Oxidation of Phytosterols in a Test Food System

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ABSTRACT: The oxidative stability of phytosterols in canola, coconut, peanut, and soybean oils was examined under simulated frying conditions of 100, 150, and 180°C for 20 h. The degree of oxidative decomposition was assessed by the loss of phytosterols, accumulation of phytosterol oxides, and the change in fatty acid profiles. The phytosterol oxides produced in the oils were identified using mass spectroscopy. Oils with higher levels of polyunsaturated fatty acids showed greater amounts of sterol loss; however, the sterol loss was less complete than in the more saturated oils. A greater variety of sterol oxides was observed at the lower temperatures of 100 and 150°C compared to 180°C. This study demonstrates that under conditions similar to frying, there is a loss of phytosterols and polyunsaturated fatty acids. The accumulation of phytosterol oxides may be temperature-limited because of further breakdown into products not measurable by typical gas chromatography–mass spectrometry techniques.

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KEY WORDS: Canola oil, coconut oil, frying vegetable oils, heating effects, peanut oil, phytosterol oxides, soybean oil.

Phytosterols, or plant sterols, are minor components of vegetable origin. They function as structural components in membrane lipids and as precursors to steroid hormones. They are analogous and similar in chemical structure to cholesterol in animal lipids. Because of these similarities, cholesterol and phytosterols are expected to undergo similar chemical reactions including oxidation. Cholesterol has been found to undergo oxidative changes, with the resulting products causing deterioration of lipid quality, producing cytotoxic and mutagenic compounds and affecting cholesterol metabolism (1). A number of research studies have quantified the generation of cholesterol oxides in fats, cell components, and finished food products (2–4). These reports focused on the tendency of cholesterol-containing foods and ingredients to oxidize during processing. Reports on phytosterol oxidation are limited to isolated pure sterol compounds and certain edible oils (5–7). As the Western diet has changed from animal fats as the major frying fat sources to vegetable oils, an increase in the amount of phytosterols ingested has occurred (8).

Autoxidation of sterols is theorized to be a free radical process (3). The conditions of common food-processing operations involve contact with light, heat, air, water, and metal ions. All of these conditions are suited to initiate the chain of events leading to free radical formation. Some processes, such as deep frying, intentionally induce oxidation to produce or protect preferred flavor components (9). The higher polyunsaturated fatty acid (PUFA) levels in vegetable oil compared to animal fats provide the opportunity for greater exposure of the phytosterols present to oxidation by free radicals (10).

Earlier research attributing cholesterol as the cause of arterial lesions in rabbits has since been more closely examined, repeated, and discussed as to the validity of the data (11). More recently, investigators have concluded that the presence of cholesterol oxidation products (COPS) in the cholesterol fed to the animals was the major cause of the arterial lesions (12). COPS have been shown to be absorbed from foods by humans (13). Cholesterol oxides are implicated in the onset of arteriosclerosis and coronary heart disease in humans, whereas the epoxides of cholesterol have been found to be mutagenic (14,15).

As phytosterols differ from cholesterol in structure only by the addition of small hydrocarbon moieties on the side chain, research is needed to establish the conditions that will initiate the oxidation of phytosterols and to determine if phytosterol oxidation products are similar in toxicity to COPS. Studies at the cellular level indicate that damage does occur but is less severe with phytosterol oxides than with COPS (16). The basic four-ring structure where most of the oxidation of cholesterol occurs is the same in phytosterols.

The increased use of vegetable oils for frying in place of animal fat and animal fat blends provides increased opportunities for the production of phytosterol oxidation products from oil-containing foods. This study examined the effects of temperature, oxygen, and metal contact surfaces on the oxidation of vegetable oils containing varying levels of PUFA and phytosterols. Canola, coconut, peanut, and soybean oils were subjected to heat treatments at temperatures comparable to those used for deep-fat frying and evaluated for phytosterol content, fatty acid content, and the presence of phytosterol oxides.

