Studies on Phytosterol Oxides. II: Content in Some Vegetable Oils and in French Fries Prepared in These Oils[1]

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ABSTRACT: Hydrogenated rapeseed oil/palm oil blend, sunflower oil and high-oleic sunflower oil, and French fries fried in these oils were assessed for contents of sterol oxidation products. Different oxidation products of phytosterols (7α- and 7β-hydroxy-sito- and campesterol, 7-ketosito- and 7-ketocampesterol, 5α,6α-epoxy-sito- and campesterol, 5β,6β-epoxy-sito- and campesterol, dihydroxysitosterol and dihydroxycampesterol) were identified and quantitated by gas chromatography (GC) and GC–mass spectroscopy. Rapeseed oil/palm oil blend contained 41 ppm total sterol oxides before frying operations. After two days of frying, this level was increased to 60 ppm. Sunflower oil and high-oleic sunflower oil had 40 and 46 ppm sterol oxides, respectively, before frying operations. After two days of frying operations, these levels increased to 57 and 56 ppm, respectively. In addition to campesterol and sitosterol oxidation products, small amounts of 7α- and 7β-hydroxystigmasterol were detected in the oil samples. Total sterol oxides in the lipids of French fries fried at 200°C in rapeseed oil/palm oil blend, sunflower oil, and high-oleic sunflower oil were 32, 37, and 54 ppm, respectively. The levels of total oxidized sterols, calculated per g sample, ranged from 2.4 to 4.0 ppm. In addition to the content of phytosterol oxides, full scan mass spectra of several oxidation products of stigmasterol are reported for the first time.

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KEY WORDS: Campesterol oxides, frying oils, high-oleic sunflower oil, rapeseed oil/palm oil blend, sitosterol oxides, stigmasterol oxides, sunflower oil, vegetable oils.

Vegetable oils contain varieties of sterols, commonly known as phytosterols (1), whereas in animal fats cholesterol predominates. Cholesterol is susceptible to oxidation, and a large number of cholesterol oxides in foods and biological materials have been identified (2–4). Phytosterols are structurally analogous to cholesterol, and different oxidation products of phytosterols have been reported in foods that contain vegetable oils (5). During refining of vegetable oils, various chemical, physical, and temperature treatments are applied. Each of these refining processes exerts effects on the sterol composition (1). A few studies have shown that crude rapeseed oil, as well as rapeseed and soybean oils during industrial refining, contain 7-hydroxysitosterol and 7-ketositosterol. However, these studies mainly demonstrated qualitative data on these oxides (6,7). Several model studies on the oxidation of sitosterol, and one study with stigmasterol in different fats and oils media, have been published (8–11). Modification of sterols to different polar and nonpolar oxidation products during refining of vegetable oils, and in the food products prepared in these vegetable oils, has recently been reviewed (12).

Lee *et al*. (13) investigated sterol epoxides in lipids from French fries prepared in mixed animal fats and vegetable oils. The content of mixed cholesterol and sitosterol epoxides was measured by a colorimetric method and by high-performance liquid chromatography (HPLC) with refractive index detection. None of the methods, however, was able to resolve cholesterol and sitosterol epoxides. The quantity of total sterol epoxides ranged from 162 to 439 μg/g lipid in the colorimetric method, but much lower amounts of these components were found in the same samples, ranging from 2 to 65 μg/g lipid, when quantitated by HPLC. In another study from the same laboratory, α-epoxide, β-epoxide, 7α-hydroxy, and 7β-hydroxy sterols (mixture of cholesterol and phytosterol oxides) were quantitated by HPLC (14). The total amounts of these sterol oxides in the lipids of French fries, prepared in mixed beef tallow and hydrogenated vegetable oils, from five restaurants ranged from 3 to 111 μg/g lipid. Park and Addis (15) used HPLC to determine the content of 7-keto-, 7α-hydroxy, and 7β-hydroxy cholesterol in French fries prepared in tallow. Zhang *et al*. (16) surveyed cholesterol oxidation products in French fries that were sampled from a restaurant every day during a period of 30 d. The level of total cholesterol oxides varied daily from 11 to 50 ppm in samples analyzed by gas chromatography (GC) and GC–mass spectroscopy (MS). The major sterol oxides from one restaurant were 5α-cholestane-3β, 5,6β-triol, 7-ketocholesterol, and 25-hydroxycholesterol. Although the report mentioned that the French fries were prepared in animal–vegetable shortening, no separate results on phytosterol oxides, if present, were reported.

