Storage, Heating, and Tocopherols Affect Cholesterol Oxide Formation in Food Oils

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The influence of fatty acid composition and tocopherol on cholesterol oxidation in fish, flax, sunflower, and palm oil to which cholesterol was added were examined during storage and heating. The contents of cholesterol oxides in all oils increased ($p < 0.05$) during storage and heating. Total cholesterol oxides in fish oil were higher ($p < 0.05$) than that in vegetable oils. Addition of α-tocopherol to oils reduced the concentration of cholesterol oxides in fish oil ($p < 0.05$) but not in vegetable oils. Palm oil was the most stable oil. These results suggest a relationship between fatty acid composition and cholesterol oxidation, particularly with respect to the polyunsaturated fatty acid content.

Keywords: *Oil; cholesterol oxide; tocopherols*

INTRODUCTION

Cholesterol can undergo autoxidation in air to form cholesterol oxide products (COPs). The production of cholesterol oxides has been reported in many types of foods, including heated fats and oils (Park and Addis, 1986a,b; Zhang et al., 1990; Yan and White, 1990), deep-fried foods (Bascoul et al., 1986), meat products (Park and Addis, 1987; Zubillaga and Maerker, 1991), and milk and egg products (Naber et al., 1985; Nourooz-Zadeh et al., 1988). The presence and quantity of COPs in foods resulting from processing have received considerable attention in the recent years because of their adverse human health effects (Addis, 1986; Jacobson, 1987; Hubbard et al., 1989).

In food, cholesterol exists in a close proximity to other lipids and fatty acids. Thus, the rate of cholesterol oxidation is altered by other lipid and nonlipid molecules (Nawar, 1991). The polyunsaturated fatty acid (PUFA) content of oils has been reported to enhance the production of COPs (Li et al., 1994). PUFAs are high in fish, seafood ($20:5n-3$, $22:5n-3$, $22:6n-3$), leaf vegetables and pods ($18:3n-3$), and some oil seeds ($18:3n-3$; Leaf and Weber, 1988). These PUFAs are very susceptible to peroxidation, even under mild ambient conditions, and are easily incorporated into the chain reaction of lipid peroxidation to yield free radicals and peroxy radicals (Hsieh and Kinsella, 1989). It is, therefore, logical to suspect that the oxidation of cholesterol might be accelerated in the presence of peroxidized lipids. Ohshima et al. (1993) found that a continuous increase in the amount of cholesterol oxides was accompanied by a remarkable concurrent decrease in PUFA residues. Therefore, compounds that inhibit unsaturated fatty acid oxidation may reasonably be expected to inhibit cholesterol oxidation.

There have been many reports on the protective effect of tocopherols as food antioxidants (Dougherty, 1988).

Tocopherols are the major antioxidants in vegetable oils (Sherwin, 1978) and are capable of quenching free radicals. Thus, tocopherols protect phospholipids and cholesterol against oxidation and subsequent breakdown to potentially harmful, chemically reactive products (Faustman, 1989; Hudson and Mahgoub, 1981). Dietary tocopherol supplementation has been shown to improve the oxidative stability of chicken muscle, eggs (Asghar et al, 1990; Cherian et al., 1996), and pork (Monahan, 1992). Considering the importance of PUFAs and tocopherols in lipid oxidation, the objective of this study was to determine the effect of fatty acid composition and tocopherols on the cholesterol oxidation in food oils during storage and heating.

MATERIALS AND METHODS

Reagents. Cholesterol and cholesterol oxide standards ($>99\%$ purity), including 7α-hydroxycholesterol, 7β-hydroxycholesterol, α-epoxide, β-epoxide, cholestanetriol, 7-ketocholesterol, and 6-ketocholestanol, were purchased from Sigma Chemical Company (St Louis, MO) and Steraloids Inc. (Bayonne, NH). Tocopherol isomer standards were purchased from Sigma Chemical. Celite 545 and Calcium phosphate ($CaHPO_4 \cdot 2H_2O$) were purchased from Fisher Scientific Company (Malvern, PA) and silicic acid (100 mash) from Aldrich Chemical Company (Milwaukee, WI). Acetonitrile and methanol were HPLC grade from BDH Inc. (Toronto, ON), and all other solvents used were glass distilled.