EXPERIMENTAL PROCEDURES

Project design. The goals of this project were to determine the effects of selected processing conditions on the oxidation of phytosterols and PUFA and to examine the breakdown

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products of phytosterol oxidation. During frying, fat-containing foods are subjected to heat with oil being used as the heat transport medium. To imitate this, we designed model test conditions to simulate the use of oils of varying composition and applied different temperature conditions in the presence and absence of metal ions and oxygen. The inherent levels of the major phytosterols, campesterol, stigmasterol, and β-sitosterol, and the fatty acid profiles were measured using gas chromatography (GC). The extent of oxidation was measured by examining changes in the phytosterols and fatty acids following the application of the experimental treatments. Peanut oil was used to optimize the test conditions, whereas oils of varying composition were used to determine the effects of oil composition on phytosterol oxidation. As oxidation products occur as minor constituents during the processing of oil-containing foods, an enrichment model was also used in this study to facilitate recovery of the phytosterol oxides for analysis. The β-sitosterol was used as the enrichment sterol. Oxidation compounds were identified in the treated samples using GC–mass spectrometery (GC–MS). Following oxidation and prior to GC–MS analysis, solid-phase extraction (SPE) was used to remove any unoxidized phytosterols and to further concentrate the levels of phytosterol oxides.

Samples. Canola (Ventura Foods, Baton Rouge, LA) and soybean oils (Procter & Gamble, Cincinnati, OH) were purchased from local grocery stores. Coconut oil was USP grade (Humco Laboratories, Texarkana, TX). Peanut oil (Twelve Baskets Sales and Marketing, Mableton, GA) was provided by the USDA, ARS, Market Quality and Handling Branch Unit at North Carolina State University (Raleigh, NC).

Reagents. Solvents and chemicals were of analytical grade and were purchased from EM Science (Gibbstown, NJ) with some exceptions. Absolute ethanol was purchased from Spectrum Chemical Corporation (Gardena, CA). Pyridine and boron trifluoride (14% in methanol) were purchased from Sigma Chemical Corporation (St. Louis, MO). Regisil, a derivatizing reagent composed of bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS), was purchased from Regis Technologies (Morton Grove, IL). The internal standard, 5-α-cholestane, and standards of the cholesterol oxides, 7-ketocholesterol and 5-α, 6-α-epoxycholesterol were purchased from Sigma. Standards of fatty acid methyl esters (FAME) were purchased from Alltech Corporation (Deerfield, IL). A sterol standard mixture consisting of cholesterol, brassicasterol, campesterol, stigmasterol, and β-sitosterol was purchased from the Matreya Company (Pleasant Gap, PA).

Design of the oxidation test system. In an effort to establish the conditions sufficient to cause the oxidation of the phytosterols present, three test systems were examined. These involved the heating of peanut oil to induce oxidation in (i) an Oxidative Stability Instrument (OSI) (Omnion, Rockland, MA) at 100°C, with the airflow set at 5 psi, (ii) using an Active Oxygen Method apparatus (AOM) set at 98.7°C, with the airflow at 20 L/min, and (iii) heating of peanut oil in a forced draft oven (Grieve Corp., Round Lake, IL) using aluminum pans. The AOM was built at Southern Testing Laboratories (Wilson, NC) to the specifications of Method Cd 12-57 of the AOCS (17).

Application of the oxidation test system. Canola, coconut, peanut, and soybean oils were oxidized by heating in metal pans using a forced draft oven. Samples were heated in aluminum weighing dishes for 20 h at 100, 150, and 180°C. The first temperature was chosen, as that is the temperature at which the OSI and AOM are normally run; also, this is a low temperature that oil-containing foods might be subjected to during processing. The 180°C temperature was chosen to simulate deep-fat frying, and 150°C was chosen as a midpoint between the OSI/AOM determination and frying temperatures. Following each heat treatment, a portion of each oil was evaluated for fatty acid content using GC. A second portion was cold-saponified, extracted, derivatized, and analyzed for the phytosterol content using GC.

Fortification of samples to monitor phytosterol oxide production. In another series of analyses, the four oils in the study, canola, coconut, peanut, and soybean were fortified by adding an additional 90 mg/100 g of β-sitosterol before heat treatment for analysis by GC–MS. The oils were then used in the test system described above to determine the types of phytosterol oxides produced under these conditions. The addition of phytosterol was made in order to raise the amounts of oxidation compounds above the detection limit of the GC–MS system (0.1 mg/100 g).

