JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Determination of Toxic α-Dicarbonyl Compounds, Glyoxal, Methylglyoxal, and Diacetyl, Released to the Headspace of Lipid Commodities upon Heat Treatment

Yaping Jiang,[†,§] Matt Hengel,[†] Canping Pan,[‡] James N. Seiber,[†] and Takayuki Shibamoto*[,†]

[†]Department of Environmental Toxicology, University of California, Davis, California 95616, United States

[‡]College of Science, China Agricultural University, Beijing 100094, China

ABSTRACT: Toxic α-dicarbonyl compounds, glyoxal, 2-methylglyoxal, and diacetyl, released from the headspace from butter, margarine, safflower oil, beef fat, and cheese heated at 100 and 200 °C were analyzed by gas chromatography as quinoxaline derivatives. Total amounts of α-dicarbonyl compounds ranged from 40.5 ng/g (butter) to 331.2 ng/g (beef fat) at 100 °C and from 302.4 ng/g (safflower oil) to 4521.5 ng/g (margarine) at 200 °C. The total amount of α-dicarbonyl compounds increased approximately 55- and 15-fold in the headspace of heated butter and margarine, respectively, when the temperature was increased from 100 to 200 °C. However, only slight differences associated with temperature variation were observed in the cases of safflower oil and beef fat (1.3- and 1.1-fold, respectively). Diacetyl was found in the highest amounts among all samples, ranging from 13.9 ± 0.3 ng/g (butter) to 2835.7 ng/g (cheese) at 100 °C and from 112.5 ± 102 ng/g (safflower oil) to 2274.5 ± 442.6 ng/g (margarine) at 200 °C, followed by methylglyoxal, ranging from 13.0 ± 0.5 to 112.7 ± 10.1 ng/g (cheese) at 100 °C and from 34.7 ± 5.0 ng/g (safflower oil) to 1790 ± 372.3 ng/g (margarine) at 200 °C. Much less glyoxal formed, in amounts ranging from 13.6 ± 0.7 ng/g (butter) to 53.4 ± 11.2 ng/g (beef fat) at both temperatures. The amounts of α-dicarbonyl compounds released into the vapor phase from lipid commodities during heating were satisfactorily analyzed.

KEYWORDS: *α-dicarbonyl compounds, diacetyl, glyoxal, headspace, heated butter and cooking oils, methylglyoxal*

INTRODUCTION

There have been many studies on volatile chemicals formed in foods and beverages. These chemicals have been investigated from many different viewpoints, including flavor, taste, antioxidant, anti-inflammatory properties, and antimicrobial qualities as well as from the perspective of toxicity associated with various diseases.[1] Among over 1000 volatile chemicals identified in cooked foods, the analysis of low molecular weight carbonyl compounds, such as acetaldehyde, acrolein, glyoxal, methylglyoxal, and diacetyl, formed in lipids or lipid-rich foods is one of the most difficult procedures because they are highly volatile, reactive, and water-soluble.[2] However, determination of these chemicals in foods is extremely important to adequately assess food safety.

α-Dicarbonyl compounds, including glyoxal, methylglyoxal, and diacetyl, are known to form from lipids upon oxidation[3,4] and are also known as important precursors of heterocyclic flavor chemicals in the Maillard reaction.[5] It has recently been reported that the release of these chemicals from foods during cooking plays an important role in consumer evaluations of the acceptability of a food because some of these chemicals may be toxic by inhalation and cause adverse effects to humans.[6,7]

Inhalation of glyoxal vapor reportedly causes local irritation of the eyes and respiratory organs as well as hyperemia and foamy secretions in the lungs, and oral exposure to glyoxal may produce gastrointestinal irritation and congestion in the gastrointestinal tract, lung, kidney, and adrenal glands.[8] Glyoxal also has potential tumor-promoting activities.[8,9] Methylglyoxal, which is a metabolic byproduct of glycolysis formed during food processing, was found in food products of both animal and plant origin.[10]

