Formation of Genotoxic Dicarbonyl Compounds in Dietary Oils upon Oxidation

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ABSTRACT: Dietary oils—tuna, salmon, cod liver, soybean, olive, and corn oils—were treated with accelerated storage conditions (60°C for 3 and 7 d) and a cooking condition (200°C for 1 h). Genotoxic malonaldehyde (MA), glyoxal, and methylglyoxal formed in the oils were analyzed by GC. Salmon oil produced the greatest amount of MA (1070 ± 77.0 ppm of oil) when it was heated at 60°C for 7 d. The highest formation of glyoxal was obtained from salmon oil heated at 60°C for 3 d. More glyoxal was found from salmon and cod liver oils when they were heated for 3 d (12.8 ± 1.10 and 7.07 ± 0.19 ppm, respectively) than for 7 d (6.70 ± 0.08 and 5.94 ± 0.38 ppm, respectively), suggesting that glyoxal underwent secondary reactions during a prolonged time. The amount of methyglyoxal formed ranged from 2.03 ± 0.13 (cod liver oil) to 2.89 ± 0.11 ppm (tuna oil) in the fish oils heated at 60°C for 7 d. Among vegetable oils, only olive oil yielded methylglyoxal (0.61 ± 0.03 ppm) under accelerated storage conditions. When oils were treated under cooking conditions, the aldehydes formed were comparable to those formed under accelerated storage conditions. Fish oils produced more MA, glyoxal, and methylglyoxal than did vegetable oils because the fish oils contained higher levels of long-chain PUFA, such as EPA and DHA, than did the vegetable oils. A statistically significant correlation ($P < 0.05$) between the α-tocopherol content and the oxidation parameters was obtained from only MA and fish oils heated at 60°C for 3 d.

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It is well known that oxidative damage, in particular, lipid peroxidation, is strongly associated with various diseases (1). Also, the effects of lipid peroxidation on food quality and food safety have been reported (2,3). In addition, many reports have appeared on the toxicity of oxidized fats and the formation of toxic compounds from oxidized oils (4–6). For example, oxidized methyl linoleate, containing 4-hydroxy-2-nonenal as the major component, caused lymphocyte necrosis in the thymus and Peyer's patches in mice (7). Palm oil oxidized by heat caused reduced rates of pregnancy (by 55%) in rats (8). Lipid peroxidation yields reactive oxygen species (ROS) such as the hydroxyl radical, which leads to the formation of toxic chemicals from lipids, including malonaldehyde (MA). The toxicity of lipid peroxidative products is caused by the interaction of these secondary products, such as MA, rather than ROS directly, because ROS are not readily absorbed by the intestines (9).

Among the many products of lipid peroxidation, dicarbonyl compounds, such as MA, glyoxal, and methylglyoxal, have received much attention because they are implicated in various diseases (10,11). Pancreatic lesions consisting primarily of atrophied exocrine cells with loss of zymogen granulation occurred in 8-wk-old female Swiss mice that received 500 μg MA/g body weight (12). Methylglyoxal is also reported to have biological implications (13). Development of stomach neoplasms was observed in 6% of experimental animals (mice) that were administered 10 μg MA/g body weight (14). Methylglyoxal inhibited protein, DNA, and RNA synthesis in villus and crypt cells as well as colonocytes (15). A study using outbred male Wistar rats indicated that glyoxal exerts tumor-promoting activity on rat glandular stomach carcinogenesis (16). These reports clearly indicate that some dicarbonyl compounds produced from lipids by oxidation caused genotoxicities in experimental animals.

In the present study, genotoxic MA, glyoxal, and methylglyoxal formed in oxidized dietary oils were analyzed by GC to assess the role of these compounds in food safety.

MATERIALS AND METHODS

Chemicals and reagents. BHT, MA tetrabutylammonium salt, α-tocopherol, SDS, 1,2-phenylenediamine, and methylhydrazine were purchased from Sigma Chemical Co. (St. Louis, MO). Starch solution (1%) and 0.01 N sodium thiosulfate solution were bought from LabChem, Inc. (Pittsburgh, PA). Standard quinoxaline and 2-methylquinoxaline were synthesized by a previously reported method (17).

Dietary oils. Olive oil, soybean oil, corn oil, tuna oil, cod liver oil, and salmon oil were provided by Arista Industries, Inc. (Wilton, CT).