Analysis. (i) Analysis of fatty acid profiles. Samples were analyzed before and after treatment for fatty acids as FAME. Samples were hydrolyzed and methylated using boron trifluoride (14% in methanol) according to AOCS Method Ce 1b-89 (17). The methyl esters were analyzed using a Perkin-Elmer Autosampler System gas chromatograph (Perkin-Elmer, Norwalk, CT) equipped with a flame-ionization detector. A fused-silica capillary column RTX-2330 (Restex, Bellefonte, PA) of 105 m × 0.25 mm with 0.20 mm film thickness was used. Helium was used as the carrier gas at a flow rate of 0.7 mL/min. Temperature programming was used with an initial temperature of 100°C and held for 2 min. The temperature was increased at 10°C per min to 200°C and held for 0.5 min. The temperature was then increased by 4°C per min to a final temperature of 260°C and held for 8.5 min. The injector temperature was 230°C, and the detector was run at 265°C. The split ratio of the injection was 3:1. The identification of the FAME was done by comparison of sample peaks to the retention times of FAME standards. Fatty acid content was expressed as the weight percentage of the total FAME. Normalization technique was used to calculate the response factor for all identified fatty acid peaks based on the composition of the FAME standards used as the reference (17).

(ii) Analysis of phytosterols. All oil samples were analyzed before heat treatment for their inherent phytosterol content (campesterol, stigmasterol, and β-sitosterol) to establish the starting levels. These samples were saponified and extracted according to AOAC Method 970.50 (18). The total unsaponifiables recovered were derivatized to trimethylsilyl

(TMS)-ethers for subsequent analysis by GC where comparisons were made with the retention times of phytosterol standards derivatized in the same manner as the samples described in the section discussing preparation of the TMS-ether derivatives.

After heat treatment, samples were cold-saponified and extracted according to Dutta and Åppelqvist (19). This was done to avoid preparation artifacts caused by oxidation occurring as a result of additional heat and air exposure and to ensure that all oxides produced were, in fact, a result of the treatment effects. The extract was taken to dryness under nitrogen and analyzed for intact sterols after derivatization to TMS-ethers.

(iii) Analysis of phytosterol oxides. Oil samples that had previously been enriched by the addition of β-sitosterol were subjected to the heat treatments using the oven and the metal pans. The samples were then cold-saponified and extracted as described for the heat-treated samples in the previous section. Extracts were purified by SPE of unoxidized phytosterols and enriched in sterol oxides using 0.5-g silica cartridges (JT Baker, Phillipsburg, NJ) (17). The final eluant was concentrated under nitrogen to dryness. TMS-ethers of the oxides were prepared as described in the next section and analyzed by GC–MS. The application of SPE provides an additional step to remove unoxidized sterols and to concentrate the oxides that are generally present in small quantities (ppb vs. ppm for sterols).

(iv) Preparation of TMS-ether derivatives of sterols and their oxides. After extraction and drying, the samples containing phytosterols or the oxides were brought up in 1 mL of hexane containing 20 ppm of 5-α-cholestane as the internal standard. Solutions were transferred to GC vials, and 100 μL each of pyridine and Regisil was added. The vials were capped, vortexed, and placed in a heating block at 50°C for 1 h. Sterols and sterol oxides were then analyzed by GC and GC–MS, respectively.

(v) Capillary column GC analysis of unoxidized phytosterols. The TMS-ether derivatives were analyzed using the PerkinElmer Autosampler System GC described above. A fused silica capillary column RTX-50 (Restex) 30 m × 0.32 mm with 0.25 μm film thickness was used. Helium was used as the carrier gas at a flow rate of 2.1 mL/min. Temperature programming was used with an initial temperature of 100°C and held for 0.2 min. The temperature was increased at 10°C per min to a final temperature of 290°C and held for 8 min. The injector temperature was 230°C, and the detector was 300°C. Identification of the phytosterols was done by comparison of the retention time of the sample peaks to the standard sterol mixture derivatized using the same technique used for the samples. Response factors were calculated using 5-α-cholestane as an internal standard.

(vi) Capillary column GC–MS analysis of phytosterol oxides. GC–MS analyses were performed on a Hewlett-Packard model 5890 gas chromatograph (Hewlett-Packard, Avondale, PA) coupled to a VG Triol mass spectrometer with a LAB-BASE™ data system (Fisons Instrument, VG MASS LAB, Manchester, England). The GC conditions were identical to those described in the section above. The mass spectra were recorded at an electron energy of 70eV, and the ion source temperature was 200°C.

