Formation of Toxic Aldehydes in Cod Liver Oil After Ultraviolet Irradiation

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Formaldehyde, acrolein, malonaldehyde (MA), acetaldehyde and propanal produced from cod liver oil upon ultraviolet irradiation (λ_{max} = 300 nm) were derivatized into nitrogen- or sulfur-containing compounds and then analyzed by capillary gas chromatography with a nitrogen-phosphorus detector or a flame photometric detector. Acrolein and MA were formed at levels of 10.9 ± 3.06 and 190.2 ± 38.4 nmol/mg of fish oil, respectively. Maximum levels of formaldehyde, acetaldehyde and propanal formed were 7.0 ± 0.90, 49.1 ± 4.5 and 35.8 ± 4.0 nmol/mg of oil, respectively. Formation of propanal in large quantities corresponded to the high content of ω-3 fatty acids in cod liver oil.

KEY WORDS: Acetaldehyde, acrolein, cod liver oil, formaldehyde, malonaldehyde, photooxidation.

The lipid content of fish is highly variable, both among species and within a given species (1). Lipid content in edible flesh ranges from a minimum of 0.5% to a maximum of 25%. It occurs primarily as triglycerides, with phospholipids accounting for around 0.5% of muscle (2). The major polyunsaturated fatty acids (PUFAs) in fish oils are eicosapentaenoic acid (C20:5, ω-3, approx. 10%) and docosahexaenoic acid (C22:6, ω-3, 30–33%) (3). The highly unsaturated fatty acids render fish tissue and fish oil extremely susceptible to autooxidation and rapid deterioration.

PUFAs produce toxic aldehydes, including formaldehyde, acetaldehyde, acrolein and malonaldehyde (MA), by action of heat, light metals or enzymes (4,5). Among the lipid peroxidation products, β-dicarbonyl such as MA and α,β-unsaturated aldehydes such as acrolein and 4-hydroxynonenal have received much attention as biologically active agents (6). For example, MA elicits toxic activities by reacting with biological nucleophiles such as DNA (7). Acrolein is reportedly mutagenic toward *Salmonella typhimurium* strains (8,9). Measurement of the levels of these toxic aldehydes formed in fish oil as a result of ultraviolet (UV) exposure may further improve understanding of the link between formation of these compounds and the toxic effects of solar irradiation.

In the present study, the formation of acrolein, MA, formaldehyde, acetaldehyde and propanal from cod liver oil upon UV irradiation were investigated by means of the derivatization methods developed previously (10,11). Fish oil was chosen as a model study because of its high content of ω-PUFA.

MATERIALS AND METHODS

Materials. Cod liver oil (consisting of approximately 70% ω-3 fatty acids, including ω-3 octadecatetraenoic, eicosapentaenoic and docosahexaenoic), was purchased from Sigma Chemical Co. (St. Louis, MO). 2-Methylpyrazine, and cysteamine·HCl were obtained from Aldrich Chemical Co. (Milwaukee, WI). N-Methylhydrazine was purchased from Fluka Chemical Corp. (Ronkonkoma, NY). Standard 1-methyl-2-pyrazoline and 1-methylpyrazole were prepared as described previously (11,12).

UV-irradiation of cod liver oil. The oil (55.8 mg) was dissolved in 2 mL of dichloromethane and coated on the inside of 20-cm × 2-cm i.d. pyrex tubes. Coating was achieved by evaporating the solvent under a nitrogen stream while rotating the tubes. A fish oil-coated tube wrapped with aluminum foil was placed in a freezer (−5°C) for 24 h as a control. A sample tube was sealed and irradiated with eight UVB (λ_{max} = 300 nm) lamps for various periods of time.

Analysis of acrolein and MA formed from cod liver oil. An irradiated or control sample was rinsed with 2 mL of dichloromethane and transferred into a test tube containing 6 mL of dichloromethane and 16 μL of N-methylhydrazine. The reaction mixture was stirred with a magnetic stirrer at room temperature for 1 h and then acetylacetone (50 μL) was added to remove the excess unreacted N-methylhydrazine (12). A sample was brought to a final volume of 10 mL by adding dichloromethane in a volumetric flask, and then a 150-μL dichloromethane solution of 2-methylpyrazine (2.2 μg/mL) was added as a gas chromatographic (GC) internal standard. Samples were analyzed for acrolein as 1-methyl-2-pyrazoline and MA as 1-methylpyrazole by GC with a nitrogen-phosphorus detector (NPD). GC calibration curves for acrolein and MA analyses were prepared as described previously (11,12).