Oxidation of oils. An oil (1 g) was heated in an 8-mL amber vial at 60°C for 3 and 7 d using a thermostatic oven (Precision, Jouan, Inc., Winchester, VA). A tightly Teflon-sealed cap was used in an 8-mL amber vial to prevent the escape of volatile MA, glyoxal, and methylglyoxal during storage. The same oil was also heated to 200°C for 1 h under aerobic conditions. The oxidized samples were kept in a freezer (–5°C) until analysis.

Measurement of PV of oxidized oils. PV were measured according to AOAC method 965.33 with minor modifications

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Abbreviations: MA, malonaldehyde; 1-MP, 1-methylpyrazole; NPD, nitrogen phosphorus detector; ROS, reactive oxygen species; SPE, solid-phase extraction.

(18). An oil (100 μL) was mixed with 10 mL of chloroform/acetic acid (2:3, vol/vol) and 0.5 mL of aqueous saturated potassium iodide in a 25-mL Erlenmeyer flask. After the mixture was shaken for 1 min, deionized water (10 mL) and 0.5 mL of a 1% starch solution were added. The solution was titrated with 0.01 N sodium thiosulfate solution. A blank determination was conducted daily and was subtracted from a sample titration. The PV was calculated as

$$\text{milliequivalents to moles of } O_2 \text{ (mequiv } O_2\text{/kg)} = S \times N \times 1000\text{/g of sample} \quad [1]$$

where S = amount of sodium thiosulfate solution used (mL), and N = normality of thiosulfate solution.

Analysis of α-tocopherol in oils. An oil (100 μL) was dissolved into 0.5 mL of 2-propanol. The solution was placed in a C_{18} solid-phase extraction (SPE) cartridge (Varian, Harbor City, CA) and then eluted with 5 mL of methanol under reduced pressure using a vacuum manifold (Alltech Associates, Inc., Deerfield, IL). The SPE cartridge was preconditioned by rinsing with 1 vol each of ethyl acetate and methanol, in a series, prior to use. The eluent was concentrated under a purified nitrogen stream to 0.5 mL in volume. α-Tocopherol was analyzed by a Waters Model 501 HPLC equipped with a Capcell Pak C_{18} reversed-phase column (Shiseido Inc., Tokyo, Japan) and a UV detector (set at 290 nm). The flow rate of the methanol mobile phase was 1.0 mL/min with an isocratic mode. The sample injection volume was 20 μL.

Analysis of MA. MA was analyzed after it was derivatized into 1-methylpyrazole (1-MP) by a method slightly modified from previous reports (19–21). An aqueous solution (5 mL) containing 100 μL of an oxidized or nonoxidized oil (blank), 0.5 mL of 1% SDS, and 30 μL of methylhydrazine was stirred with a magnetic stirrer at room temperature for 1 h. The reaction solution was placed in a C_{18} SPE cartridge and then eluted with 5 mL of ethyl acetate under reduced pressure. The SPE cartridge was preconditioned by rinsing with 1 vol each of ethyl acetate, methanol, and deionized water, in series, prior to use. The eluent was concentrated under a purified nitrogen stream to 0.5 mL. The volume of the ethyl acetate eluent was brought to 5 mL with ethyl acetate, and then 10 μL of 2-methylpyrazine solution (10 mg/mL ethyl acetate) was added as a GC internal standard. MA was analyzed as 1-MP by GC with a nitrogen phosphorus detector (NPD) (refer to the subsequent *Instrumentation* section for the detailed GC conditions). A typical gas chromatogram of ethyl acetate extract obtained from salmon oil stored at 60°C for 7 d is shown in Figure 1. A gas chromatograph/mass spectrometer (GC/MS) was used to confirm the identity of the 1-MP. The mass spectral data of 1-MP are as follows: *m/z* (relative intensity) = 82 [M^+, 100], 54 [31], 53 [14], and 41 [14].