Experimental Design. Duplicate 40-g samples of each oil [fish (FO), flax (LO), sunflower (SO), and palm (PO)] were weighed into a 50-mL culture tube, 400 mg of pure cholesterol (checked for cholesterol oxides by thin-layer chromatography) were added to each tube, tocopherol (16 mg) was added to one of the tubes, and the samples were well mixed. The oil samples were of edible quality and were obtained from the local market. Samples were divided into three equal aliquots for the storage study. Samples ($n = 6$) stored at room temperature and open to air were mixed daily to ensure uniformity of light (normal daylight cycle) and air access. One sample was taken from each tube for analysis of cholesterol oxides and two samples were taken from each tube for analysis of tocopherols and fatty acids on days 0, 15, and 35 during the period of storage. For the heat-treatment experiment, six tubes for each oil were prepared and 400 ppm α-tocopherol was added to three tubes of each oil. Samples were heated in an air convection oven at 110 °C for 22 h and analyzed. One

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Table 1. Major Fatty Acid Composition of Food Oils[a]

	oil							
fatty acid	FO	FO+T	LO	LO+T	SO	SO+T	PO	PO+T
14:0	9.0	9.2	0.0	0.0	0.2	0.1	1.1	1.1
16:0	19.0	19.0	5.7	5.5	6.9	6.8	43.2	43.2
18:0	3.4	3.4	2.7	2.7	5.0	4.9	4.6	4.6
16:1	11.7	12.1	0.1	0.0	0.2	0.2	0.2	0.2
18:1n−9	9.4	8.9	11.5	11.4	18.6	18.6	39.0	39.0
18:2n−6	1.6	1.4	16.1	16.8	65.0	64.8	9.9	9.9
18:3n−3	1.4	1.4	63.4	62.8	0.8	0.7	0.4	0.4
18:4n−3	3.5	3.6	0.0	0.0	0.0	0.0	0.0	0.0
20:5n−3	14.5	14.7	0.0	0.0	0.6	0.6	0.0	0.0
22:6n−3	12.8	12.9	0.0	0.0	0.0	0.0	0.0	0.0
SAFA	33.7	34.1	8.4	8.2	12.0	11.9	49.0	49.0
MUFA	28.4	27.8	12.2	12.1	21.6	22.1	40.7	40.8
total n−6	2.9	2.8	16.1	16.8	65.0	64.8	9.9	9.9
total n−3	35.0	35.3	63.4	62.9	1.4	1.3	0.4	0.4
PUFA	37.9	38.1	79.5	79.6	66.4	66.2	10.3	10.3

[a] FO = fish oil; FO+T = fish oil + α-tocopherols; LO = flax oil; LO+T = flax oil + α-tocopherols; PO = palm oil; PO+T = palm oil + α-tocopherols; SO = sunflower oil; SO+T = sunflower oil + α-tocopherols; SAFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; results are expressed as percent of total fatty acid methyl esters with standard errors of means.

Table 2. Initial Tocopherol Contents in Different Food Oils[a]

	tocopherol isomers (ppm)			
treatment	α-T	γ-T	δ-T	Total -T
FO	11.8 ± 1.4	5.0 ± 0.5	2.1 ± 0.15	18.9 ± 1.8
FO+T	333.9 ± 20	7.3 ± 2.2	3.4 ± 2.1	344.6 ± 20
LO	91.1 ± 5.6	631.6 ± 17	170.9 ± 6.9	893.6 ± 26
LO+T	415.5 ± 10	626.3 ± 21	169.3 ± 4.5	1211.1 ± 25
SO	211.5 ± 5.5	25.2 ± 1.2	0.0	236.7 ± 5.3
SO+T	522.3 ± 6.3	31.3 ± 8.7	0.0	553.6 ± 12
PO	102.3 ± 9.7	7.9 ± 1.7	0.0	110.2 ± 11
PO+T	426.4 ± 6.2	10.3 ± 1.8	0.0	436.7 ± 6.4

[a] Mean ± SD (n = 6); FO = fish oil; FO+T = fish oil + tocopherol; LO = flax oil; LO+T = flax oil + tocopherol; SO = sunflower oil; SO+T = sunflower oil + tocopherol; PO = palm oil; PO+T = palm oil + tocopherol; α-T = α-tocopherol; γ-T = γ-tocopherol; δ-T = δ-tocopherol.

sample for cholesterol oxides from each tube and two samples for fatty acids and tocopherols from each tube were analyzed before and after heating.

