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

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