Analysis of glyoxal and methylglyoxal. Glyoxal and methylglyoxal were analyzed after they had been derivatized into quinoxaline and 2-methylquinoxaline, respectively, by a method slightly modified from previous reports (17). An aqueous solution (5 mL) containing 100 μL of an oil, 0.5 mL

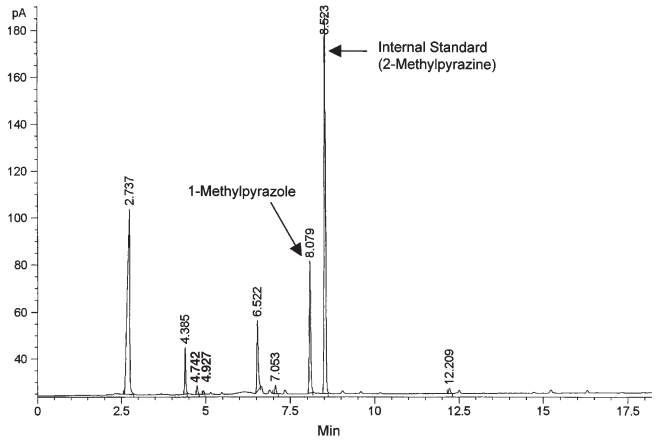

FIG. 1. A typical gas chromatogram of the ethyl acetate extract obtained from salmon oil heated at 60°C for 7 d (see Materials and Methods section for GC conditions). Malonaldehyde was determined as 1-methylpyrazole (retention time of 8.079 min).

of aqueous 1% SDS solution, and 100 μL of 1,2-phenylenediamine solution (10 mg/mL), in which the pH was adjusted to 7.0 with 0.1 N sodium hydroxide solution, was stirred with a magnetic stirrer at room temperature for 2 h. The reaction solution was loaded onto a C_{18} SPE cartridge and then eluted with 5 mL of ethyl acetate under reduced pressure. The SPE cartridge was preconditioned by rinsing with 1 vol each of ethyl acetate, methanol, and deionized water, in series, prior to use. The eluent was condensed to 1.5 mL in volume with a purified nitrogen stream. After 20 μL of 2-methylpyrazine solution (0.2 mg/mL ethyl acetate) was added as a GC internal standard, the solution was brought to 2 mL in volume with ethyl acetate. Glyoxal and methylglyoxal were analyzed as quinoxaline and 2-methylquinoxaline by GC with an NPD (refer to the subsequent *Instrumentation* section for detailed GC conditions). A typical gas chromatogram of ethyl acetate extract obtained from salmon oil stored at 60°C for 3 d is shown in Figure 2. A GC/MS was used to confirm the identity of quinoxaline and 2-methyl quinoxaline. The mass spectral data of quinoxaline are as follows: *m/z* (relative intensity) = 130 [M^+, 100], 103 [61], 76 [49], and 50 [21]. The mass spectral data of 2-methylquinoxaline are as follows: 144 [M^+, 100], 117 [81], 76 [41], and 50 [23].

Recovery efficiency test of MA from SPE. An aqueous solution (5 mL) containing 100 mM of MA tetrabutylammonium salt, 2 mL of phosphate buffer (pH 7.4), and 10 μL of methylhydrazine was stirred for 1 h at room temperature using a magnetic stirrer. The reaction solution was loaded onto a C_{18} SPE cartridge and then eluted with 5 mL of ethyl acetate under reduced pressure. The SPE cartridge was preconditioned by rinsing with 1 vol each of ethyl acetate, methanol, and deionized water, in series, prior to use. After 10 μL of 2-methylpyrazine solution (10 mg/mL ethyl acetate) was added as a GC internal standard, the solution was brought to 5.0 mL in volume with ethyl acetate. Glyoxal and methylglyoxal were analyzed as quinoxaline and 2-methylquinoxaline by GC with an NPD.

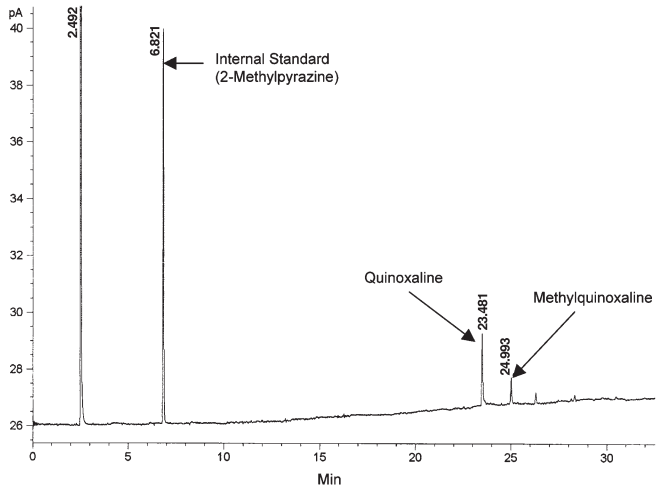

FIG. 2. A typical gas chromatogram of the ethyl acetate extract obtained from salmon oil heated at 60°C for 3 d (see Materials and Methods section for GC conditions). Glyoxal and methylglyoxal were determined as quinoxaline (retention time of 23.481 min) and methylquinoxaline (retention time at 24.993 min), respectively.