Fatty Acid Analysis. The fatty acid methyl esters were prepared from oil samples by transesterification with BF3-methanol (Morrison and Smith, 1964). Analyses were performed on a Varian 3600 gas chromatograph (GC; Varian Associates Inc., Walnut Creek, CA) equipped with an 8200 autosampler, an on-column injector, and a flame ionization detector (FID) as described by Ajuyah et al. (1991).

Tocopherol Assay. A weighed amount (1 g) of oil in duplicate was placed into a 10-mL tissue culture tube. Samples were diluted with 1 mL of hexane and thoroughly mixed. Twenty microliters of mixture were placed into a 2-mL autosampler vial and 200 μL of the internal standard (rac-5,7-dimethyltocol, 10 μg/mL in methanol) were added. A 25-μL volume was injected onto HPLC column. A Varian 5000 liquid chromatograph system with a Shimadzu Sil-9A model autosampler was used to determine α-, γ-, and δ-tocopherol concentrations in the oil samples. Separation was achieved on a 3-μm Supelcosil, RPLC-18 column (150 × 4.6 mm i.d.; Supelco Canada Ltd., Oakville, ON) with a guard column (5 cm, 20−40 μm LC-18 packing) and a mobile phase of methanol: acetonitrile (50:50 v/v) at a flow rate of 1.5 mL/min. Tocopherol isomers were detected with a Shimadzu RF-535 fluorescence detector at an excitation wavelength of 295 nm and an emission wavelength of 330 nm. A Shimadzu EZchrom chromatography data system (Shimadzu Scientific Instruments Inc., Columbia, MD) was used to integrate peak areas.

Table 3. Changes of Total Tocopherol Content during Storage in Different Food Oils[a]

	storage		
oil	0 days	15 days	35 days
FO	18.9[a]	1.51[a]	0.0[a]
FO+T	344.6[a]	102.4[b]	0.0[c]
LO	893.6[a]	840.8[a]	681.2[b]
LO+T	1211.1[a]	1135.4[b]	791.7[c]
SO	236.7[a]	215.5[a]	141.0[b]
SO+T	553.6[a]	479.2[b]	374.2[c]
PO	110.2[a]	62.0[a]	62.3[a]
PO+T	436.7[a]	290.8[b]	249.0[b]

[a] FO = fish oil; FO+T = fish oil + tocopherol; LO = flax oil; LO+T = flax oil + tocopherol; SO = sunflower oil; SO+T = sunflower oil + tocopherol; PO = palm oil; PO+T = palm oil + tocopherol; means within same row with no common superscripts (a−c) differ significantly ($p < 0.05$); standard error of mean (SEM) was pooled: 19.24.

Cholesterol Oxidation Products Analysis. The column chromatography method for isolation of cholesterol oxides developed by Park and Addis (1985) and Zubillaga et al. (1991) was used with some modifications. A homogenous mixture containing silicic acid (100 mesh), Celite 545 (Fisher Scientific), and CaHPO$_4$·2H$_2$O (10:9:1, w/w/w) was made into a slurry with chloroform and packed into a glass column (12 mm i.d. × 30 cm) to a height of 15 cm. The column was washed with 10 mL of hexane:ethyl acetate (9:1, v/v; solvent I) before samples were applied (Li et al., 1996a). Oil samples (0.3 g) dissolved in 5 mL of solvent I and 10 μg of 6-ketocholestanol, as an internal standard, were applied onto the column. Neutral lipids and cholesterol were eluted by passing 50 mL of solvent-I and then 35 mL of hexane:ethyl acetate (8:2, v/v; solvent II) through the column. Elution of cholesterol oxides was accomplished with 40 mL of acetone:ethyl acetate:methanol (50:50:5, v/v/v; solvent III) at a flow rate of ∼1 mL/min.