Analysis of formaldehyde, acetaldehyde and propanal formed from cod liver oil. Pyridine (3 mL) and 0.2 mL of cysteamine solution (0.6 mmol) were added to the irradiated and control samples. The pH of each sample solution was adjusted to 8.5–9.5 with 1 NNaOH, and then the mixtures were stirred at 0°C for 1 h. The reaction mixtures were brought to 10 mL with absolute ethanol, and a 100-μL dichloromethane solution of 2,4,5-trimethythiazole (10 μg/mL) was added as a GC internal standard. The samples were analyzed for formaldehyde as thiazolidine, acetaldehyde as 2-methylthiazolidine, and propanal as 2-ethylthiazolidine by GC with a flame photometric detector (FPD). GC calibration curves were prepared as previously described (12).

Instrumental. A Hewlett-Packard (HP) Model 5880A GC equipped with an NPD and a 30-m × 0.25 mm i.d. bonded-phase DBWAX fused-silica capillary column (J&W Scientific, Folsom, CA) was used for the quantitation of 1-methyl-2-pyrazoline and 1-methylpyrazole. The GC peak areas were integrated with an HP 5880A integrator. The oven temperature was held at 60°C for 5 min and then programmed to 190°C at 5°C/min. Injector and detector temperatures were 250°C and 300°C, respectively. Linear helium carrier gas flow rate was 42 cm/s with a split ratio of 21:1.

An HP 5890 GC equipped with an FPD and a 30-m × 0.25-mm i.d. bonded-phase DB-1 fused-silica capillary column (J&W Scientific) was used for the quantitation of thiazolidine, 2-methylthiazolidine and 2-ethylthiazolidine. The GC peak areas were integrated by a Spectra-

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TABLE 1

Malonaldehyde (MA) and Acrolein Formed in Cod Liver Oil After Ultraviolet Irradiation of Various Durations

Exposure duration (h)	Amounts (nmol/mg fish oil)[a]	
	MA	Acrolein
0	3.8 ± 0.2	1.65 ± 0.12
2	190.2 ± 38.4	6.04 ± 1.44
4	78.9 ± 9.24	6.60 ± 0.40
6	69.5 ± 14.9	10.90 ± 3.06
8	46.2 ± 1.75	4.64 ± 0.11
10	24.2 ± 2.57	2.81 ± 0.37

[a]Values are mean ± standard deviation (n = 4) and are corrected for control.

TABLE 2

Toxic Aldehydes Formed in Cod Liver Oil After Ultraviolet Irradiation of Various Durations

Exposure duration (h)	Amounts (nmol/mg fish oil)[a]		
	Formaldehyde	Acetaldehyde	Propanal
0	n.d.[b]	3.5 ± 0.8	n.d.
2	0.46 ± 0.20	22.4 ± 1.7	35.8 ± 4.00
4	1.20 ± 0.13	36.2 ± 6.4	32.4 ± 4.00
6	2.60 ± 0.31	41.7 ± 8.5	27.9 ± 1.16
8	1.03 ± 0.09	37.3 ± 7.2	19.6 ± 3.20
10	2.00 ± 0.73	35.0 ± 7.1	15.8 ± 4.10
12	7.01 ± 0.90	49.1 ± 4.5	14.7 ± 0.98
15	4.68 ± 1.30	23.5 ± 5.4	12.5 ± 0.66

[a]Values are mean ± standard deviation (n = 5) and are corrected for control.
[b]n.d., none detected.

Physics SP 4290 integrator (San Jose, CA). Oven temperature was programmed from 50°C to 200°C at 8°C/min. Injector and detector temperatures were 250°C and 230°C, respectively. Linear helium carrier gas flow rate was 33 cm/s with a split ratio of 34:1.

An HP 5890 GC interfaced to a VG Trio II mass spectrometer with a VG II-250 computer data system was used for mass spectrometric confirmation of the derivatives. The ionization voltage was 70 eV, and the ion source temperature was 150°C. The column and oven conditions for gas chromatography/mass spectrometry (GC/MS) were as described for the GC analyses.

RESULTS AND DISCUSSION

Amounts of acrolein and MA found in irradiated cod liver oil in the present study are shown in Table 1. Maximum formation of acrolein (10.9 ± 3.06 nmol/mg) was obtained after 6 h of irradiation, whereas a maximum of MA (190.2 ± 38.4 nmol/mg) was obtained after 2 h of irradiation. The formation of MA declined steadily after 2 h of irradiation. A similar phenomenon was observed when squalene was irradiated under the same conditions (13), except for the time required to reach maximum formation (6 h). Trace levels of MA are always found in lipid samples, suggesting that autooxidation produces MA (14).