Recovery efficiency test of glyoxal and methylglyoxal from SPE. An aqueous solution (5 mL) containing 100 μL of corn oil, 10 μL of glyoxal, 10 μL of methylglyoxal, 0.5 mL of 1% SDS, and 100 μL of 1,2-phenylenediamine solution (10 mg/mL water) was stirred for 2 h at room temperature using a magnetic stirrer. The solution was neutralized with 110 mL of 0.1 N sodium hydroxide solution prior to stirring. The reaction solution was loaded onto a C_{18} SPE cartridge and then eluted with 5 mL of ethyl acetate under reduced pressure. The SPE cartridge was preconditioned by rinsing with 1 vol each of ethyl acetate, methanol, and deionized water, in series, prior to use. The eluent was condensed to 1.5 mL with a purified nitrogen stream. After 20 μL of 2-methylpyrazine solution (0.2 mg/mL ethyl acetate) had been added as a GC internal standard, the solution was brought to 2.0 mL with ethyl acetate. Glyoxal and methylglyoxal were analyzed as quinoxaline and 2-methylquinoxaline by GC with an NPD.

Instrumentation. An Agilent Technologies Model 6980N gas chromatograph equipped with a 30 m × 0.25 mm (d_f = 0.25 μm) ZB-WAX fused-silica capillary column (Phenomenex, Torrance, CA) and an NPD were used for quantitative analysis of 1-MP, quinoxaline, and 2-methylquinoxaline. The GC oven temperature was programmed from 60 to 130°C at 3°C/min for 1-MP analysis. The GC oven temperature was held at 80°C for 3 min and then programmed to 180°C at 4°C/min for quinoxaline and 2-methylquinoxaline analysis.

A Hewlett-Packard model 5890 series II gas chromatograph interfaced to an HP 5971 mass spectrometer was used to confirm 1-MP, quinoxaline, and 2-methylquinoxaline. The GC conditions were the same as those just described. The mass spectra were obtained by EI ionization at 70 eV at an ion source temperature of 250°C.

Data analysis. All measurements were in triplicate, and results were expressed as mean ± SD (n = 3). Linear correlation coefficients (R) between α-tocopherol content and oxidation parameters (PV, amount of MA, amount of glyoxal, and amount of methylglyoxal) were calculated using JMP IN software (version 4.0) (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Prior to the present study, 1-MP derived from MA was recovered using a liquid–liquid continuous extractor with dichloromethane (20–22). Liquid–liquid continuous extraction provides satisfactory recovery efficiency (82–91%) (23), but the process is somewhat tedious. Moreover, because halogenated solvents such as dichloromethane damage the NPD, the solvent must be changed to a nonhalogenated one such as ethyl acetate before the sample is injected into the gas chromatograph. Therefore, SPE was used to recover 1-MP in the present study. The result of the recovery efficiency test of MA as 1-MP was 111.3 ± 2.0%. The recovery efficiencies of glyoxal and methylglyoxal were 45.8 ± 1.7 and 82.0 ± 2.7%, respectively. The recovery of glyoxal was rather low. However, the values of SD were relatively low, indicating that the reproducibility of the recovery is satisfactory.

Using elevated temperatures is a common practice to examine the storage stability of foods. For example, changes in the chemical compositions and properties of Australian honeys was investigated at 50°C (24,25). Antioxidative activity of TBHQ was investigated using canola oil heated at 65°C (26). The oxidative status of high-heat (45°C) and medium-heat (25°C) whole milk powder was investigated by measuring the formation of TBARS (27). Therefore, the formation of MA, glyoxal, and methylglyoxal was investigated at accelerated storage conditions of 60°C. Also, the investigation was conducted at a cooking temperature of 200°C.