Diacetyl is listed in the FDA GRAS (Generally Regarded As Safe) List and has been used as a flavoring primarily in butter, popcorn, caramel, coffee, and cream soda.[11] It is also present in natural products such as essential oils and fruits.[12] A recent review describes the possible toxic effects of diacetyl related to lung disease.[13] After NIOSH investigated lung disease associated with the butter flavoring used in popcorn,[14] the safety level of diacetyl in ambient air has been targeted for some restriction by scientists.[15] These findings indicate that determining levels of food-associated chemicals in the vapor phase is important to a comprehensive assessment of their toxicity.

In the present study, vapor phase glyoxal, methylglyoxal, and diacetyl formed from heated lipids and a cheese were analyzed by GC-NPD to assess their role in toxicity to consumers, chefs, and others involved in food preparation and consumption.

MATERIALS AND METHODS

Chemicals and Reagents. Glyoxal (ethanedial), 2-methylglyoxal (2-oxopropanal), diacetyl (butane-2,3-dione), *o*-phenylenediamine dihydrochloride, quinoxaline, 2-methylquinoxaline, and 2,3-dimethylquinoxaline were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Sodium hydroxide, anhydrous sodium sulfate, dichloro-

Received: November 6, 2012
Revised: January 11, 2013
Accepted: January 14, 2013
Published: January 14, 2013

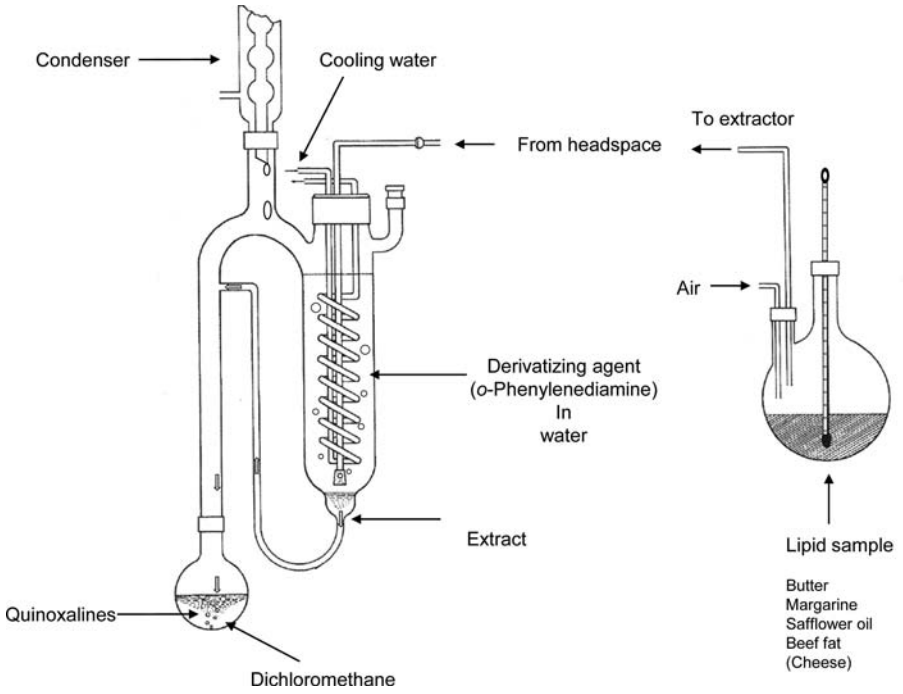

Figure 1. Apparatus used to extract quinoxalines from the headspace of heated lipid samples.

methane, and ethyl acetate were bought from Fisher Scientific (Fair Lawn, NJ, USA).

Commodity Samples. Lipid commodities used were butter, margarine, safflower oil, and beef fat. One kind of cheese (American cheese) was used as a lower lipid food sample. Butter, margarine, safflower oil, and cheese samples were purchased from a local market and used immediately.