(vii) Statistical analysis. A General Linear Model was used to analyze the data as a randomized complete block design with treatments blocked by temperature. Mean differences were determined using Duncan's Multiple Range Test for separation of means showing significant differences ($P < 0.05$) (20).

RESULTS AND DISCUSSION

Oxidation systems. This research initially evaluated the ability of standard systems, such as the OSI method and the AOM, that have traditionally been used to monitor oxidation in edible oils for their ability to produce measurable amounts of phytosterol oxides. Previous studies have shown that cholesterol will oxidize at ambient storage conditions in air over long periods of time (i.e., several years), whereas phytosterols appear to be more resistant (1). In a preliminary study, peanut oil was heated in an OSI for up to 96 h; the decrease in phytosterols levels was only 36% for campesterol and 30% for β-sitosterol, indicating that their oxidation was not complete (data not shown). Based on this result, it was felt that a comparison of several oxidation systems needed to be evaluated in order to select a model that would represent a worse-case scenario. As indicated in the previous section, three test systems (OSI, AOM, and a forced-draft oven) were selected to test. It was also felt that contact with a metal surface would be necessary; therefore, the oven system was modified to substitute aluminum pans for the glass tubes of the OSI and the AOM in this study.

The data collected from this evaluation of a test system showed that the inherent phytosterols studied decreased in a manner similar to that observed for the linoleic acid content in the peanut oil (Table 1). For example, β-sitosterol decreased by 33.2% when heated 48 h in an oven but only by 4.72% in the AOM for the same amount of time. Similar results were seen when the OSI was used. After 100 h, the AOM treatment produced a decrease in the β-sitosterol content of 30.8%, whereas 70.2% was lost in oven-heated samples in metal pans over the same time period.

TABLE 1
Levels of Phytosterols (mg/100 g) and Linoleic Acid (%) in Peanut Oil After Heating at 100°C Using Test Systems

Treatment	Time (h)	Campesterol	Stigmasterol	β-Sitosterol	Linoleic acid
None	0	30.3	14.8	122.1	29.9
AOM[a]	48	22.4	10.4	116.3	29.7
OSI[b]	48	19.4	9.9	83.7	n.a.[d]
Oven[c]	48	15.7	6.4	81.5	16.8
AOM[a]	100	15.3	7.7	84.5	18.3
Oven[c]	100	7.1	2.8	36.4	3.5

[a]AOM refers to samples heated using an Active Oxygen Method Apparatus.
[b]OSI refers to samples heated using an Oxidative Stability Unit.
[c]Oven refers to samples heated in aluminum pans in a forced draft oven.
[d]n.a. indicates the sample was not analyzed for that parameter.

The addition of metal contact was effective in increasing the rate and severity of the oxidation of the PUFA. Table 1 shows the loss of linoleic acid from peanut oil following heating in the AOM and in the metal pans in an oven. After 48 h of AOM treatment, the linoleic acid level was not significantly different from the unheated oil. However, heating in metal pans for 48 h produced a decrease of 43.8% in the linoleic acid level. After 100 h of oven treatment, only 3.54% of the linoleic acid remained. Similar treatment with the AOM technique at 100 h showed a decrease in the linoleic acid of 39.4%, which is close to the loss with the oven treatment at 48 h.

Table 1 shows that of these test systems used to examine oxidation of phytosterols, the oven method was the most effective in causing loss of phytosterols and oxidation of PUFA. The conditions of the OSI and the AOM were not sufficient to cause significant losses of phytosterols or the oxidation of the PUFA. The additional metal catalysis and surface exposure to air increased the oxidation of the unsaturated fatty acids in the oil. The oven oxidation proceeded nearly twice as fast as shown by the two treatments at 48 h. A comparison of the values at 100°C shows that the oxidation was much more complete; the linoleic acid was nearly gone (Table 1). Based on this research, additional studies were done using the test system consisting of oils exposed to oxidizing conditions using metal pans in a forced draft oven.