In a parallel study, we investigated the content of different oxidation products of campesterol and sitosterol in potato chips fried in different vegetable oils (17). During that study,

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several components could not be identified owing to nonavailability of oxidation products of phytosterols, other than campesterol and sitosterol. As part of the present study, different oxidation products of stigmasterol were synthesized. Stigmasterol, a desmethylsterol widely distributed in vegetable oils, differs structurally from sitosterol by having an additional double bond at the C_{22} position (1). The few reports mentioned above on phytosterol oxidation products deal mainly with sitosterol oxides, and one report deals with both sitosterol and campesterol oxides (18). Only one report has been published on stigmasterol oxidation in model systems (11). Eight possible oxidation products of stigmasterol were isolated by preparative thin-layer chromatography (TLC) in that study. The oxidation products of stigmasterol were generated by heating 5% stigmasterol in purified triolein at 180°C for 48 h. These products were stigmasta-3,5,22-triene, stigmasta-3,5,22-trien-7-one, stigmasta-4,22-dien-3-one, stigmasta-4,6,22-trien-3-one, 5,6-epoxy-stigmasterol, stigmasta-5,22-diene-3β,7β-diol, stigmasta-5,22-diene-3β,7α-diol, and 5α-stigmast-22-ene-3β,5,6β-triol. However, only a few of these components, which are mainly nonpolar compounds, were identified by mass spectral analyses. These identified components were 3β-hydroxy-pregn-5-en-20-one, stigmasta-4,22-dien-3-one, stigmasta-3,5,22-trien-7-one, and stigmasta-3,5,22-triene.

Growing interest in vegetable oils for utilization as frying media has led to the development of varieties of vegetable oils with increased content of monounsaturated fatty acid (19). This investigation was undertaken as a part of a multinational EU (European Union) project on the content of phytosterol oxides in a blend of hydrogenated rapeseed oil/palm oil, in sunflower oil, and in high-oleic sunflower oil before and during industrial preparation of French fries. Quantitative data on different polar phytosterol oxides in some refined vegetable oils are presented in this report. Identification and quantitation of different phytosterol oxides in the lipids of French fries prepared in these oils are also reported. In addition, several oxidation products of stigmasterol were synthesized, and the full-scan mass spectra of those authentic samples of stigmasterol oxides as their trimethylsilyl (TMS) ether derivatives are reported for the first time in this paper.

EXPERIMENTAL PROCEDURES

Samples. Samples of French fries, produced in different vegetable oils, were prepared by the Raisio Group, Finland (20). The French fries were prefried at 180°C for *ca*. 30 s in an industrial frying process and were frozen after production. The frozen samples were packed in 500-g plastic bags and stored at –20°C until they were transported to Sweden in frozen condition and maintained at –20°C until analysis. Prior to extraction, samples were prepared for consumption by heating the fries at 200°C for 15 min in a laboratory oven.

Reagents. A standard sample of sitosterol (65% sitosterol and 30% campesterol) was purchased from Research Plus Inc. (Bayonne and Denville, NJ); 7α- and 7β-hydroxycholesterol and 5β,6β-epoxycholesterol were purchased from Steraloids Inc. (Wilton, NH); campesterol, stigmasterol, 7-ketocholesterol, 5α,6α-epoxycholesterol, 19-hydroxycholesterol, 5α-cholestane, and stearic acid were purchased from Sigma Chemical Company (St. Louis, MO); *m*-chloroperbenzoic acid was purchased from Fluka Chemie AG (Buchs, Switzerland). Acetone, sodium chloride, sodium hydroxide, sodium sulfate, and trisodium phosphate were purchased from Merck (Darmstadt, Germany); sodium bicarbonate was purchased from Kebo Lab AB (Stockholm, Sweden); potassium hydroxide was purchased from EKA (Bohus, Sweden). Cerium sulfate hydrate was from Aldrich-Chemie (Steinheim, Germany), and phosphomolybdic acid hydrate was purchased from Aldrich Chemical Company Ltd. (Dorset, England). Dichloromethane was purchased from Fisons (Loughborough, England); hexane, chloroform, diethyl ether, and acetic acid were purchased from Prolabo (Paris, France); cyclohexane was purchased from Riedel-de Haen AG (Seelze, Germany); and ethanol was purchased from Kemetyl (Stockholm, Sweden). All chemicals and solvents used were of analytical grade.

Synthesis of phytosterol oxides. For the preparation of campesterol, sitosterol, and stigmasterol oxides, different methods were used, and detailed descriptions of synthesis are given elsewhere (17).

Extraction of lipids from French fries. In brief, a 40-g sample of French fries was homogenized by an Ultra-Turrax T25 homogenizer (Jankel & Kunkel GmbH, Staufen, Germany) with 100 mL of hexane/2-propanol (HIP) (21) at maximum speed for 30 s. The homogenates were filtered. This procedure was repeated. The combined filtrates were collected in a separatory funnel and were mixed with 100 mL of 6.67% anhydrous Na_2SO_4 dissolved in water. The upper phase was collected and evaporated to dryness under vacuum at 30°C on a rotary evaporator. The required amount of lipids was saponified, according to the method described below, for further analysis or dissolved in chloroform and stored at –20°C until further analysis.