The final eluate was dried under nitrogen, and the cholesterol oxidation products were converted to TMS derivatives with 200 μL of pyridine and 100 μL of Sylon BFT (BSTFA: TMCS, 99:1; Supelco Inc., Bellefonte, PA) at 80 °C for 1 h (Nawar et al., 1991).

Gas Chromatography and Mass Spectrometry. Analysis of cholesterol oxides was performed on a Varian 3400 GC with an on-column injector and a FID detector. A 25 m × 0.20 mm i.d. bonded phase 5% phenylsilicone column with 0.33-μm film thickness (HP Ultra 2; Hewlett-Packard, Avondale, PA) was used. The initial oven temperature of 90 °C was held for 0.5 min, increased to 290 °C at 30 °C/min, and held at 290 °C for 20 min. The injector temperature was programmed from 100 to 290 °C at 150 °C/min and held at 290 °C for 25 min. The detector temperature was 290 °C. Helium was used as a carrier gas at a head pressure of 20 psi and 30-mL/min flow rate to the detector as a make-up gas. Cholesterol oxides were identified by comparison of their retention times against authentic standards. A response factor for each oxysterol measured was obtained by dividing peak-area ratio by weight ratio of oxysterol to 6-ketocholestanol in the prepared standard mixture (Li et al., 1996a). Peak areas were integrated with a Shimadzu EZChrom chromatography data system (Shimadzu Scientific Instruments).

To confirm the identity of COPs isolated from oil samples, an HP 5890 II Series GC equipped with an on column injector, a 7673 autosampler, and a 5971A mass selective detector was used. Cholesterol oxides were separated on a DB5-MS capillary column (J&W Scientific, Inc., Rancho Cordova, CA; 30 m × 0.25 mm i.d. × 0.25-μm film thickness). The initial column temperature of 80 °C was held for 0.5 min, increased to 280 °C at 40 °C/min, and held at 280 °C for 18 min. The injector temperature was programmed from 90 to 280 °C at 150 °C/min and held at 280 °C for 25 min. The GC-MS interface temperature was maintained at 280 °C and 0.5 μL of sample was injected onto a DB-5 GC column. Helium was used as

Table 4. Changes in Total Tocopherol Content (ppm) in Unheated versus Heated Oils[a]

	oil							
treatment	FO	FO+T	LO	LO+T	PO	PO+T	SF	SF+T
unheated	18.0[a]	344.6[a]	896.3[a]	1191.1[a]	110.0[a]	436.7[a]	236.7[a]	553.6[a]
heated	3.7[c]	163.9[b]	665.4[b]	848.8[b]	35.9[b]	219.3[b]	125.3[b]	256.4[b]

[a] FO = menhaden oil; FO+T = menhaden oil + tocopherol; LO = flax oil; LO+T = flax oil + tocopherol; PO = palm oil; PO+T = palm oil + tocopherol; SF = sunflower oil; SF+T = sunflower oil + tocopherol; pooled standard error of mean = 15.18; means within row with no common superscripts (a−c) differ significantly ($p < 0.05$).

Table 5. Effects of Storage and α-Tocopherol on Cholesterol Oxidation Products (Micrograms per Gram of Oil) in Different Food Oils[a]

	storage			
oil	0 days	15 days	35 days	overall[b]
FO	11.2[y]	62.1[a,y]	133.1[a,x]	68.8a
FO+T	8.5[z]	41.6[b,y]	73.4[b,x]	41.2b
LO	nd	1.9[c,x]	9.9[d,x]	3.9de
LO+T	nd	1.9[c,x]	8.5[d,x]	3.5de
SO	nd	4.7[c,y]	25.5[c,x]	10.1c
SO+T	nd	3.6[c,y]	21.3[c,x]	8.3cd
PO	nd	2.8[c,x]	4.1[d,x]	2.3e
PO+T	nd	1.3[c,x]	4.4[d,x]	1.9e
overall[c]	2.5[z]	14.9[y]	35.2[x]	