Beef Fat. The beef fat was obtained from the renal periphery of the beef carcass, bought from the Department of Animal Science, University of California, Davis, CA USA. The fat tissue was ground with small amounts of dry ice in a blender. The pulverized, solidified fat was heated at 70−80 °C. The nonfat materials denatured were removed from the melted fat. The refined beef fat was stored at 4 °C until used.

Sample Preparation. A lipid commodity sample (50 g) was placed in a three-neck round-bottom flask (1 L). The flask was connected with glass tubing to a simultaneous purging and solvent extractor as shown in Figure 1.[16] Each sample was heated at 100 and 200 °C for 1 h. The volatiles formed in the headspace were purged by a purified air stream (7.2 mL/min) into a 250 mL aqueous solution (pH, 12) containing 1.5 g of *o*-phenylenediamine dihydrochloride. The temperature reached the set point within 5 min in all samples, but the purging started at room temperature.

The quinoxaline derivatives, quinoxaline, 2-methylquinoxaline, and 2,3-dimethylquinoxaline, formed in the water trap were simultaneously and continuously extracted with 100 mL of dichloromethane for 3 h. The dichloromethane extract was dried over anhydrous sodium sulfate for 12 h. After sodium sulfate was filtered off, dichloromethane was removed by a rotary flash evaporator. The residual sample was dissolved in 0.5 mL of ethyl acetate, and then 20 μL each of 2-methylpyrazine (508 mg/L) and benzothiazole (95.8 mg/L) ethyl acetate solutions were added as internal standards. The sample solution was adjusted to exactly 1.0 mL by purging with purified nitrogen gas. The ethyl acetate sample solution (2 μL) was injected into a gas chromatograph equipped with a nitrogen−phosphorus detector (GC-NPD) for analysis of quinoxalines. The experiments were repeated three times.

Analysis of Headspace Samples. An Agilent model HP 6890 series gas chromatograph (GC) equipped with a 30 m × 0.25 mm i.d. DB-WAX fused silica capillary column and a NPD was used for the quantitative analysis of quinoxalines. The oven temperature was held at 40 °C for 2 min and then programmed to rise to 170 °C at 4 °C/min and held for 15 min. Helium carrier rate was 1.5 mL/min. The injector was operated at 260 °C in splitless mode. The detector temperature was 300 °C.

Identification of quinoxaline, 2-methylquinoxaline, and 2,3-dimethylquinoxaline in a sample was performed using an HP model 6890 gas chromatograph interfaced to a 5973 mass selective detector (GC-MS). The GC column and conditions were the same as those described above. Chemicals in the headspace sample were identified by comparison with the Kovats gas chromatographic retention index I and by the mass spectral fragmentation pattern of each component compared with those of authentic compounds.

Preparation of a Standard Curve for Quantitative Analysis. The standard calibration curves for the three quinoxaline derivatives were prepared at eight concentrations (0.5, 1, 5, 10, 50, 100, 500, and 1000 mg/L) using standard ethyl acetate solutions. The curves were prepared according to the internal standard method (508 mg/L of 2-methylpyrazine and 95.8 mg/L of benzothiazole).[17]

Recovery Tests for Glyoxal, 2-Methylglyoxal, and Diacetyl. Standard glyoxal, methylglyoxal, and diacetyl (4 μg/g each) were spiked into a 50 g butter sample at room temperature in the apparatus shown in Figure 1, and then the sample was heated at 100 °C for 1 h. The purging conditions were the same as those of food commodity samples. The three derivatives recovered were quantitatively analyzed according to the method described above using the calibration curves. The experiments were repeated three times. Butter was used as a representative sample.

To examine the reaction yields and extraction efficiencies, glyoxal, methylglyoxal, and diacetyl were spiked (0.02 and 0.1 mg/L) to an aqueous solution of *o*-phenylenediamine (1.5 g/250 mL) in the water trap. The trap was extracted with 100 mL of dichloromethane for 3 h. The three derivatives recovered were quantitatively analyzed as described above. The experiments were repeated three times.

