Studies on Phytosterol Oxides. I: Effect of Storage on the Content in Potato Chips Prepared in Different Vegetable Oils[1]

Paresh Chandra Dutta* and Lars-Åke Appelqvist

Department of Food Science, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

ABSTRACT: Potato chips fried in palm oil, sunflower oil, and high-oleic sunflower oil were studied for the content of different phytosterol oxides during 0 to 25 weeks of storage in the dark. Oxidation products of sitosterol (24α-ethyl-5-cholesten-3β-ol) and campesterol (24α-methyl-5-cholesten-3β-ol) were synthesized to help identify the phytosterol oxides. The oxides of phytosterols were analyzed by preparative thin-layer chromatography, solid-phase extraction, capillary column gas chromatography (GC), and GC-mass spectrometry. Epimers of 7-hydroxysitosterol and 7-hydroxycampesterol; 7-ketositosterol and 7-ketocampesterol; epimers of 5,6-epoxy-sitosterol and 5,6-epoxy-campesterol; 24α-ethylcholestane-3β,5,6β-triol (dihydroxysitosterol) and 24α-methylcholestane-3β,5,6β-triol (dihydroxycampesterol) were detected and quantitated in the samples of chips fried in different vegetable oils. Potato chips fried in palm oil had the lowest level of total sterol oxides, ranging from 5 to *ca.* 9 ppm in the lipids from time 0 to 25 wk of storage. The level of total sterol oxides in chip samples fried in sunflower oil ranged from 46 to 47 ppm, and the lipids in samples fried in high-oleic sunflower oil ranged from 35 to 58 ppm from 0 time to 25 wk of storage. During 25 wk of storage no considerable increase in sterol oxides was observed in the samples of chips fried in palm oil and sunflower oil. The chip samples fried in high-oleic sunflower oil had slightly higher levels of sterol oxides after 10 and 25 weeks of storage. In addition to the levels of individual sterol oxides, a new method for enrichment of phytosterol oxides from the unsaponifiables and full-scan mass spectra of various oxidation products of sitosterol and campesterol are reported in this paper.

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KEY WORDS: Campesterol oxides, frying vegetable oils, high-oleic sunflower oil, palm oil, sitosterol oxides, storage effect, sunflower oil.

Abundant information exists on the formation of cholesterol oxidation products in foods and their biological implications (1), but there is relatively little on such products generated from phytosterols (2–4). Owing to structural similarities between cholesterol and phytosterols, phytosterols also form oxidation products, similar to those formed from cholesterol (2–3). Sitosterol absorption in humans ranges from 1.5 to 10%, while absorption of cholesterol ranges from 20 to 60% (2). The authors cautioned that the limited absorption of sitosterol may be reflected in limited absorption of oxidized phytosterols; however, certain population groups might be more susceptible to possible adverse effects of oxidized phytosterols. Patients afflicted with β-sitosterolemia accumulate high concentrations of plant sterols, especially β-sitosterol and campesterol, in plasma, red blood cells, adipose tissue, and skin. In infants and children, phytosterol-rich diets can induce 5- to 15-fold higher plasma phytosterol levels compared with those in adults (2). The authors assumed that humans absorb phytosterol oxides and that subsequent metabolic conversions of these oxides may be of toxicological significance based on the limited studies discussed in that review. Also the toxic effects of phytosterol oxides on intestinal tissue should not be ignored because 5,6-epoxy- and 7-ketosterols are potential alkylating agents, which might react with intestinal nucleophiles, thereby exerting toxic effects. In addition, microbial modifications of these oxides in the colon may produce toxic products, and these possibilities require study. The authors noted that the problems associated with the analyses of cholesterol oxides in foods also apply to the phytosterols (2). Also the biological effects of phytosterol oxides have been neglected so far because most of the research work was done on cholesterol oxidation products because of health concerns (3).

Few studies have been conducted on phytosterol oxides in foods of plant origin (3). Only two reports have been published on the phytosterol oxides in potato chips (5,6). An investigation was conducted on the content of oxidized phytosterols in potato chips, fried in cottonseed oil and packaged in aluminum foil for storage at 40°C for 95 d, and at 23°C for 150 d (5). The oxidation products found were a result of elevated temperature because there were no detectable oxidation products during prolonged storage at 23°C, in contrast to chips stored at 40°C, which had 28 ppm (total) of the three sitosterol oxidation products analyzed. Although the samples

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*To whom correspondence should be addressed at Department of Food Science, Swedish University of Agricultural Sciences, Box 7051, S-750 07 Uppsala, Sweden.

stored at 40°C already had elevated peroxide values and a rancid taste after 24 d of storage, no sterol oxidation products were detected at this stage (5). In another report, the content of sterol oxides in potato chips, fried in unspecified oils, was presented (6). Different oxidation products, generated from cholesterol, campesterol and sitosterol, were quantitated, and the total level of all oxidation products was 48 ppm in the chip sample. In that report, it was suggested that abusive storage conditions in supermarkets (clear packages exposed to light, temperature and moisture) might stimulate the formation of sterol oxides in the samples.

As a part of a multinational European Union (EU) project for utilization of high-oleic sunflower oil for industrial frying operations, potato chips fried in palm oil, sunflower oil, and high-oleic sunflower oil were compared for the content of different phytosterol oxides. Owing to unavailability of standard phytosterol oxides, several polar oxidation products from campesterol and sitosterol needed to be synthesized in this laboratory. In addition to the content of different phytosterol oxides, a new method of enrichment of phytosterol oxides from unsaponifiables, analysis by gas chromatography (GC) and GC–mass spectrometry (MS), and data on mass spectra of several polar oxidation products of campesterol and sitosterol are discussed in this report.