Effects of the oxidation test system on selected oils. The changes in fatty acid composition of the four test oils were monitored with heating at 100, 150, and 180°C for 20 h using a forced-draft oven and metal pans. The oxidation of the plant sterols was monitored by the loss of the compounds, campesterol, stigmasterol, and β-sitosterol at each temperature. Table 2 lists the amounts of sterols remaining in each oil after heating. Figure 1 shows the loss of linoleic acid in each oil sample with increasing temperature. Linoleic acid was plotted as representative of the PUFA content of each oil following heat treatment. The oils examined were canola, a very highly unsaturated oil, and coconut, an oil very low in unsaturated fatty acids. Peanut and soybean oils were chosen as common oils used for deep frying that contain relatively high amounts of unsaturated fatty acids, but less than canola oil (21). The time of heating was chosen as that close to the oxidative stability index for peanut oil previously determined by OSI defining the resistance of the sample to oxidation (18.9 h) (data not shown). At this point, the oil would have lost its functionality as defined by the oxidative stability index.

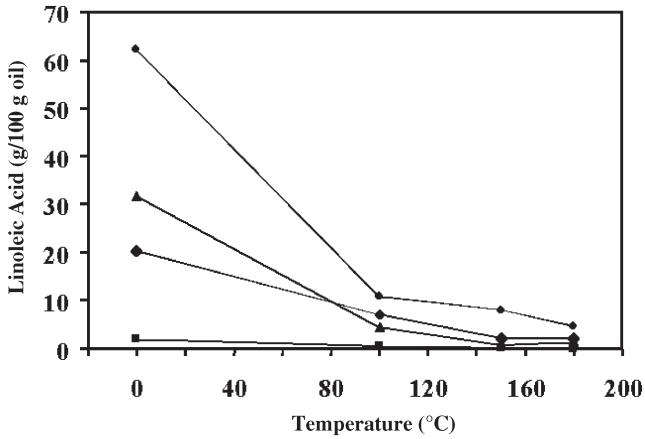

FIG. 1. Changes in linoleic acid content in oils tested with heat treatments: ◆ Canola oil, ■ coconut oil, ▲ peanut oil, ● soybean oil.

Canola oil was the most highly unsaturated of the four oils tested. It contained less than 10% saturated fatty acids. Most of these fatty acids (60%) were monounsaturated; therefore, it was not the highest in PUFA of the oils in the study. Canola oil contained the highest levels of phytosterols, 550 mg of phytosterols per 100 g of oil compared to soybean with 317 mg/100 g and peanut oil with 167 mg/100 g. The fatty acids in coconut oil were 98% saturated, and the phytosterol content was 57.5 mg/100 g, the lowest of the oils tested. Soybean oil contained the highest level of PUFA with 62%, whereas monounsaturated fatty acids constituted 22%; the peanut oil contained 32% PUFA. Comparison of the rates of linoleic acid decrease (Fig. 1) revealed that oils higher in linoleic acid content showed higher rates of decrease compared to more saturated oils. However, at 180°C, the amount of linoleic acid remaining in the oils was less than 5% in each case.

In Table 2, the data for the plant sterols campesterol and β-sitosterol recovered from canola oil after heat treatment showed decreases with increasing temperature. Canola oil does not naturally contain stigmasterol. The losses at 100°C were 32% for campesterol and 33% for β-sitosterol. At 150°C, the losses were even greater at 94% for campesterol and 95% for β-sitosterol. The losses at 180°C were slightly more than those at 150°C, but at both of these temperatures, few of the original sterols remained.

To compare an oil lower in sterols, as well as low in PUFA, coconut oil was included in the study. The total losses in

TABLE 2
Levels of Phytosterols (mg/100 g) in Oils Tested After Heating 20 h in Forced Draft Oven Using Metal Pans[a]