Methods of saponification for sterol oxide analyses are described elsewhere, except that before saponification 10 μg 19-hydroxycholesterol was added and before TMS derivatization 10 μg 5α-cholestane was added (17). TLC enrichment of sterol oxides from total unsaponifiables by solid-phase extraction (SPE), preparation of TMS-ether derivatives of sterol oxides, capillary column GC analysis of sterol oxides, and GC–MS for identification of sterol oxides are described in detail elsewhere (17).

RESULTS AND DISCUSSION

The most crucial step in the analysis of sterol oxides is the enrichment of the sterol oxide fraction from the unoxidized sterols. While oxidation products of cholesterol are well separated from the unoxidized form in capillary columns, it may still be necessary to enrich the oxidized cholesterol (2) because the amounts of oxidized cholesterol are often negligi-

ble compared with unoxidized cholesterol. Separation of the complex mixture of phytosterol oxides in the capillary columns is not so straightforward as for cholesterol oxides. The unoxidized phytosterols overlap with the oxidation products that arise from different phytosterols. It is therefore of utmost importance that the unoxidized sterols be separated from the oxidized sterols before the GC and GC–MS analyses are accomplished. The elution order of different phytosterol oxides in the capillary column and the GC conditions used were similar to those for cholesterol oxides. However, separation between 19-hydroxycholesterol, often used as an internal standard in cholesterol oxide analyses, and 7α-hydroxycampesterol was not at baseline. Therefore, internal standards other than 19-hydroxycholesterol can be used. 5α-Cholestane is also used as internal standard during analysis of oxidation products of cholesterol; however, it can be added only prior to silylation because of its nonpolar nature. The elution pattern of the sterol oxides analyzed in this study is in the order 7α-hydroxy-, 7β-hydroxy-, 5β,6β-epoxy-, 5α,6α-epoxy-, triols, and 7-ketosterols. Separation between the epimers of epoxides was not achieved at baseline in the column used, and therefore, the amounts of these oxides are presented together in the tables. Baseline separation between 7β-hydroxystigmasterol and 5α,6α-epoxycampesterol was also difficult to achieve. The amounts of these two components were calculated from the ratio of the peak heights. The detection limits of different sterol oxides by GC analyses were at 0.1 ppm in the lipids. Because a large number of oxidation products may arise from mixed phytosterols, capillary columns with different polarity and dimensions can be tested to achieve better separation.

Mass spectral data on campesterol and sitosterol oxides are described elsewhere (17). To our knowledge, full-scan mass spectra have not been published on the polar oxidation products of stigmasterol as their TMS-ether derivatives, but mass spectral data on ion fragmentation of some nonpolar oxidation products of stigmasterol were presented in tables (11). Therefore, it is of interest to present the full-scan mass spectra of the authentic samples of some polar oxidation products of stigmasterol. The mass spectra of 7α-hydroxy- and 7β-hydroxystigmasterol as TMS-ether derivatives are presented in Figure 1A and B. In contrast to epimers of 7-hydroxycampe-