Statistical Processing. The results of the present study were averaged, and the comparison between experimental groups was drawn through an ANOVA based on the SAS system. After the ANOVA, the level of significance was computed using Duncan's multiple-range test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

In the present study, the widely used derivative quinoxalines for α-dicarbonyl compounds analysis were used.[2] The limit of detection (LOD) was 0.5 ng for quinoxaline, 1.0 ng for 2-methylquinoxaline, and 0.3 ng for 2,3-dimethylquinoxaline. The limit of quantitation (LOQ) was 1.32 ng for quinoxalines, 1.04 ng for methylquinoxaline, and 1.54 ng for 2,3-dimethylquinoxaline. Linearity values (R^2) of standard curves for quantitative analysis were 0.9938−0.9997 for quinoxaline, 0.9987−0.9999 for 2-methylquinoxaline, and 0.9925−0.9999 for 2,3-dimethylquinoxaline.

Diacetyl exhibited the greatest recovery efficiency (75.6 ± 4.5%), followed by 2-methylglyoxal (70.9 ± 3.8%) and glyoxal (66.3 ± 11.6%), from the headspace of the spiked butter sample heated at 100 °C for 1 h. The amounts of the three α-dicarbonyl compounds formed in the headspace of butter heated at 100 °C for 1 h (blank) were subtracted from each corresponding compound formed in the spiked sample for the recovery calculation.

Results of the recovery tests from an aqueous solution containing *o*-phenylenediamine are shown in Table 1. The recoveries of glyoxal and methylglyoxal as quinoxalines derivatives in an aqueous solution (250 mL) were >75%, except that diacetyl spiked with 20 μg/L had 62.7% recovery.

Table 1. Recovery Efficiencies of Glyoxal, Methylglyoxal, and Diacetyl from Aqueous Solutions as Corresponding Quinoxaline Derivatives[a]

	amount spiked	
	20 μg/g	100 μg/g
glyoxal	118.5 ± 1.4	75.4 ± 11.8
methylglyoxal	78.1 ± 5.4	77.7 ± 1.9
diacetyl	62.7 ± 8.2	75.6 ± 4.5

[a]Values are the mean ± RSD (n = 3).

A typical gas chromatogram of a headspace sample obtained from a butter sample heated at 200 °C is shown in Figure 2. The peak at the retention time of 33.3 min is quinoxaline, that at 35.1 min is 2-methylquinoxaline, and that at 38.2 min is 2,3-dimethylquinoxaline. Peaks at 36.6 and 39.6 min are tentatively identified as 2-ethylquinoxaline (formed form ethylglyoxal) and 2-ethyl-3-methylquinoxaline (formed from 2,3-pentadione), respectively; positive identification was not attempted due to lack of standard chemicals.

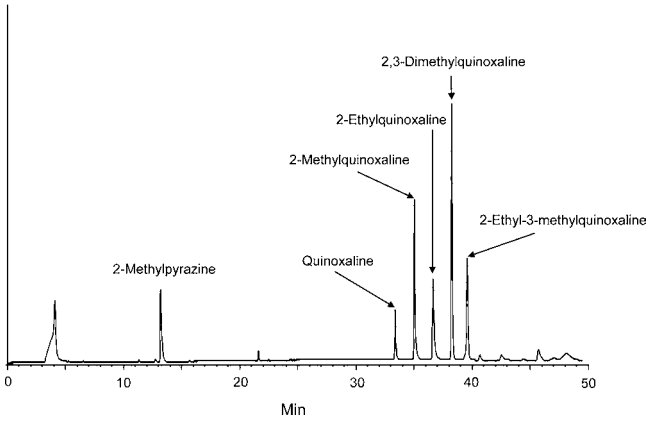

Figure 2. Typical gas chromatogram of a headspace sample obtained from butter heated at 200 °C for 1 h.