[a] FO = fish oil; FO+T = fish oil + tocopherol; LO = flax oil; LO+T = flax oil + tocopherol; SO = sunflower oil; SO+T = sunflower oil + tocopherol; PO = palm oil; PO+T = palm oil + tocopherol; a-d Means within same column with no common superscripts (a−e) differ significantly ($p < 0.05$); means within same row with no common superscripts (x−z) differ significantly ($p < 0.05$); standard error of mean (SEM) for eight treatments during storage was pooled: 3.37; nd = not detected. [b] Standard error of mean (SEM) for the overall was pooled: 1.95. Standard error of mean (SEM) for the overall was pooled: 1.19.

Table 6. Effect of Storage and Tocopherols on the Major Cholesterol Oxide Formation in Different Food Oils[a]

COPs (ppm)	oil							
	FO	FO+T	LO	LO+T	SO	SO+T	PO	PO+T
				0 Days of Storage				
7α-OH	nd	nd	nd	nd	nd	nd	nd	nd
7β-OH	nd	nd	nd	nd	nd	nd	nd	nd
7-keto	6.2	6.3	nd	nd	nd	nd	nd	nd
α-epoxide	1.9	0.9	nd	nd	nd	nd	nd	nd
β-epoxide	4.4	2.0	nd	nd	nd	nd	nd	nd
				15 Days of Storage				
7α-OH	1.4[a]	nd	0.3[d]	0.4[d]	1.1[b]	0.7[c]	0.4[d]	0.2[d]
7β-OH	nd	nd	0.1	nd	0.2	nd	0.4	0.3
7-keto	24.5[a]	11.6[b]	0.6[c]	0.9[c]	1.8[c]	1.4	nd	nd
α-epoxide	23.7[a]	15.9[b]	0.5[c]	1.1[c]	1.2[c]	0.9[c]	nd	nd
β-epoxide	21.3[a]	14.0[b]	0.3[c]	0.3[c]	1.0[c]	0.7[c]	nd	nd
				35 Days of Storage				
7α-OH	3.3	1.5	1.4	1.0	2.5	1.8	0.5	0.2
7β-OH	2.2	0.7	0.5	0.3	2.0	1.8	0.6	0.2
7-keto	64.2[a]	31.9[b]	3.2[d]	3.8[d]	10.8[c]	10.3[c]	1.1[e]	0.8[e]
α-epoxide	30.1[a]	20.8[b]	1.8[d]	2.1[cd]	3.6[c]	3.4[c]	1.3[d]	0.9[d]
β-epoxide	34.3[a]	21.3[b]	1.8[d]	1.6[d]	5.3[c]	4.3[c]	0.9[d]	1.0[d]

[a] FO = fish oil; FO+T = fish oil + α-tocopherol; LO = flax oil; LO+T = flax oil + α-tocopherol; SO = sunflower oil; SO+T = sunflower oil + α-tocopherol; PO = palm oil; PO+T = palm oil + α-tocopherol; 7α-OH, 7α-hydroxycholesterol; 7β-OH, 7β-hydroxycholesterol; 7-keto, 7-ketocholesterol; means within same row with no common superscripts (a−c) differ significantly ($p < 0.05$); nd = not detected.

carrier gas at a flow rate of 1 mL/min. The analyses were performed with the detector in the scan mode and an electron-impact ionization potential at 70 eV, as described earler (Li et al., 1996b).

Statistical Analysis. One-way analysis of variance (ANOVA) was used to analyze the differences in fatty acids among different oils during storage. Data for cholesterol oxides and tocopherols were analyzed by three-way ANOVA with sources of variation of oils ($o = 4$), tocopherol ($t = 2$), and days of storage ($s = 3$). Means of effects (oil, tocopherol, and storage) were separated by the Student-Newman-Keuls (SNK) test (Steel and Torrie, 1980). Data related to cholesterol oxides of heated oil were analyzed by two-way ANOVA within heating. Means of interaction were compared using the PDIFF function of the SAS program (SAS Institute, 1985).