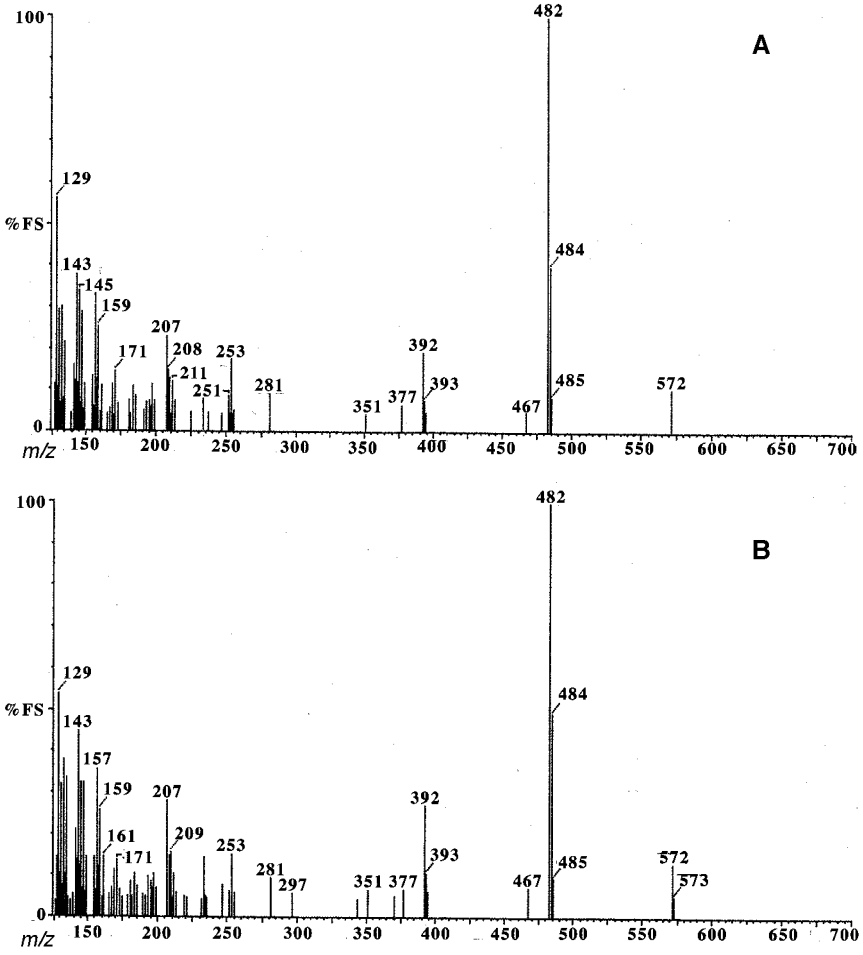

FIG. 1. Mass spectrum of an authentic sample of trimethylsilyl-ether derivative of (A) 7α-hydroxystigmasterol showing the molecular ion at 572 and a base peak at 482 ($M^+ - 90$), and (B) 7β-hydroxystigmasterol showing the molecular ion at 572 and a base peak at 482 ($M^+ - 90$). Abbreviation: % FS, percentage full scale.

and 7-hydroxysitosterols (17), the molecular ion at *m/z* 572 (M^+) was observed at relatively higher intensity for both epimers. The intensities of the molecular ions were at 10.4 and 13.1%, respectively, for 7α- and 7β-hydroxystigmasterol. The base peak was observed at *m/z* 482 ($M^+ - 90$) for both epimers, which was also observed for epimers of 7-hydroxycampe- and 7-hydroxysitosterols (17). The intensity of the other major typical ion fragments was generally higher than those for 7-hydroxycampe- and 7-hydroxysitosterols (17). The typical fragments were at *m/z* 557 ($M^+ - 15$, 2.5–1.5%), 467 ($M^+ - 90 - 15$, 5.1–7.6%), 392 ($M^+ - 180$, 19.9–27.1%), 377 ($M^+ - 180 - 15$, 6.7–7.1%), 343 ($M^+ - 90$ – side chain, 3.6–4.3%), and 253 ($M^+ - 180$ – side chain, 17.9–15.4%), along with a few other unspecified peaks at *m/z* 233 (8.1–14.2%) and 159 (25.1–25.5%). This pattern of fragmentation agrees with the data on 7-hydroxycampesterol and 7-hydroxysitosterol, and 7-hydroxycholesterol (22,23), except for the intensity of the peaks.

The mass spectrum of the TMS-ether derivative of 7-ketostigmasterol is presented in Figure 2. The molecular ion at *m/z* 498 (M^+, 76.3%) was quite high and was almost similar in intensity to that observed for 7-ketocampe- and 7-ketositosterol (17). However, the base peak was observed at *m/z* 269 ($M^+ - 90$ – side chain) in contrast to 7-ketocampe- and 7-ketositosterol, where the base peak was observed at *m/z* 174, an unspecified peak (17). This peak at 174 and another unspecified peak at *m/z* 142 seem to be typical fragments for TMS-ether derivatives of 7-ketosterols (23) and were also present at 50.5 and at 10.4%, respectively. The other typical peaks are at *m/z* 483 ($M^+ - 15$, 7.1%), 408 ($M^+ - 90$, 41.9%), 393 ($M^+ - 90 - 15$, 11.6%), and 359 (M^+ – side chain, 67.7%). Several other unspecified peaks at *m/z* 481, 442, 429, 161, and 159 were also present and were observed for both 7-ketocampe- and 7-ketositosterol, as reported previously (22).

Mass spectra of 5α,6α-epoxy- and 5β,6β-epoxystigmasterol are presented in Figure 3A and B. The molecular ions at *m/z* 500 were present at 36.7 and 32.1%, respectively, for 5α,6α-epoxy- and 5β,6β-epoxystigmasterol. In contrast to epimers of epoxycampe- and epoxysitosterol (17), the base peak was at 143 for 5α,6α-epoxystigmasterol and at 147 for 5β,6β-epoxystigmasterol, although the peak at 143 was present at 61% for 5β,6β-epoxystigmasterol. The other characteristic ion fragments for both epimers were observed at *m/z* 485 ($M^+ - 15$, 15.9–6.6%), 482 ($M^+ - 18$, 9.5–11.7%), 410 ($M^+ - 90$, 39.5–22.6%), 395 ($M^+ - 90 - 15$, 11.9–8.5%), 377 ($M^+ - 90 - 18$, 7.4–5.1%), and 271 (M^+ – side chain – 90, 43.4–31.6%). Additional unspecified fragments at *m/z* 471 (4.2–5.7%), 382 (9.7–53.4%), and 355 (7.1–5.8%) were observed, and these peaks were also observed at different intensities for epoxycampe- and epoxysitosterols (22).