Oil	Treatment (°C)	Campesterol	Stigmasterol	β-Sitosterol
Canola	None	226.5^{A}	$<1.0^{A}$	323.4^{A}
	100	154.2 ± 28.8^{B}	$<1.0^{A}$	215.8 ± 15.3^{B}
	150	13.7 ± 1.3^{C}	$<1.0^{A}$	17.8 ± 0.5^{C}
	180	13.1 ± 2.0^{C}	$<1.0^{A}$	11.5 ± 1.7^{D}
Coconut	None	6.7^{A}	11.5^{A}	39.3^{A}
	100	6.2 ± 0.0^{A}	8.5 ± 0.5^{B}	33.0 ± 0.2^{B}
	150	0.3 ± 0.0^{B}	0.3 ± 0.0^{D}	0.4 ± 0.1^{D}
	180	0.2 ± 0.0^{B}	0.7 ± 0.0^{C}	0.8 ± 0.1^{C}
Peanut	None	30.3^{A}	14.8^{A}	122.1^{A}
	100	12.0 ± 0.1^{B}	5.3 ± 0.0^{D}	37.1 ± 0.2^{B}
	150	3.8 ± 0.1^{D}	6.5 ± 0.2^{C}	17.8 ± 0.4^{C}
	180	5.0 ± 0.1^{C}	10.2 ± 1.1^{B}	5.1 ± 0.0^{D}
Soybean	None	68.8^{A}	64.0^{A}	183.8^{A}
	100	30.4 ± 0.5^{B}	20.6 ± 1.3^{B}	69.8 ± 1.5^{B}
	150	16.4 ± 0.4^{C}	19.7 ± 0.4^{B}	28.0 ± 0.1^{C}
	180	9.9 ± 0.2^{D}	1.5 ± 0.0^{C}	11.9 ± 0.3^{D}

[a]Numbers followed by the same capital letter were not significantly different between treatments of that oil at $P < 0.05$ ($n = 3$) for that column.

sterols were greater in canola and soybean oils than in the more saturated oils. After treatment at 100°C, only 8% of the campesterol, 26% of the stigmasterol, and 16% of the β-sitosterol were lost from coconut oil. When coconut oil was heated at 180°C, the losses were more complete; only 3, 6, and 2% of the campesterol, stigmasterol, and β-sitosterol, respectively, remained.

The observed trend is that oils higher in PUFA still retained more sterols after heat treatment at 100 and 150°C than the saturated oils. However at 180°C, the losses were over 90% in almost every case, as listed in Table 2. Of the oils tested, soybean oil had the highest level of PUFA. The decrease in the sterol concentrations in the soybean oil was nearly linear with increasing heat treatment. In soybean oil, losses of campesterol were 56, 76, and 86% at 100, 150, and 180°C, respectively. Similar decreases were seen with β-sitosterol, whereas stigmasterol decreased 68 and 69% at 100 and 150°C, respectively, and near total loss (98%) at 180°C.

The peanut oil used in the study contained only half the concentration of linoleic acid found in soybean oil (31.63% vs. 62.05%). Sterol concentrations were found to have decreased more in the peanut oil with the heat treatment as compared to soybean oil (Table 2). The initial temperature of 100°C produced losses of 60, 64, and 88% for campesterol, stigmasterol, and β-sitosterol, respectively. Equivalent losses of sterols occurred at the higher temperatures, 150 and 180°C. For example, β-sitosterol losses were 97% at 150°C and 96% at 180°C. The slightly higher values for campesterol and stigmasterol after treatment at 180°C compared to those at 150°C may be indicative of interference in the chromatogram rather than genuine differences in oxidation.

As previously discussed, the data show that the higher the PUFA content, as represented by linoleic acid, the less the absolute oxidation of phytosterols with heat treatment at 100°C. In a study of the production of COPS as related to PUFA in fish oil model systems, Li and coauthors (22) found that the amount of COPS produced increased with increasing PUFA content. They did not report values for intact cholesterol. In our work, at frying temperatures of 180°C, losses of greater than 90% were seen for all the sterols regardless of the oil present.

Phytosterol oxides. The GC–MS analysis revealed that phytosterol oxides were produced in all the oils spiked with β-sitosterol as listed in Table 3. Using published mass spectra from the literature (7,19), identifications of various oxides were made. As expected, the recoverable levels of the oxides were low. The amount of oxides in Table 3 did not add up to the amount of sterols lost. This may be because of a number of factors. At a minimal detection level of 0.10 mg/100 g, the background spectra from the column bleed begin to overwhelm the analyte spectra. Sterols may also have been broken down to smaller components than oxides that were not detectable by GC–MS. This is especially applicable at the higher temperatures. Kim and Nawar (23) in their study of COPS attributed the difference to polymer formation rather than oxide formation by the cholesterol.