RESULTS AND DISCUSSION

Fatty acid compositions of four oils are summarized in Table 1. The PUFA contents of each oil as a percentage of total fatty acids were 37.9, 79.5, 66.4, and 10.3% for FO, LO, SF, and PO, respectively. FO contained mainly long-chain PUFAs (LCPUFAs); that is, 20:5n−3 and 22:6n−3 at 14.5 and 12.8%, respectively. LO and SO contained 18:3n−3 and 18:2n−6 at 63.4 and 65%, respectively. PO contained palmitic acid (16:0, 43.2%), and total saturates were higher in PO than other oils. PO contained higher levels monoenoic acid (40.7%) when compared to the other oils (Li et al., 1996a).

Total tocopherol content and major tocopherol isomers were different for each oil source (Table 2). LO contained a high level of natural tocopherols (~890 ppm), especially γ- and δ-tocopherol, whereas FO contained only ~19 ppm. The order of magnitude of the natural

tocopherol contents was LO > SO > PO > FO. SO and PO contained α-tocopherol at 211 and 102 ppm, respectively. The level of tocopherol decreased ($p < 0.05$) with time storage (Table 3) and heating (Table 4). No tocopherols were detected in the fish oil after 35 days and trace levels of tocopherols were detected after heating. However, the vegetable oils contained a high level of tocopherols, particularly LO after 35 days of storage and heating. The extent of the decline was positively correlated to the initial concentration of tocopherols in the oils, with the greatest loss occurring in oils with the highest initial content of tocopherols with both storage and heating.

Changes in total cholesterol oxides during storage are shown in Table 5. Cholesterol oxides were not detected initially in the oils except in FO (6−11 ppm). The contents of cholesterol oxides in all oils increased ($p < 0.05$) with storage. FO had the largest level of cholesterol oxides, over 30-fold more than PO at 35 days, followed by SO. SO produced more cholesterol oxides than LO during storage, although LO had a much higher concentration of oxygen-sensitive PUFAs, such as 18:3 n-3. For PO, very low levels of cholesterol oxides were formed under this experimental condition. It may be that LCPUFA (20:5n−3 and 22:6n−3) greatly influenced cholesterol oxidation. The differences in susceptibility of cholesterol oxidation between FO and other vegetable oils may be attributed to different degrees of unsaturation of triacylglycerols. This hypothesis is supported by the fact that a small amount of cholesterol oxides accumulated in PO, which is rich in saturated

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Table 7. Effects of Heating and Tocopherols on Cholesterol Oxidation in Different Food Oils

oils[b]	major COPs (μg/g)[a]						
	total COPs	7α-OH	7β-OH	α-epoxide	β-epoxide	triol	7-keto
Unheated Oils							
FO	11.6	nd	nd	1.2	4.3	nd	6.0
FO+T	9.1	nd	nd	0.8	2.1	nd	6.1
LO	nd	nd	nd	nd	nd	nd	nd
LO+T	nd	nd	nd	nd	nd	nd	nd
SO	nd	nd	nd	nd	nd	nd	nd
SO+T	nd	nd	nd	nd	nd	nd	nd
PO	nd	nd	nd	nd	nd	nd	nd
PO+T	nd	nd	nd	nd	nd	nd	nd
Heated Oils							
FO	152.2[a]	16.8[a]	15.4[a]	31.2[a]	56.5[a]	4.4	32.4[a]
FO+T	75.2[b]	6.1[b]	6.1[b]	15.7[b]	32.0[b]	2.7	15.3[c]
LO	28.5[de]	2.3[c]	1.5[d]	6.8[cd]	11.4[cd]	nd	6.5[de]
LO+T	22.2[e]	2.0[c]	3.7[c]	5.5[cd]	8.8[de]	nd	2.3[e]
SO	56.5[c]	3.6[c]	3.0[cd]	7.7[c]	15.9[c]	nd	26.4[b]
SO+T	40.0[d]	3.4[c]	2.8[cd]	7.7[c]	12.6[cd]	nd	13.5[c]
PO	31.3[de]	2.1[c]	2.0[cd]	4.9[cd]	6.2[ef]	nd	16.0[c]
PO+T	22.0[e]	2.1[c]	1.7[d]	3.3[c]	3.6[f]	nd	11.4[cd]
SEM	4.39	0.66	0.62	1.21	1.65	1.79	