The mass spectrum of dihydroxystigmasterol (5α-stigmast-22-ene-3β,5,6β-triol) is presented in Figure 4. Detailed discussions on the derivatization of the hydroxyl groups and typical ion fragmentation of bis-TMS-ether and tris-TMS ether-derivatives of different dihydroxysterols are given elsewhere (17). No molecular ion for tris-TMS ether derivatives of dihydroxystigmasterol at *m/z* 662 (M^+) was observed. The base peak was observed at 429 (M^+ – ring A), as was observed for dihydroxycampe- and dihydroxysitosterol (17) and as was shown for cholestanetriol (23). The other major fragments from the tris-TMS-ether derivative were at *m/z* 572 ($M^+ - 90$, 58.2%), 557 ($M^+ - 90 - 15$, 17.2%), 482 ($M^+ - 180$, 77.9%), 467 ($M^+ - 180 - 15$, 21.0%), 392 ($M^+ - 270$, 10.2%), 377 ($M^+ - 270 - 15$, 5.0%), and 253 (M^+ – side chain – 270, 77.5%). As discussed in a separate paper (17), some of these fragments can also be generated from the bis-TMS-ether derivative because a small peak at *m/z* 590 (6.6%), equivalent to the molecular ion of the bis-TMS-ether derivative of dihydroxystigmasterol, was also observed. The present data are comparable to those presented for cholestanetriol (23) and for dihydroxysitosterol and cholestanetriol (24), apart from the presence of the molecular ion.

The sterol oxides present in this study were identified by comparing their relative retention times with those of authen-

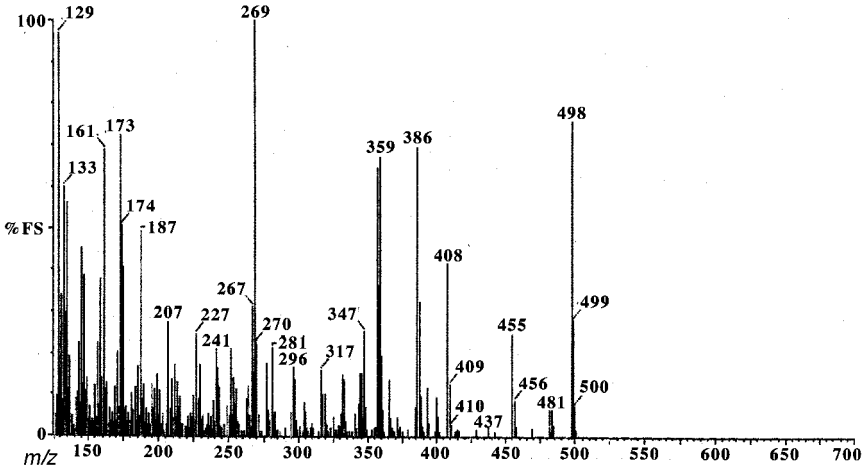

FIG. 2. Mass spectrum of an authentic sample of trimethylsilyl-ether derivative of 7-ketostigmasterol showing the molecular ion at 498. For abbreviation see Figure 1.