The oxides found are analogous to the major products produced by cholesterol when oxidized by heat and air. Phytosterols appear to follow the common routes that have been described for the oxidation of cholesterol (3). The major site of oxygen attack is on the C-7 on the B-ring to form hydroperoxides, hydroxy and keto sterols. Oxygen is capable of adding across the double bond between C-5 and C-6 to form epoxides and triols. Each of the phytosterols examined in this study, campesterol, stigmasterol, and β-sitosterol, produced recoverable amounts of hydroperoxides, epoxides, and ketones. Although the side chain is capable of taking on oxygen at C-20 or C-25, no side-chain oxidation products were recovered.

TABLE 3
Phytosterol Oxides (mg/100 g) Identified in Enriched Oil Samples After Heating 20 h in Forced-Draft Oven Using Metal Pans[a]

Oil	Phytosterol oxide	100°C	150°C	180°C
Canola	5-α,6-α-Epoxycampesterol	1.05	n.d.	n.d.
	5-β,6-β-Epoxycampesterol	2.24	n.d.	1.50
	5-α,6-α-Epoxysitosterol	3.76	n.d.	n.d.
	7-α-Hydroxysitosterol	0.76	1.53	n.d.
	7-Ketocampesterol	n.d.	0.25	n.d.
	7-Ketositosterol	n.d.	n.d.	0.63
Coconut	5-α,6-α-Epoxysitosterol	0.93	0.10	n.d.
	7-Ketositosterol	0.82	0.16	n.d.
Peanut	5-α,6-α-Epoxycampesterol	0.30	n.d.	n.d.
	5-β,6-β-Epoxycampesterol	0.28	n.d.	n.d.
	5-α,6-α-Epoxystigmasterol	0.22	0.13	n.d.
	5-β,6-β-Epoxysitosterol	1.62	0.13	n.d.
	7-Ketositosterol	1.13	n.d.	n.d.
Soybean	5-α,6-α-Epoxycampesterol	0.55	0.26	n.d.
	5-β,6-β-Epoxycampesterol	0.78	0.33	n.d.
	5-α,6-α-Epoxystigmasterol	0.36	0.17	n.d.
	5-β,6-β-Epoxystigmasterol	0.24	0.14	n.d.
	5-α,6-α-Epoxysitosterol	1.07	0.79	0.21
	7-α-Hydroxysitosterol	n.d.	0.12	1.35
	7-Ketositosterol	0.45	n.d.	n.d.

[a]n.d., indicates none detected at a limit of 0.10 mg/100 g.

The trend presented by the data in this study indicates that as the temperature increased, fewer phytosterol oxides were recovered. This implies that the extreme heat treatments resulted in total degradation of the sterol complexes. Also, the greater the proportion of unsaturated fatty acids present in the oil, the more oxides recovered. This would imply that the types and quantities of fatty acids may influence phytosterol oxidation in oils.

Approximately 60% of the sterols in a plant lipid exist as esters with fatty acids in an oil system (24). Figure 2 illustrates this complex as a generalized structure. Smith (25) has postulated that the sterol ester structure may create a physical or a steric barrier to oxidation, in that oxygen will act on the fatty acid first, slowing the attack on the sterol. Oxidation of cholesterol in meat samples was found to be promoted by the oxidation of the PUFA present (26). However, these fatty

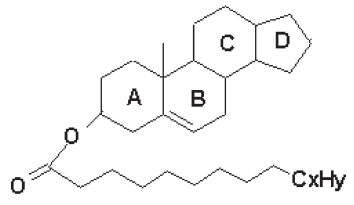

FIG. 2. Schematic diagram of a sterol bound as a fatty acid ester.

acids were not esterified to the cholesterol in that study. When the fatty acid is saturated, the oxygen will proceed to the sterol without being inhibited by the fatty acid. When the fatty acid is unsaturated, the oxygen will oxidize the fatty acid first, followed by oxidation of the sterol (25). This would indicate that a highly unsaturated oil is able to use more oxygen before the total destruction of the sterols through an oxidative pathway. Thus, when an unsaturated oil such as soybean is subjected to the oxidative stresses described above, it is possible to recover more intact sterols and more sterol oxides, when compared to a highly saturated oil such as coconut. The data in this study fit well into Smith's theory that the more unsaturated oils showed less complete loss of sterols and greater accumulation of sterol oxides than more saturated oils. The greater accumulation of sterol oxides occurred in the oils containing more PUFA, whereas greater degradation of the oxides occurred in the more saturated oils resulting in less recovery of both intact sterols and sterol oxides.

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