Determination of Malonaldehyde and Formaldehyde Formed from Fatty Acid Ethyl Esters upon Microwave and Thermal Heating

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Aqueous fresh and frozen solutions of ethyl arachidonate, ethyl linolenate, and ethyl linoleate were exposed to microwave and thermal heating to induce lipid peroxidation. Malonaldehyde and formaldehyde were derivatized to 1-methylpyrazole with *N*-methylhydrazine and to thiazolidine with cysteamine, respectively, and were then quantitated with capillary gas chromatography with a nitrogen–phosphorus detector. For both heating methods, the malonaldehyde concentration depended on the degree of unsaturation in the fatty acid. The samples containing arachidonate produced the most malonaldehyde (7.8–11.2 nmol/mg), followed by those containing linolenate (2.9–5.3 nmol/mg) and linoleate (0.7–1.7 nmol/mg). There were no significant differences in the amounts of malonaldehyde formed between the heating methods for both fresh and frozen solutions. Ethyl arachidonate and ethyl linolenate produced about the same amount of formaldehyde (5.8–10.4 nmol/mg), whereas ethyl linoleate produced slightly less (3.2–5.3 nmol/mg) formaldehyde.

It has been reported that an estimated 80 million microwave ovens are in use today in the United States, and this figure continues to increase (Rubbright, 1990). Due to the convenience of microwave cooking, more consumers are using it in preference to traditional cooking methods. The effects of microwave and conventional heating on nutrient retention and the formation of flavors and color in foods have been compared (Mudgett, 1989). The current study was designed to compare the relationship between heating methods and the production of lipid peroxidation products in fatty acid ester model systems.

Polyunsaturated fatty acid ethyl esters were chosen as model systems in this study because triglycerides contain the ester form of fatty acids in foods. Fatty acids are usually found in the ester form in such biological materials as triglycerides, phosphatidylcholine, and phosphatidylethanolamine. Malonaldehyde (MA) and formaldehyde (FA) were chosen to investigate these products of lipid peroxidation because they are commonly known to affect the nutritional quality of foods (Frankel, 1987). These aldehydes also interact with proteins, nucleic acids, and other biological materials, which can lead to membrane and enzyme damage in cells and tissue and to atherosclerosis (Wilson, 1976).

Lipid peroxidation products formed from various foods under cooking conditions have been studied using the thiobarbituric acid (TBA) assay (Pikul et al., 1985). The TBA assay, however, is never specific to the product of interest (Namiki, 1990). In the present study, gas chromatography (GC) with a nitrogen–phosphorus detector (NPD) was used to analyze MA as a *N*-methylhydrazine (NMH) derivative, 1-methylpyrazole (1-MP) (Umano et al., 1988), and to analyze FA as a cysteamine derivative, thiazolidine (TH) (Hayashi et al., 1986).

EXPERIMENTAL PROCEDURES

Materials. The highest available grade from Sigma Chemical Co. (St. Louis, MO) of ethyl arachidonate, ethyl linolenate, ethyl linoleate, and butylated hydroxytoluene (BHT) was purchased and used without further purification. Sodium dodecyl sulfate (SDS) and cysteamine hydrochloride were purchased from Aldrich Chemical Co. (Milwaukee, WI). 2-Methylpyrazine, tributylamine, TH, and 1-MP standards were also obtained from Aldrich. NMH was purchased from Fluka Chemika-BioChemika (Ronkonkoma, NY). Baker Resi-analyzed dichloromethane and chloroform were purchased from J. T. Baker (Phillipsburg, NJ). Stock solutions of 2-methylpyrazine (150 μg/mL) and tributylamine (1.0 mg/mL) were prepared in chloroform for GC internal standards. A cysteamine solution (0.6 M) was prepared in deionized water.

Sample Preparation. *Oxidation of Fatty Acid Ethyl Esters with Thermal or Microwave Heating.* Model aqueous mixtures containing 10 or 20 μL of fatty acid ethyl ester, 200 μL of 5% SDS, and 5 mL of deionized water were placed in test tubes and subjected to microwave or steam heating. Microwave heating was conducted at the high setting of a 700-W microwave oven, and solutions were exposed for 10 and 15 s. After 10 s, the samples were immediately removed from the microwave, and their temperatures were approximately 65 °C. The samples given 15 s of irradiation boiled and were approximately 85 °C after immediate removal from the microwave. Thermal heating was accomplished by exposing the sample tubes in a steam bath for 3.5 and 4.5 min to reach temperatures of 85 and 100 °C, respectively.