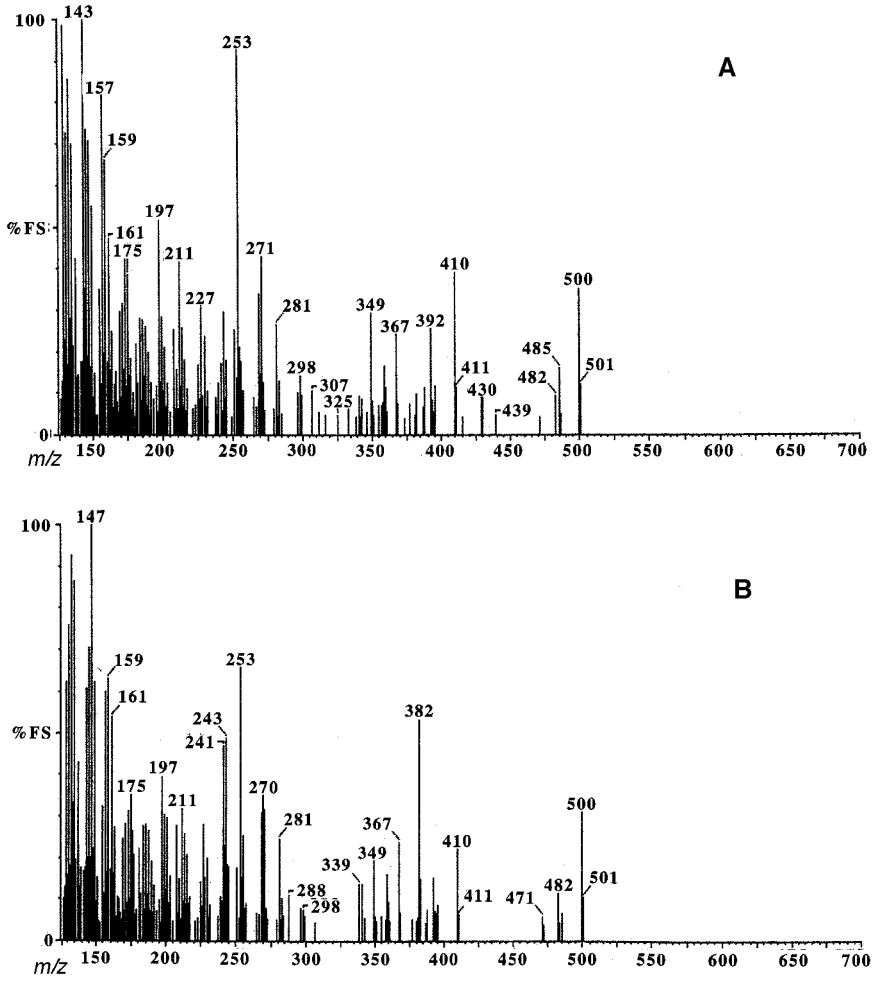

FIG. 3. Mass spectrum of an authentic sample of trimethylsilyl-ether derivative of (A) 5α, 6α-epoxystigmasterol showing the molecular ion at 500, and (B) 5β, 6β-epoxystigmasterol showing the molecular ion at 500. For abbreviation see Figure 1.

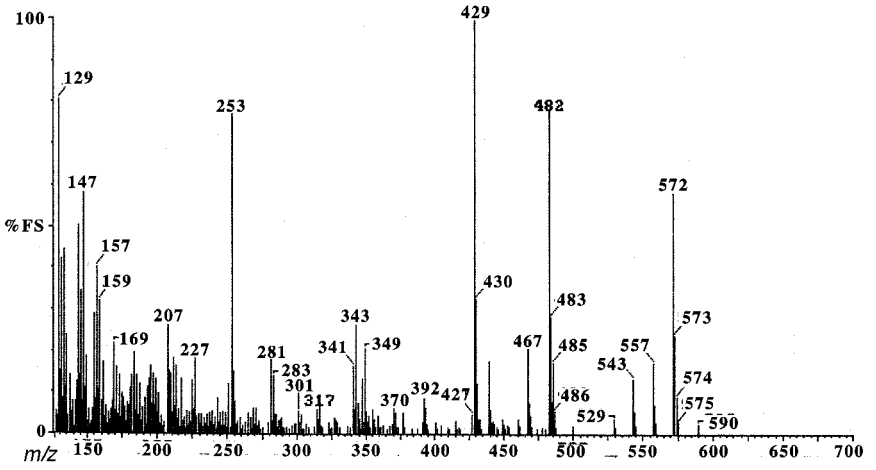

FIG. 4. Mass spectrum of an authentic sample of trimethylsilyl-ether derivative of dihydroxystigmasterol showing the base peak for tris-TMS-ether derivative at *m/z* 429 (M^+ – Ring A). For abbreviation see Figure 1.

tic sterol oxides samples and by comparing their mass spectra. However, the abundance of different ion fragments was not exactly the same as for authentic samples because the amounts of sterol oxides present in the samples were often small. For the sterol oxides present in small amounts, the identification was accomplished by examination of the mass spectrum and selective ion monitoring of a few typical peaks.