Figures 3 and 4 show the amounts of glyoxal, 2-methylglyoxal, and diacetyl found in the headspace of lipid commodities heated at 100 and 200 °C, respectively, for 1 h. The values are the mean ± SD (n = 3). Generally, amounts of α-dicarbonyl compounds found in the vapor phase of heated lipid commodities varied across the sample type and temperature. Total amounts of α-dicarbonyl compounds ranged from 40.5 ng/g (butter) to 331.2 ng/g (beef fat) at 100 °C and from 302.4 ng/g (safflower oil) to 4521.5 ng/g (margarine) at 200 °C, indicating that the formation of α-dicarbonyl compounds was positively correlated with the heating temperatures. The total amount of α-dicarbonyl compounds increased approximately 55- and 15-fold in the headspace of heated butter and margarine, respectively, when the temperature was increased from 100 to 200 °C. However, only slight differences due to temperature variation were observed in the cases of safflower oil and beef fat (1.3- and 1.1-fold increases, respectively). It is interesting that the total amount of α-dicarbonyls found in butter at 100 °C (40.5 ng/g) was much less that those in the other lipid samples (238.8−331.2 ng/g) at 100 °C, whereas it was much greater (2207.0 ng/g) than those in safflower oil (302.4 ng/g) and beef fat (347.4 ng/g) at 200 °C. This may be

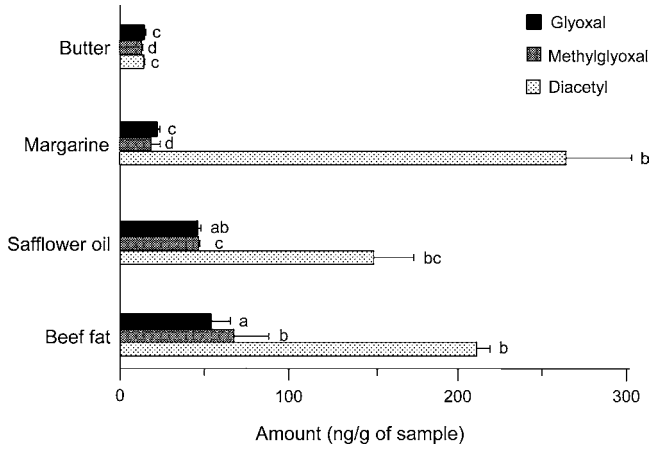

Figure 3. Amounts of α-dicarbonyl compounds recovered from lipid samples heated at 100 °C.

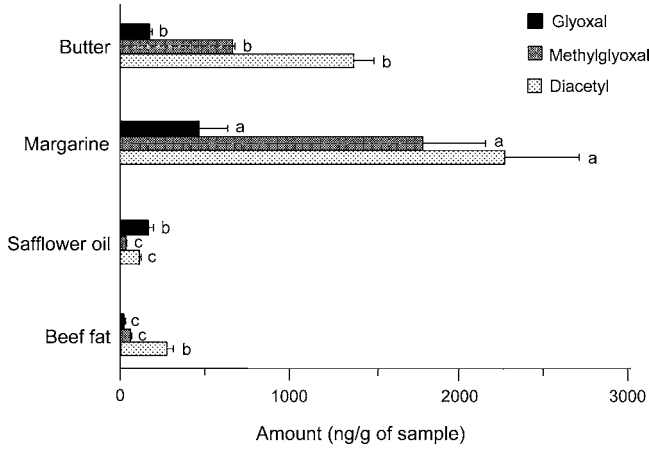

Figure 4. Amounts of α-dicarbonyl compounds recovered from lipid samples heated at 200 °C.