Frozen samples were prepared by placing test-tube samples in 95% ethanol/dry ice for 3.5 min. These samples were exposed to either microwave or steam heating. Frozen samples took about 90 s to melt by microwave and started to boil approximately 7 s after melting (97 s total). Steam heating of the frozen samples required 3.5 and 4.5 min to reach 85 and 98 °C, respectively. Control samples were left at room temperature. All microwaved and thermal-heated samples were cooled in an ice bath, and 40 μL of BHT (4% in ethanol) was added to prevent further oxidation.

Derivatization of MA in the Samples. NMH (50 μL) was added to the oxidized and control samples and stirred for 1 h at room temperature. The solution was extracted with 15 mL of dichloromethane using a continuous liquid–liquid extractor for 3 h. The solution was saturated with NaCl prior to extraction. The extract was transferred to a volumetric flask and brought to 10 mL in dichloromethane. 2-Methylpyrazine stock solution (150 μL) was added as a GC internal standard. The solution was analyzed by GC with an NPD.

Derivatization of FA in the Samples. Cysteamine solution (150 μL) was added to the oxidized and control solutions, and the pH was adjusted to 8.5 with 1 N NaOH. The solution was stirred and allowed to react for 1 h at room temperature. The resulting solution was saturated with NaCl and extracted with

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Table I. MA and FA Concentrations in Fresh and Frozen Fatty Acid Ethyl Ester Samples after Microwave and Steam-Heat Treatments

sample	ethyl arachidonate[a]		ethyl linolenate[a]		ethyl linoleate[a]	
	MA	FA	MA	FA	MA	FA
control	2.3 ± 1.5	3.8 ± 0.1	1.3 ± 0.2	3.0 ± 0.2	0.2 ± 0.2	not detected
microwave heating						
fresh samples						
heated (10 s)	8.4 ± 0.6	7.0 ± 1.7	5.3 ± 1.4	6.4 ± 2.3	1.4 ± 0.9	5.3 ± 1.0
boiled (15 s)	9.0 ± 1.8	8.8 ± 2.1	4.0 ± 2.3	6.7 ± 2.2	1.7 ± 0.6	4.4 ± 0.9
frozen samples						
melted (90 s)	11.2 ± 1.0	8.6 ± 1.4	2.9 ± 0.8	8.4 ± 0.2	1.7 ± 0.1	5.2 ± 1.0
boiled (97 s)	8.5 ± 1.7	7.2 ± 2.2	3.5 ± 0.9	5.8 ± 3.3	1.4 ± 0.5	4.8 ± 0.6
thermal (steam) heating						
fresh samples						
heated (2.5 min)	9.7 ± 0.9	9.0 ± 1.8	4.1 ± 0.5	9.6 ± 0.7	0.7 ± 0.1	4.5 ± 0.4
boiled (3.0 min)	10.8 ± 2.4	6.4 ± 0.6	4.4 ± 0.8	9.4 ± 2.0	1.0 ± 0.1	4.3 ± 1.1
frozen samples						
heated (3.5 min)	7.8 ● 1.2	9.6 ± 2.2	3.9 ± 0.3	8.8 ± 2.0	1.2 ± 0.4	3.2 ± 0.6
boiled (4.5 min)	8.8 ± 1.2	6.1 ± 0.8	5.3 ± 1.2	10.4 ± 1.2	1.2 ± 0.5	3.6 ± 0.3

[a] Values are means (nmol MA or FA/mg fatty acid ethyl ester) ± standard deviations (n = 3).

20 mL of chloroform using a continuous liquid–liquid extractor for 5 h. The extract was transferred to a volumetric flask and brought to 10 mL in chloroform. Tributylamine (400 μL) was added as a GC internal standard. The solution was analyzed by GC with an NPD.

Instruments. A Hewlett-Packard (HP) Model 5880 GC equipped with an NPD and a 30 m × 0.25 mm i.d. DB-Wax bonded phase fused silica capillary column (J&W Scientific, Folsom, CA) was used for analysis of 1-MP. The oven temperature was held at 60 °C for 2 min, programmed to 190 °C at 4 °C/min, and held at 190 °C for 10 min. The injector and detector temperatures were 270 and 300 °C, respectively. Linear velocity of helium carrier gas was 35.5 cm/s. The injector split ratio was 30:1.