The contents of major sterol oxides in the oils are presented in Table 1. The total sterol oxides content in all samples on day 0 was slightly lower than on day 2. Palm/rapeseed oil blend, sunflower, and high-oleic sunflower oil contained 41 and 60 ppm, 40 and 57 ppm, 46 and 56 ppm, on day 0 and on day 2, respectively. The major sterol oxides in palm/rapeseed oil blend on day 0 were epoxy derivatives of both campesterol and sitosterol, 17 and 11 ppm, respectively. The other components were 7α- and 7β-hydroxysitosterol, dihydroxysterols, and 7-ketositosterol at 4, 1, 3, and 2 ppm, respectively. The content of all of these sterol oxides increased to some extent on day 2. A higher level of 7α-hydroxycampesterol, at 5.1 ppm, was detected in the palm oil/rapeseed oil blend after two days of frying operations. This peak may contain other unknown components. The level of 7β-hydroxycampesterol was less than the detection level at 0.1 ppm in the same sample. The major difference in the sterol oxides in sunflower oil, compared with the rapeseed oil/palm oil blend, was the higher contents of 7α- and 7β-hydroxysitosterol, dihydroxysterols, and 7-ketositosterol, and the lower content of epoxysterols. A rather similar pattern was observed in the high-oleic sunflower oil except that this oil, compared with sunflower oil, contained larger amounts of 7-ketositosterol and epoxysterols, both at 0 and at 2 d. Small amounts of 7-hydroxystigmasterol were also detected, ranging from 0.4 to 0.8 ppm in most of the frying oils, except in the zero-time palm oil/rapeseed oil blend (Table 1).

Recent quantitative data on the polar oxidized sterols in refined vegetable oils are scarce (12). A few studies in the early 1960s and 1970s, however, demonstrated the presence of 7-hydroxysito- and 7-ketositosterol in crude and refined vegetable oils (6,7). It was demonstrated that 7-ketositosterol was generated from 7-hydroxysitosterol mainly during bleaching processes (7). However, only qualitative data, based on TLC and spectroscopic analyses, were presented in those reports (6,7). A recent study demonstrated that crude soybean oil, freshly refined soybean oil, refined soybean oil stored at 4°C for one year, and freshly opened olive oil samples did not contain any sterol oxidation products, at a detection limit of 0.2 ppm. However, olive oil stored at room temperature for 30 mon had trace amounts of sterol oxides (25).

The oxidation products of sterols can be divided into two groups in a broad sense from their chromatographic characteristics in relation to unoxidized sterols (11): (i) the nonpolar products, arising as a result of dehydration (sterenes or sterol-

TABLE 1
Levels of Sterol Oxides (ppm)[a] in the Oil Samples of Rapeseed Oil/Palm Oil Blend, Sunflower Oil, and High-Oleic Sunflower Oil at 0 and 2 d After Frying of French Fries

Oil sample	7α-OH	7β-OH	7-Keto	Epoxy[b]	Dihydroxy	Total
IFORP 0[c]						
Sitosterol	4.4	1.3	1.6	17.2	2.9	41.0
Campesterol	1.1	n.d.	n.d.	10.8	1.7	
Stigmasterol	n.d.[d]	n.d.	n.d.	n.d.	n.d.	
IFORP 2						
Sitosterol	4.6	6.3	2.7	21.6	4.0	59.4
Campesterol	5.1	n.d.	n.d.	13.1	1.4	
Stigmasterol	n.d.	0.6	n.d.	n.d.	n.d.	
IFOSO 0						
Sitosterol	5.8	6.6	12.9	5.3	4.9	39.9
Campesterol	1.7	0.5	n.d.	0.9	n.d.	
Stigmasterol	0.7	0.6	n.d.	n.d.	n.d.	
IFOSO 2						
Sitosterol	14.3	10.9	11.4	5.8	3.0	56.6
Campesterol	2.5	4.7	n.d.	2.6	n.d.	
Stigmasterol	0.6	0.8	n.d.	n.d.	n.d.	
IFOHOSO 0						
Sitosterol	7.7	5.4	14.1	7.8	5.7	46.7
Campesterol	2.8	1.0	n.d.	1.4	n.d.	
Stigmasterol	0.4	0.4	n.d.	n.d.	n.d.	
IFOHOSO 2						
Sitosterol	9.1	7.8	16.9	8.9	4.4	55.9
Campesterol	3.6	n.d.	n.d.	3.9	n.d.	
Stigmasterol	0.6	0.7	n.d.	n.d.	n.d.	

[a]Means of duplicate analyses. [b]Epoxy: includes both 5α,6α-epoxy- and 5β,6β-epoxy-sterols. [c]Abbreviations: IFORP, blend of hydrogenated rapeseed/palm oil; IFOSO, sunflower oil; IFOHOSO, high-oleic sunflower oil; 0, at day 0 (before frying); 2, after 2 d of frying (Ref. 20). [d]n.d.: <0.1 ppm, not detected.