Figure 3 shows the PV of thermally oxidized dietary oils. When the oils were heated at 60°C for 3 d, soybean oil had the highest value (110 ± 4.30 mEq O_2/kg sample). The PV of soybean oil (260 ± 7.50 mEq O_2/kg sample) was higher than that of olive oil (200 ± 1.00 mEq O_2/kg sample), even though

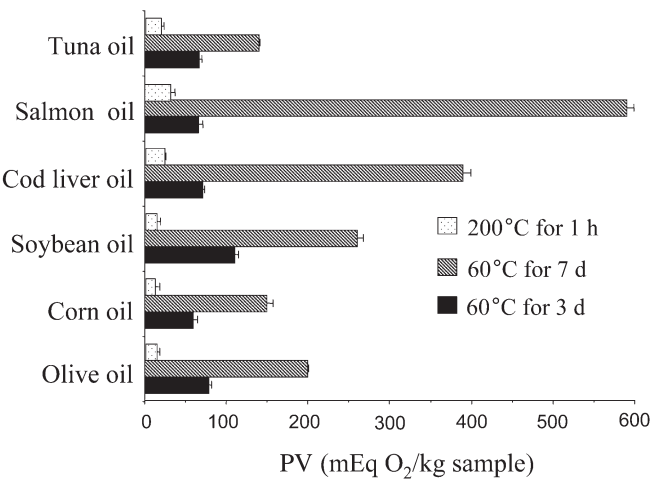

FIG. 3. PV of thermally oxidized dietary oils. Data are expressed as mean ± SD (n = 3).

the α-tocopherol content was higher in soybean oil (21.0 ± 2.80 ppm) than in olive oil (12.0 ± 1.60 ppm). It is interesting that the PV of oils heated at 200°C for a short time (1 h) were much lower than those heated at 60°C for a prolonged time (3 and 7 d). This may be due to the degradation of peroxides at a higher temperature (200°C).

Figure 4 shows the MA formed from thermally oxidized dietary oils. The MA content of natural oils ranged from 1.88 ± 0.530 (soybean oil) to 33.8 ± 11.7 ppm (salmon oil). Salmon oil seems to be most susceptible to oxidation. Salmon oil produced MA at a level of 1070 ± 77.0 ppm when it was heated at 60°C for 7 d, whereas corn oil produced only 6.65 ± 0.640 ppm under the same conditions. Fish oils produced more MA. MA formed from fish oils and vegetable oils ranged from 35.3 ± 2.80 (tuna oil) to 95.6 ± 2.30 ppm (salmon oil) and from 2.33 ± 0.25 (corn oil) to 9.56 ± 0.320 ppm (soybean oil), respectively, when they were heated at 60°C for 3 d. Also, MA formed from fish oils ranged from 157 ± 15.0 (tuna oil) to 1070 ppm ± 77.0 (salmon oil), whereas MA formed from vegetable oils ranged from 6.65 ± 0.640 (corn oil) to 35.9 ± 5.20 ppm (soybean oil) when they were heated at 60°C for 3 or 7 d. Much more MA formed from the vegetable oils heated at 200°C for 1 h than at 60°C for a prolonged time. On the other hand, the formation of MA from fish oils heated at 200°C for 1 h was somewhere between MA from fish oils heated at 60°C for 3 and 7 d. The results suggest that, in general, vegetable oils are oxidized more quickly at a high temperature.

Figure 5 shows the amount of glyoxal formed from thermally oxidized dietary oils. More glyoxal was formed from fish oils than from vegetable oils. The highest formation of glyoxal was obtained from salmon oil heated at 60°C for 3 d. It is interesting that more glyoxal was recovered from salmon and cod liver oils when they were heated for 3 d (12.8 ± 1.10 and 7.07 ± 0.19 ppm, respectively) than for 7 d (6.70 ± 0.080 and 5.94 ± 0.380 ppm, respectively). The results suggest that glyoxal underwent secondary reactions during the 7-d period and yielded secondary products, such as glyoxylic acid, CO_2 (34). In a previous study, 27 ppm of glyoxal was formed from cod liver oil irradiated with UV light for 10 h (17). Glyoxal was analyzed to examine the effects of antioxidants, including α-tocopherol, in cod liver oil oxidized by Fenton's reagent (35). Therefore, it is obvious that genotoxic glyoxal is formed in oxidized oils in ppm levels.