dx.doi.org/10.1021/jf3047303 | J. Agric. Food Chem. 2013, 61, 1067−1071

because of the different levels of saturated and unsaturated fatty acids in lipid samples. Generally, more energy is required for degradation of a fatty acid with less unsaturation than one with more unsaturation because more reaction steps are needed for fatty acid with unsaturated C−C bonds to degrade into lower molecular weight compounds.[18] The mechanisms of lipid oxidation including autoxidation, photooxidation, and thermal oxidation are well-established. Under normal conditions, the autoxidation of fatty acids occurs slowly. However, thermal oxidation of lipids, the conditions used in the present study, occurs quickly and may involve different mechanisms from those of autoxidation because the conditions of thermal oxidation are much more intense than those of autoxidation.[19] The heat treatment used in the present study, in particular at 200 °C, may have sufficient energy to produce various low molecular weight radicals, including $^{\bullet}CH_3$, $^{\bullet}CO$, and $^{\bullet}CHO$, which will subsequently produce OHC−CHO (glyoxal), OCH−CO (CH_3) (methylglyoxal), and (CH_3)CO−CO (CH_3) (diacetyl) rather than going through the normal autoxidation mechanisms.[20]

Butter, which contains the highest proportion of saturated fatty acid (60−80%) of the tested lipid samples,[21] produced the least amount of α-dicarbonyls at 100 °C. However, the results in the present study showed that the composition of unsaturated fatty acids was not correlated to the amount of α-dicarbonyl compound formation at 200 °C. For example, despite the fact that the proportion of unsaturated fatty acids was much higher in safflower oil (92.1%)[22] than in margarine (53.5%),[8] total α-dicarbonyl compound formation was much higher in margarine (4521.5 ng/g) than in safflower oil (302.4 ng/g) at 200 °C, suggesting that a high temperature, such as 200 °C, provides excess energy and is thus able to degrade lipids regardless of the levels of saturated or unsaturated fatty acids. Both saturated and unsaturated fatty acids degrade readily to form volatile carbonyls at high temperatures.[23] However, more research is needed to rationalize the relationships between the formation of α-dicarbonyl compounds and the composition of fatty acids in the lipid samples.

Amounts of glyoxal and methylglyoxal formed from each lipid at 100 °C were about equal. However, the amounts of their formation from each lipid at 200 °C were significantly different. Diacetyl was found in the greatest amounts in all samples, ranging from 13.9 ± 0.3 ng/g (butter) to 264.3 ± 38.8 ng/g (margarine) at 100 °C and from 112.5 ± 10.2 ng/g (safflower oil) to 2274.5 ± 442.6 ng/g (margarine) at 200 °C, followed by methylglyoxal, ranging from 13.0 ± 0.5 ng/g to 67.3 ± 20.5 ng/g (beef fat) at 100 °C and from 34.7 ± 5.0 ng/g (safflower oil) to 1790 ± 372.3 ng/g (margarine) at 200 °C. Glyoxal formed least, ranging from 13.6 ± 0.7 ng/g (butter) to 53.4 ± 11.2 ng/g (beef fat). In the case of safflower oil at 200 °C, glyoxal was produced more than the other two α-dicarbonyl compounds. In the case of butter, methylglyoxal (660.9 ± 14.5 ng/g) formation from the lipids was much higher than that of glyoxal (165.5 ± 27.1 ng/g) at 200 °C. On the other hand, methylglyoxal (34.7 ± 5.0 ng/g) formation was much less than that of glyoxal (155.2 ± 29.6 ng/g) at 200 °C in the case of safflower oil. The amounts of diacetyl found in the headspace of heated margarine in the present study were comparable to previously reported results.[24]

In the case of cheese, the sample started smoking before the temperature reached 100 °C and decomposition occurred when the temperature was raised above 100 °C. Therefore, sample preparation above 100 °C for cheese was impossible, and only the results obtained at 100 °C are reported. The amounts found in the headspace of cheese heated at 100 °C were 40.5 ± 4.8 ng/g for glyoxal, 112.7 ± 10.1 ng/g for methylglyoxal, and 2835.7 ± 163.6 ng/g for diacetyl. Cheese produced the greatest amount of total α-dicarbonyl compounds (2988.9 μg/kg) in the headspace of samples heated at 100 °C. The results suggest that these α-dicarbonyl compounds form also from nonlipid components, such as carbohydrate and protein. In fact, a previous paper has demonstrated that 15 samples of whole Ossolano cheese contained diacetyl at a mean concentration of 4.52 g/kg.[25]