TABLE 2
Levels of Sterol Oxides (ppm)[a] in the Lipids of French Fries Samples Fried in Rapeseed Oil/Palm Oil Blend, Sunflower Oil and High-Oleic Sunflower Oil

Sample	7α-OH	7β-OH	7-Keto	Epoxy[c]	Dihydroxy	Total
IFFRP2[d]						
Sitosterol	2.9	3.7	4.1	7.6	0.5	32.0
Campesterol	1.4	2.6	3.3	5.4	0.5	
IFFSO2						
Sitosterol	3.8	7.3	13.1	2.2	1.1	36.9
Campesterol	0.3	1.3	5.9	1.3	0.6	
IFFHOSO2						
Sitosterol	4.7	9.7	13.5	5.4	2.8	53.7
Campesterol	1.4	1.8	9.2	3.6	1.6	

[a]Means of duplicate analyses; <0.1 ppm, not detected. [b]Samples of French fries prepared for consumption by heating at 200°C for 15 min. [c]Epoxy: includes both 5α,6α-epoxy- and 5β,6β-epoxysterol. [d]Abbreviations: IFFRP2, lipids from French fries fried in a blend of palm/rapeseed oil; IFFSO 2, lipids from French fries fried in sunflower oil; IFFHOSO 2, lipids from French fries fried in high-oleic sunflower oil (Ref. 20).

hydrocarbons) or dehydrogenation (monooxygenated with single, mono-, di-, or triunsaturation) of sterols and (ii) the group of sterol oxides, generally more polar than the abovementioned group, either with an additional hydroxyl group in the ring structure or in the side chain, with two additional hydroxyl groups in the ring structure, or monooxygenated in the ring structure with additional monohydroxylation either in the ring structure or in the side chain. The mechanism and the kinetics of the formation of these sterene compounds, along with several polar oxidized sterols, including the epimers of 7-hydroxy-, 7-keto-, epimers of epoxysitosterol and dihydroxy-sitosterol, have been studied in different model systems (8–10). Some recent studies have shown the contents and the kinetics of the production of sterenes in vegetable oils (26–31). Assessment of the phytosterol oxides content in crude vegetable oils, and the kinetics of the formation of these compounds during different stages of refining, will be worthy of future investigation.

Because French fries prepared for consumption by heating at 250°C for 15 min had large amounts of sterol oxides (32), it was of interest to study the effect of temperature on the generation of these components. The contents of sterol oxides in the lipids extracted from French fries prepared by heating at 200°C for 15 min are presented in Table 2. 7-Ketositosterol and 7-ketocampesterol were the dominant sterol oxides in all samples, followed by epimers of epoxysterols. In addition to epimers of 7-hydroxysterols that originated from both campesterol and sitosterol, both dihydroxycampesterol and dihydroxysitosterol were present at relatively lower levels of 1 ppm in palm oil/rapeseed oil blend, 1.7 ppm in regular sunflower oil, and 4.4 ppm in high-oleic sunflower oil. ln general, the content of sterol oxides in French fries prepared in different vegetable oils reflects that of oils used for the frying operations.

Park and Addis (15) reported that French fries fried in tallow contained 7-ketocholesterol at 4 μg/g sample, while 7β-hydroxycholesterol ranged from 7 to 58 μg/g sample. In a later study from the same laboratory (16), the contents of cholesterol oxidation products in French fries, fried in animal–vegetable shortenings, were investigated. Oxidation products of cholesterol alone were reported in that study. The ranges of total cholesterol oxidation products varied from 11 to 39 ppm during a 30-d survey of fries collected daily from one restaurant. The identified cholesterol oxides were 7α-, 7β-hydroxycholesterol, 7-ketocholesterol, α-epoxycholesterol, β-epoxycholesterol, 5α-cholestane-3β,5,6β-triol, and 25-hydroxycholesterol.

The content of sterol oxides (cholesterol and phytosterol oxidation products were presented together) in freshly prepared French fries, prepared in mixed beef tallow and hydrogenated vegetable oils, was studied previously (14). The sterol oxides reported were α-epoxide, β-epoxide, 7α-hydroxy-, and 7β-hydroxysterol. The levels of α-epoxide ranged from traces to 19 ppm, β-epoxide ranged from traces to 27 ppm, 7α-hydroxysterol ranged from 0 to 21 ppm, and 7β-hydroxysterol ranged from 0 to 81 ppm in the French fries samples from five different restaurants (14).

The levels of phytosterol oxides in this study, recalculated from ppm in lipids to ppm in samples, are 2.4, 2.8, and 4.0 ppm in French fries prepared in rapeseed oil/palm oil blend, sunflower oil, and high-oleic sunflower oil, respectively. These values are calculated on the basis of average lipid contents of French fries at *ca*. 7.5%. The levels of sterol oxides in the French fry samples in this study are relatively low in comparison with other studies of French fries, as discussed above. It seems from this study, and from an earlier report (32) from this laboratory, that final frying temperature plays an important role in the generation of sterol oxides because, in that earlier report, the French fries were prepared at 250°C and generated larger amounts of sterol oxides. To reach a definite conclusion on the effect of temperature, studies are underway in this laboratory to evaluate the effects of frying temperature and heating time on the generation of phytosterol oxides in French fries.

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