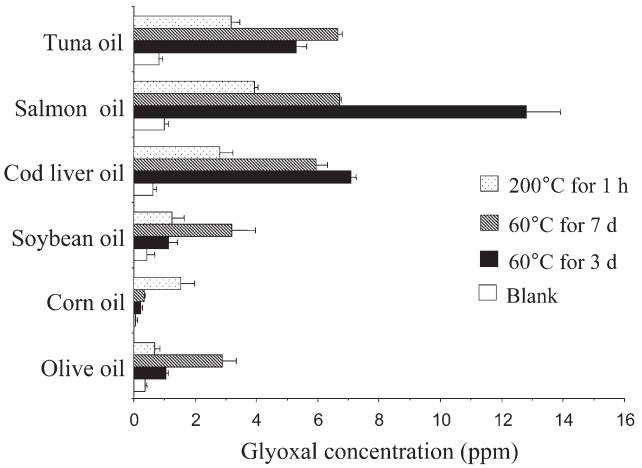

FIG. 5. Results of glyoxal analysis in thermally oxidized dietary oils. Data are expressed as mean ± SD (n = 3).

Figure 6 shows the amount of methylglyoxal formed from thermally oxidized dietary oils. Fish oils also produced much more methylglyoxal than did vegetable oils. For example, the level of methylglyoxal ranged from 2.03 ± 0.130 (cod liver oil) to 2.89 ± 0.110 ppm (tuna oil) in the fish oils heated at 60°C for 7 d. On the other hand, only 0.61 ± 0.030 ppm methylglyoxal formed in olive oil heated at the same conditions. Soybean and corn oils did not produce a detectable amount of methylglyoxal under accelerated storage conditions (heated at 60°C for 3 and 7 d). However, vegetable oils produced an appreciable amount of methylglyoxal (e.g., 0.38 ± 0.160 ppm from corn oil) when they were heated at a cooking condition (200°C for 1 h). Cod liver oil also produced 5.7 ppm of methylglyoxal with 10 h of UV irradiation (17). Therefore, genotoxic methylglyoxal is also formed in dietary oils upon oxidation.

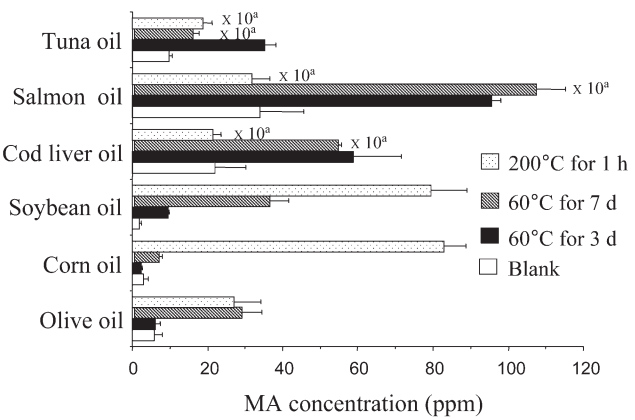

FIG. 4. Results of MA analysis in thermally oxidized dietary oils. [a]Actual values are the values in the figure × 10. Data are expressed as mean ± SD (n = 3).

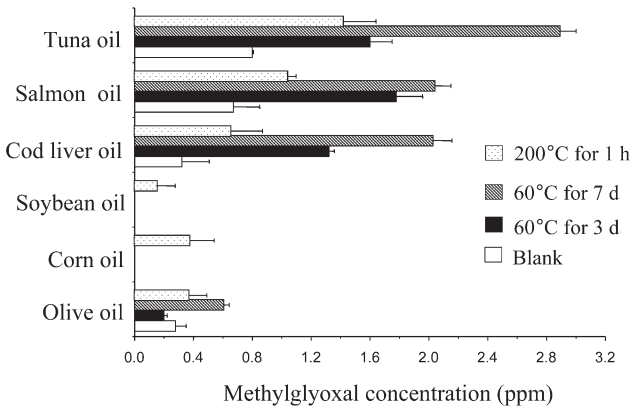

FIG. 6. Results of methylglyoxal analysis in thermally oxidized dietary oils.

Figure 7 shows the α-tocopherol content in dietary oils. α-Tocopherol content ranged from 12.0 ± 1.60 (olive oil) to 700 ± 66.0 ppm (tuna oil) in the dietary oils used in the present study. α-Tocopherol is a well-known, naturally occurring antioxidant that has been used as a standard antioxidant in numerous lipid peroxidation studies (28–30). Therefore, it is important to know the α-tocopherol content in dietary oils to evaluate their oxidative damages caused by heat.