The gas chromatographic method used for glyoxal, 2-methylglyoxal, and diacetyl analysis in the present study was highly selective, sensitive, and relatively simple, and the amounts of α-dicarbonyl compounds released into the vapor phase from lipid commodities during heating were satisfactorily analyzed. The results of the present study demonstrate that lipid-rich commodities can produce toxic α-dicarbonyl compounds during high-temperature treatments. The total amount of α-dicarbonyl compounds formed from lipid commodities significantly increases when the heat temperature reaches the smoke point. Toxic chemicals formed in food are subsequently released into the ambient air and can eventually be inhaled by people. There have been several reports of the analysis of α-dicarbonyl compounds in a whole food. For example, α-dicarbonyl compounds in various foods and beverages, such as soft drinks, coffee, vinegars, honey, and fried potatoes, were reported at levels of milligrams per liter or milligrams per kilogram.[26] However, there are few reports of the analysis α-dicarbonyl compounds in the headspace of heated foods. It should be noted that the assessment of possible adverse effects caused by inhaled chemicals formed from food should be done using data obtained from the analysis of chemicals in a headspace rather than in a food. However, toxicological evaluations of these chemicals were not within the scope of the present study.

AUTHOR INFORMATION

Corresponding Author

*Phone: +1 (530) 752-4523. Fax: +1 (530) 752-3394. E-mail: tshibamoto@ucdavis.edu.

Present Address

[§]College of Science, China Agricultural University, Beijing 100094, China.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank our colleague Hae Won Jang for her technical support.

REFERENCES

(1) Tanaka, A.; Horiuchi, M.; Umano, K.; Shibamoto, T. Antioxidant and anti-inflammatory activities of water distillate and its dichloromethane extract from licorice root (*Glycyrrhiza uralensis*) and chemical composition of dichloromethane extract. *J. Sci. Food Agric.* **2008**, *88*, 1158−1165.

(2) Shibamoto, T. Analytical methods for trace levels of reactive carbonyl compounds formed in lipid peroxidation systems. *J. Pharm. Biomed. Anal.* **2006**, *41*, 12−25.

(3) St. Angelo, A. J. Lipid oxidation in foods. *Crit. Rev. Food Sci. Nutr.* **1996**, *36*, 175−224.