An HP 5890 GC equipped with an NPD and a 30 m × 0.25 mm i.d. DB-1 bonded phase fused silica capillary column (J&W Scientific) was used for the analysis of TH. The oven temperature was held at 80 °C for 2 min, programmed to 180 °C at 8 °C/min, and held at 180 °C for 10 min. The injector and detector temperatures were 250 and 300 °C, respectively. Linear velocity of helium carrier gas was 25.5 cm/s. The injector split ratio was 55:1.

Qualitative and Quantitative Analysis of 1-MP and TH. Authentic 1-MP and TH were used to prepare GC calibration curves for quantitative analysis of MA and FA, respectively (Ettre, 1967). 2-Methylpyrazine and tributylamine were used as GC internal standards for MA and FA determination, respectively.

An HP Model 5890 GC interfaced to a VG Trio II mass spectrometer with a VG 11-250 computer data system was used for mass spectrometric identification of the GC components. The ionization voltage was 70 eV, and the ion source temperature was 150 °C. The column and oven conditions for GC/MS were as described for the GC/NPD analysis.

RESULTS AND DISCUSSION

The amounts of MA and FA found in the fresh and frozen fatty acid ethyl ester samples exposed to either microwave or steam heating are shown in Table I. Microwaved and thermal-heated samples produced significantly higher amounts of lipid peroxidation products than the controls. Ethyl arachidonate produced the most MA (7.8–11.2 nmol of MA/mg of fatty acid ethyl ester), followed by ethyl linolenate (2.9–5.3 nmol/mg) and ethyl linoleate (0.7–1.7 nmol/mg). This finding is reasonable because arachidonate has four methylene interrupted double bonds that are susceptible to hydrogen abstraction at the C-7, C-10, and C-13 positions (Frankel, 1987). The presence of products containing multiple methylene interrupted double bonds can lead to further degradation of the remaining lipid to form other carbonyl compounds such as formaldehyde.

FA was detected as TH for the first time as a peroxidative product of fatty acid esters from microwave and thermal-heat exposure. The results indicate that ethyl arachidonate and ethyl linolenate produced slightly higher amounts (5.8–10.4 nmol of FA/mg of fatty acid ethyl ester) than ethyl linoleate (3.2–5.3 nmol/mg).

The amounts of MA and FA produced under microwave and thermal heating from fresh samples are also shown in Table I. Microwaving fresh samples for 10 s warmed the samples, whereas a 15-s microwave time caused the solution to boil. The results from the steam heating to 85 °C were used to compare the MA and FA production with the microwave-heated samples for 15 s. The amounts of MA and FA produced were the same for 10 s of microwave heating and thermal heating to 85 °C. Subjecting the sample to steam at 98 °C to examine the effect of the maximum temperature attained did not change the amounts of MA or FA formed. Samples subjected to thermal heating at the temperature equivalent of microwave irradiation did not show significant changes in MA and FA concentrations. The amount of MA produced in these systems is a function of the amount of saturation in the lipid.

Microwave and thermal heating methods produce varying results in cooked foods. Schiller et al. (1973) showed that egg yolks cooked in a conventional oven produced a TBA number that was twice that of egg yolks cooked in a microwave oven. However, the low-energy form of microwave radiation has not been known to induce lipid peroxidation. Rather, the heat created in microwave cooking causes lipid peroxidation. Myers and Harris (1975) investigated the effects of microwave and conventional heating on the fatty acid compositions of meats and poultry by GC analysis and reported no significant differences between the two heat treatments. In addition, Newburg and Concon (1980) concluded that MA concentrations are affected by cooking conditions when they detected 60- and 22-fold increases in MA from microwaved and boiled chicken, respectively, compared to raw chicken.

Frozen samples were used to simulate food-defrosting practices at home. THe results show similar trends for both frozen and fresh lipid-containing solutions. With the exception of the melting of the frozen arachidonate, the amounts of MA produced by different frozen polyunsaturated ethyl esters were similar to those of the fresh

samples (i.e., arachidonic > linolenate > linoleate). Pikul et al. (1985) reported that chicken meat frozen for 6 months had a significantly higher MA value after conventional-oven cooking than after microwave cooking. However, the type of cooking used for fresh chicken meat and for chicken meat that had been frozen for 3 months was less important and produced about the same amount of MA. These workers attributed the oxidative decomposition primarily to the storage time rather than the method of cooking. For our frozen samples, the fatty acid ethyl ester was microwaved or steam-heated immediately after being frozen. Our results show no differences in MA formation either between the fresh and frozen samples or between the two cooking methods, and the trends observed were similar to the results for either fresh or short-term frozen (3 months) samples reported by Pikul et al. (1985).