[a] COPs = cholesterol oxidation products. [b] FO = fish oil; FO+T = fish oil + tocopherol; LO = flax oil; LO+T = flax oil + tocopherol; PO = palm oil; PO+T = palm oil + tocopherol; SO = sunflower oil; SO+T = sunflower oil + tocopherol; 7α-OH, 7α-hydroxycholesterol; 7β-OH, 7β-hydroxycholesterol; 7-keto, 7-ketocholesterol; 25-OH, 25-hydroxycholesterol; SEM = standard error of mean; means within same column with no common superscripts (a–f) differ significantly ($p < 0.05$); nd = not detected.

fatty acids. Ohshima et al. (1993) reported that cholesterol oxidation in fish products proceeds in conjunction with oxidative decomposition of coexisting PUFAs of fish oils.

Changes in the concentration of oxidized cholesterol products in the oil occurred during storage and the pattern of these changes differed according to the type of cholesterol oxides being determined (Table 6). The most prevalent oxides formed were 7-ketocholesterol, which confirmed its use as marker of cholesterol autoxidation (Maerker, 1987), and epoxides (α and β). This result was in accordance with published data (Fontana et al., 1993). As reported by Maerker (1987), the ratio of 7α-hydroxycholesterol-to-7β-hydroxycholesterol,-to-7-ketocholesterol was ~1:2:6. No detectable level of 25-hydroxycholesterol and cholestanetriol were found in the oil samples during storage.

Cholesterol oxides in unheated versus heated oils are summarized in Table 7. With the exception of FO, unheated oils had no detectable oxides, but after heating, cholesterol oxides were increased up to 22 to 152 ppm. 7-Ketocholesterol and β-epoxide are predominant in heated oil, and heating generated more cholesterol oxides. FO and SO with added tocopherol contained ($p < 0.05$) lower levels of cholesterol oxides. Addition of tocopherol in LO and PO did not result in any differences (Table 7). Note that heated FO showed the presence of cholestanetriol (Table 7), the most toxic derivative of cholesterol (Blankeship et al., 1991). These studies indicate that temperature is a strong factor in lipid autoxidation. The present study suggests that both cooking temperature and storage environment are equally important factors in producing cholesterol oxides in food oils.

It has been reported that tocopherols delay cholesterol oxidation and protect lipid from autoxidation (Rankin and Pike, 1993). Adding α-tocopherol to oils significantly ($p < 0.05$) reduced the concentrations of cholesterol oxides in FO but had no significant effect on vegetable oils. This result might be related to the high levels of natural tocopherol content in vegetable oils (Table 2). Tocopherols used at relatively low concentrations (100–300 ppm of fat weight) are effective antioxi-

dants in a wide variety of food products, and their effectiveness is known to diminish when used at high levels (>500 ppm of fat weight, Dougherty, 1988). Jung and Min (1990) demonstrated that optimum concentrations of α-, γ-, and δ-tocopherol increased oxidative stability in soybean oil at 100, 250, and 500 ppm, repectively. Sagers (1991) demonstrated that although α-tocopherol inhibited cholesterol autoxidation in an aqueous model system at 0.02 and 0.2%, it exhibited pro-oxidant properties at 2.0%.

Conclusion. Fatty acid composition and tocopherol content in food oils influenced the stability of cholesterol. Fish oil was easily oxidized. Levels of cholesterol oxides in oils were increased by storage and accelerated by the presence of highly long chain PUFAs, whereas tocopherols effectively delayed cholesterol oxidation. Products from sources that are low or deficient in natural antioxidants, like fish oil, can be improved by tocopherol supplementation. Heating is a predominant factor of lipid oxidation. The results of this study suggest that cholesterol oxide formation in commercially processed and stored foods can be prevented by addition of vitamin E or a natural form of tocopherols, which would benefit the food industry and human health.

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