(4) Vercelotti, J. R.; St. Angelo, A. J.; Spanier, A. M. Lipid oxidation in foods: an overview. In *Lipid Oxidation in Foods*; St. Angelo, A. J., Ed.; ACS Symposium Series 500; American Chemical Society: Washington, DC, 1992; pp 1−11.
(5) Wang, Y.; Ho, C.-T. Flavour chemistry of methylglyoxal and glyoxal. *Chem. Soc. Rev.* **2012**, *41*, 4140−4149.
(6) Odake, S.; Van Ruth, S. M.; Akuzawa, R. Flavor release of diacetyl and 2-heptanone from skimmed and full fat milk under mouth conditions. *Food Sci. Technol. Res.* **2006**, *12*, 256−260.
(7) Yoshikawa, T. Disease. In *Peroxide lipid in Biological Systems*; Uchiyama, M., Matsuo, M., Sagai, M., Eds.; Scientific Society Press: Tokyo, Japan, 1985; pp 289−313.
(8) Kielhorn, J.; Pohlenz-Michel, C.; Schmidt, S.; Mangelsdorf, I. *Glyoxal, Concise International Chemical Assessment Document 57*; World Health Organization: Geneva, Switzerland, 2004; http://whqlibdoc.who.int/publications/2004/924153057X.pdf (accessed Sept 24, 2012).
(9) Furihata, C.; Yoshida, S.; Matsushima, T. Potential initiating and promoting activities of diacetyl and glyoxal in rat stomach mucosa. *Jpn. J. Cancer Res.* **1985**, *76*, 809−814.
(10) Statement on methylglyoxal. Committee on toxicity of chemicals in food, consumer products and the environment. Committee on toxicity, 2009; http://cot.food.gov.uk/pdfs/cotstatementmg200904.pdf (accessed Sept 24, 2012).
(11) Arctander, S. *Perfume and Flavor Chemicals*; published by the author: Montclair, NJ, 1969; p 872.
(12) Esteve, M. J.; Frigola, A.; Rodrigo, M. C.; Rodrigo, M. Use of polarography as quality-control method for determining diacetyl in citrus and vegetable juices, yoghurt and butter. *Food Addit. Contam.* **2002**, *19*, 519−523.
(13) Kovacie, P.; Cooksy, A. L. Electron transfer as a potential cause of diacetyl toxicity in popcorn lung disease. *Rev. Environ. Contam. Toxicol.* **2010**, *204*, 133−148.
(14) *NIOSH Evaluates Worker Exposures at a Popcorn Plant in Missouri*; U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, DHIHS (NIOSH) Publication 2002-128; National Institute for Occupational Safety and Health: Cincinnati, OH, 2002.
(15) Egilman, D. S.; Schilling, J. H.; Menendez, L. A proposal for a safe exposure level for diacetyl. *Int. J. Occup. Environ. Health* **2011**, *17*, 122−134.
(16) Umano, K.; Shibamoto, T. Analysis of headspace volatiles from overheated beef fat. *J. Agric. Food Chem.* **1987**, *35*, 14−18.
(17) Ettre, L. S. Interpretation of analytical results. In *The Practice of Gas Chromatography*; Ettre, L. S., Zlatkis, A., Eds.; Interscience Publishers: New York, 1967; p 402.
(18) Katragadda, H. R.; Fullana, A.; Sidhu, S.; Carbonell-Barrachina, A. A. Emissions of volatile aldehydes from heated cooking oils. *Food Chem.* **2010**, *120*, 59−65.
(19) Schaich, K. M. Lipid oxidation: theoretical aspect. In *Bailey's Industrial Oil and Fat Products*, 6th ed.; Shahidi, F., Ed.; Wiley: New York, 2005; pp 269−355.
(20) Niyati-Shirkhodaee, F.; Shibamoto, T. Gas chromatographic analysis of glyoxal and methylglyoxal formed from lipids and related compounds upon ultraviolet irradiation. *J. Agric. Food Chem.* **1993**, *41*, 227−230.
(21) Bobe, G.; Zimmerman, S.; Hammond, E. G.; Freeman, A. E.; Porter, P. A.; Luhman, C. M.; Beitz, D. C. Butter composition and texture from cows with different milk fatty acid composition fed fish oil or roasted soybeans. *J. Dairy Sci.* **2007**, *90*, 2596−2603.
(22) Bozan, B.; Temelli, F. Chemical composition and oxidation stability of flax, safflower and poppy seed and seed oils. *Bioresour. Technol.* **2008**, *99*, 6354−6359.
(23) Frankel, E. N.; Neff, W. E.; Selke, E.; Weisleder, D. Photosensitized oxidation of methyl linoleate: secondary and volatile thermal decomposition products. *Lipids* **1982**, *17*, 11−18.
(24) Rincon-Delgadillo, M. I.; Lopez-Hernadez, A.; Wijaya, I.; Rankin, S. A. Diacetyl levels and volatiles profiles of commercial starter distillates and selected dairy foods. *J. Dairy Sci.* **2012**, *95*, 1128−1139.
(25) Zeppa, G.; Conterno, L.; Gerbi, V. Determination of organic acids, sugars, diacetyl, and acetoin in cheese by high-performance liquid chromatography. *J. Agric. Food Chem.* **2001**, *49*, 2722−2726.
(26) Degen, J.; Hellwig, M.; Henle, T. 1,2-Dicarbonyl compounds in commonly consumed foods. *J. Agric. Food Chem.* **2012**, *60*, 7071−7079.