Previous reports show that MA is produced in large quantities from lipids oxidized by Fe^{2+}/H_2O_2 (Tamura and Shibamoto, 1991; Tamura et al., 1991) or irradiated by UV light (Dennis and Shibamoto, 1990). Both metal-induced and UV-irradiation-induced oxidization formed at least 10 times more FA than the heat treatments used in this study. The lower amounts of MA and FA formed from thermal and microwave exposure may be due to their high volatility, which allows them to escape from the system upon heating.

Although the model system used here is simpler than the complex and heterogeneous food system, the results of this work suggest that the amounts of MA and FA produced from microwave and steam heating are approximately the same. Actual foods contain amino acids, proteins, and other nucleophiles that may cause secondary reactions with MA and FA and thereby lower their presence. Further studies are necessary to understand lipid peroxidation induced by different types of cooking methods.

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LITERATURE CITED

Dennis, K. J.; Shibamoto, T. Gas Chromatographic Analysis of Reactive Carbonyl Compounds Formed from Lipids upon UV-Irradiation. *Lipids* **1990**, *25* (8), 460–464.

Ettre, L. S. *Practice of Gas Chromatography*; Ettre, L. S., Zlatkis, A., Eds.; Interscience: New York, 1967; p 402.

Frankel, E. Secondary Products of Lipid Oxidation. *Chem. Phys. Lipids* **1987**, *44*, 73–85.

Hayashi, T.; Reece, C. A.; Shibamoto, T. Gas Chromatographic Determination of Formaldehyde via Thiazolidine Derivative. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 101–105.

Mudgett, R. E. Microwave Food Processing. *Food Technol.* **1989**, 117–126.

Myers, S. J.; Harris, N. D. Effect of Electronic Cooking on Fatty Acids in Meats. *J. Am. Dietet. Assoc.* **1975**, *67*, 232–234.

Namiki, M. Antioxidants/Antimutagens in Foods. *CRC Crit. Rev. Food Sci. Nutr.* **1990**, *29* (4), 273–300.

Newburg, D. S.; Concon, J. M. Malonaldehyde Concentrations in Food are Affected by Cooking Conditions. *J. Food Sci.* **1980**, *45*, 1681–1687.

Pikul, J.; Leszcynski, D. E.; Kummerow, F. A. Oxidation Products in Chicken Meat After Frozen Storage, Microwave and Convection Oven Cooking, Refrigerated Storage and Reheating. *Poult. Sci.* **1985**, *64* (1), 93–100.

Rubbright, H. A. Looking into the Future of Microwave Technology. *Microwave World* **1990**, *11* (4), 19–23.

Schiller, E. A.; Pratt, D. E.; Reber, E. Lipid Changes in Egg Yolks and Cakes Baked in Microwave Ovens. *J. Am. Dietet. Assoc.* **1973**, *62*, 529–533.

Tamura, H.; Shibamoto, T. Gas Chromatographic Analysis of Malonaldehyde and 4-Hydroxy-2-(E)-nonenal Produced from Arachidonic Acid and Linoleic Acid in a Lipid Peroxidation Model System. *Lipids* **1991**, *26* (2), 170–173.

Tamura, H.; Kitta, K.; Shibamoto, T. Gas Chromatographic Analysis of Lipid Peroxidation Products Formed from Fatty Acids in a Fe^{2+}/H_2O_2 Oxidation System. *J. Agric. Food Chem.* **1991**, *31*, 439–442.

Umano, K.; Dennis, K. J.; Shibamoto, T. Analysis of Free Malondialdehyde in Photoirradiated Corn Oil and Beef Fat via a Pyrazole Derivative. *Lipids* **1988**, *23*, 811–814.

Wilson, R. B. Lipid Peroxidation and Atherosclerosis. *CRC Crit. Rev. Food Sci. Nutr.* **1976**, *7* (4), 325–338.

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