Our previous study showed no formation of acrolein from linoleic acid and squalene upon UV irradiation, whereas significant amounts of acrolein were recovered from UV-irradiated linolenic acid and arachidonic acid (15). Formation of acrolein in large amounts from heated cooking oils was previously reported (16). It has been proposed that acrolein is formed from dehydration of glycerol (17), from aldol condensation of formaldehyde and acetaldehyde (18), or from a free-radical mechanism with homolytic fission of R-O bonds of triglycerides (16). Decreases in MA and acrolein after reaching maximum levels may be due to losses in MA and acrolein as a result of volatilization, polymerization or binding to other components and the glass surface.

Quantities of formaldehyde, acetaldehyde and propanal produced from irradiated cod liver oil are shown in Table 2. Figure 1 shows typical chromatograms of control and irradiated cod liver oil after reacting with cysteamine. Maximum levels of 7.0 ± 0.90, 49.1 ± 4.5 and 35.8 ± 4.0 nmol/mg fish oil of formaldehyde, acetaldehyde and propanal were reached after 12, 12 and 2 h of irradiation, respectively.

The amounts of formaldehyde recovered seem to vary over time. This may be due to its reactivity to the glass surface of the apparatus (12). However, formaldehyde formation increases significantly after 10 h of irradiation. It may also undergo secondary reaction after prolonged time periods, and its recovery decreases significantly after

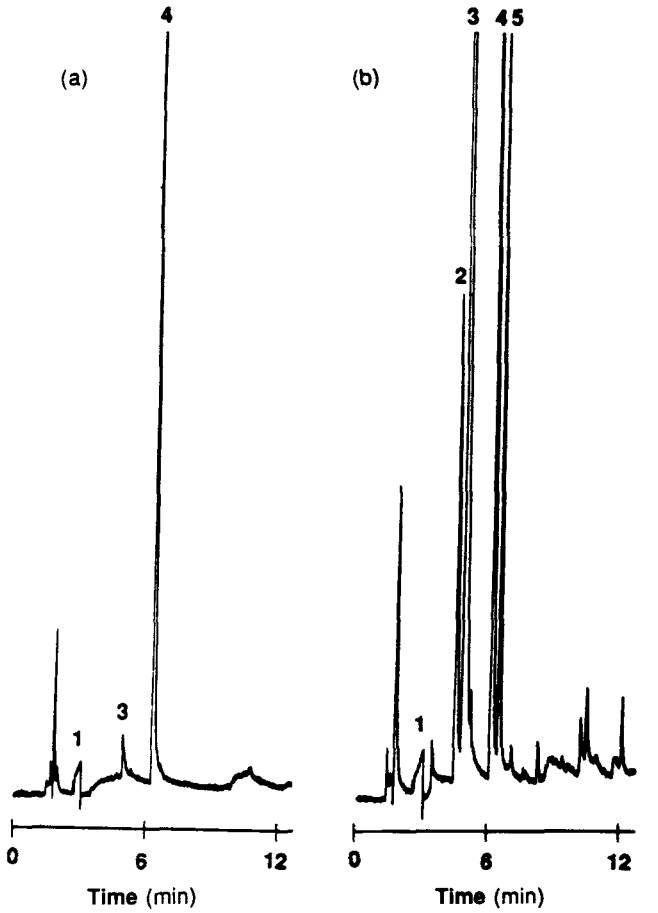

FIG. 1. Gas chromatograms of control sample (a) and sample irradiated with ultraviolet for 12 h after reacting with cysteamine (b). 1: Unreacted cysteamine, 2: thiazolidine, 3: 2-methylthiazolidine, 4: internal standard (trimethylthiazole), 5: 2-ethylthiazolidine.

12 h of irradiation. Arachidonic acid also forms formaldehyde upon UV irradiation (15).

Acetaldehyde formation occurred quickly upon irradiation. Formation of acetaldehyde was also observed upon UV irradiation of arachidonic acid, but quantities were not measured due to the lack of an appropriate analytical method (15).

Formation of propanal from lipid upon UV irradiation has never been reported before the present study. However, linolenic acid and its ethyl ester reportedly produced propanal in significant quantities upon oxidation with Fe^{2+}/H_2O_2 (19). Propanal formed in a short time, and the amount formed declined steadily with increasing irradiation time, suggesting that it underwent secondary reaction during UV irradiation and its recovery decreased over time. Formation of propanal may be due to the presence of ω-3 PUFA. It has been proposed that propanal is formed from an ω-3 moiety of fatty acids through a hydroperoxy cyclic peroxide intermediate (19). It is also generally recognized that the oxidative cleavage of a double bond produces aldehydes or ketones (20), and that the amount of various aldehydes formed from fatty acids may correspond to the amount of possible fatty acid precursors in oils (21). Therefore, formation of propanal clearly corresponds to the amount of ω-3 PUFA present in cod liver oil in the current study.

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