The α-tocopherol content did not correlate with the PV among the six dietary oil samples heated at 60°C either for 3 ($R = -0.53$, $P = 0.27$) or 7 d ($R = -0.26$, $P = 0.62$). When the three fish oils were heated at 60°C for 7 d, the α-tocopherol content seemed to correspond, although not significantly, to the PV ($R = -0.98$, $P = 0.11$). The statistically significant correlation ($P < 0.05$) between the α-tocopherol content and the oxidation parameters was obtained only from MA and fish oils heated at 60°C for 3 d ($R = -0.99$, $P = 0.02$). All other correlations between the α-tocopherol content and the oxidation parameters were not statistically significant, although there was borderline significance between the α-tocopherol content and MA in the fish oils heated at 60°C for 7 d ($R = -0.99$, $P = 0.06$). A decrease of α-tocopherol was reportedly accompanied by an increase of MA when rapeseed oil was thermally oxidized at 170°C (32). Vitamin E (α-tocopherol) significantly influenced the antioxidant capacity of virgin olive, olive, and sunflower oils after frying (33).

Overall, the α-tocopherol content in the original oils did not significantly correspond to the oxidation parameters of the oxidized oils except in the case of MA. Perhaps the α-tocopherol was rapidly consumed and therefore ineffective. In addition to α-tocopherol, these oils also contain natural antioxidants, such as flavonoids, ascorbic acid, and carotenes (31), which may play a more significant role than α-tocopherol in the PV of oxidized oils. More detailed experiments are necessary to examine the role of antioxidants in the oxidation of lipids.

The formation mechanisms of these compounds are rather complex. There are many hypotheses about the mechanisms of MA formation in oxidized oils. For example, it has been proposed that MA forms from the decomposition of prostaglandin-like endoperoxide intermediates (36). This mechanism requires at least three methylene-interrupted double bonds in a molecule. However, it was later found that MA also formed from a FA with one or two double bonds (33). Glyoxal and methylglyoxal were formed from low-M.W. carbonyl compounds, including acetaldehyde, acrolein, propanal, and acetone on 6 h of UV irradiation (17).

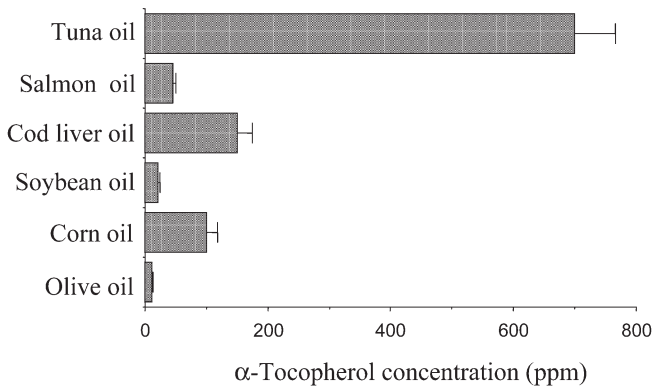

FIG. 7. Results of α-tocopherol analysis in dietary oils. Data are expressed as mean ± SD ($n = 3$).

It is well known that numerous carbonyl compounds are produced from long-chain PUFA (38,39). Therefore, the formation of MA, glyoxal, and methylglyoxal may be influenced by the content of long-chain PUFA, EPA, and DHA, rather than the TG content. A previous report showed a positive correlation between the rate of oxidation and the numbers of double bonds in the FA of a TG (40). PUFA are present in greater amounts in fish oils than in vegetable oils. Cod liver oil consists of 12.6% EPA and 10.6% DHA. Salmon oil contains 4.5% EPA and 17% DHA. Tuna oil has 6.7% EPA and 28.8% DHA. On the other hand, vegetable oils (soybean, corn, and olive) contain neither EPA nor DHA (41).

The results of the present study indicate that the formation of genotoxic dicarbonyl compounds is influenced by storage and cooking conditions. The kind of dietary oils also plays an important role in the production of these chemicals. The oils containing higher levels of long-chain PUFA, such as fish oils, produced more genotoxic dicarbonyl compounds. Further investigation to clarify formation mechanisms of these compounds is in order. However, the investigation of these genotoxic dicarbonyl compounds from dietary oils performed in the present study is one avenue to assess food safety.

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