EXPERIMENTAL PROCEDURES

Samples. Samples of potato chips produced in palm oil, sunflower oil, and high-oleic sunflower oil were prepared by Raisio Group, Raisio, Finland (7). After production, the samples of chips were stored at room temperature in the dark for different periods, and then they were stored at −20°C until further analysis.

Reagents. Standard samples of mixed sitosterol and campesterol were 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, 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). The following solvents and chemicals were of analytical grade: acetone, sodium chloride, sodium hydroxide, sodium sulfate, and trisodium phosphate (Merck, Darmstadt, Germany); sodium bicarbonate (Kebo Lab AB, Stockholm, Sweden); and potassium hydroxide (EKA, Bohus, Sweden). Cerium sulfate hydrate was obtained from Aldrich-Chemie (Steinheim, Germany), and phosphomolybdic acid hydrate 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).

Synthesis of different phytosterol oxides. For the preparation of 5α,6α- and 5β,6β-epoxysterols, a modification of the method by Fieser and Fieser (8) was used. That is, 2 g of mixed sitosterol/campesterol was dissolved in 30 mL dichloromethane in a conical flask, and one equivalent of *m*-chloroperbenzoic acid dissolved in 26 mL of dichloromethane was added dropwise to the flask with stirring on a magnetic stirrer at room temperature. The stirring continued for 4 h. Thereafter, the solvent was evaporated in a rotary evaporator (Rotavapor, Büchi, Switzerland) under reduced pressure at 30°C. The residue was dissolved in diethyl ether. After transferring the ether solution into a separatory funnel, 10 mL of 10% sodium bicarbonate was added, and the contents were mixed vigorously. The lower phase was discarded, and the diethyl ether phase was washed twice with 10 mL water. The diethyl ether phase was dried by adding anhydrous sodium sulfate and then evaporated to dryness under nitrogen. The residue was dissolved in chloroform, and the epoxysterols were further purified by preparative thin-layer chromatography (TLC) as described below.

For autoxidation of phytosterols to produce mixed phytosterol oxides, a modification of the method of Chicoye *et al*. (9) was used. In brief, a dispersing medium was prepared by mixing a solution of 250 mg stearic acid, dissolved in 37% ethanol, with a solution of 300 mg trisodium phosphate, dissolved in 37% ethanol, with stirring. An 80-mL portion of this medium was diluted to 500 mL with distilled water in a 1-L conical flask. One g of mixed sitosterol and campesterol was dissolved in 75 mL boiling ethanol and gradually added to the dispersion medium with stirring. The solution was stirred for 4 h at 70°C, and then the temperature was raised to 90°C and the solution was held for an additional 1 h. After cooling, the solution was acidified to pH 6.0. The resulting dispersion was poured into a separatory funnel and extracted with 200 mL of diethyl ether. The ether phase was washed once with 150 mL 2% KOH and twice with 150 mL distilled water. The ether phase was dried by adding anhydrous sodium sulfate and evaporated to dryness in a rotary evaporator at 30°C under reduced pressure. The residue was dissolved in hot ethanol, and the oxysterol fraction was enriched by repeated crystallization of unoxidized sterols at 4°C. The oxysterol-enriched fraction was further purified for the isolation of different sterol oxide fractions by preparative TLC as described below.

Oxidation of sterols in air. To prepare dihydroxysterols, a modification of the method of Daly *et al*. (10) was used. In brief, 1 g of mixed sitosterol and campesterol was placed on a glass plate in an oven at 100°C for 46 h, and then the temperature was raised to 120°C and held for an additional 2 h. The samples were dissolved in hot ethanol, and the oxysterols were enriched by repeated crystallization of unoxidized sterols at 4°C. The dihydroxysterols were further purified by preparative TLC as described below.

Extraction of lipids from potato chips . Extraction of lipids from chips was carried out by following a method slightly modified from Hara and Radin (11). In brief, 2.5 g of chips was homogenized by an Ultra-Turrax T25 homogenizer

(Jankel & Kunkel GmbH, Staufen, Germany) with 50 mL of hexane/2-propanol (3:2, vol/vol) at maximum speed for 30 s, then the homogenate was filtered, and the filtrate was collected in a separatory funnel. The residue was reextracted as above. The pooled filtrates were mixed with 50 mL of 6.67% anhydrous sodium sulfate in water, and the upper phase was collected and evaporated to dryness under vacuum at 30°C in in 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 analyses.

Cold saponification of the total lipids for sterol oxide analysis. Ca. 0.5 g of lipids was mixed well with 5 mL of 2 M potassium hydroxide in ethanol in a glass tube and left overnight (about 18 h) in the dark at room temperature (12). After addition of 10 mL dichloromethane and 10 mL water, the tube was shaken vigorously. The water phase was removed, and the organic phase was repeatedly washed with 10 mL water until a clear solution was obtained. The solvent was removed under nitrogen, and the unsaponifiables were dissolved in chloroform for enrichment of sterol oxides, either by preparative TLC or by solid-phase extraction (SPE) as described below.

TLC. For both analytical and preparative purposes, precoated TLC plates with silica gel 60, 20 × 20 cm, 0.25-mm thickness were used (Merck). For analytical purposes for sterol oxides, high-performance thin-layer chromatography (HPTLC) plates with silica gel 60 $F_{254,}$ 10 × 10 cm, 0.1-mm thickness (Merck) were used. For preparative purposes for the oxidized sterols, *ca*. 20 mg of the sample was applied as a 16-cm band on the TLC plate by an automatic applicator, CAMAG Linomat-III (CAMAG, Muttenz, Switzerland). Along the side of the sample band, a 0.5-cm band of a standard cholesterol oxide sample was also applied. The plate was developed in diethyl ether/cyclohexane (9:1, vol/vol) (13), up to the top of the plate. After a brief drying of the plate, the part of the plate that contained the sample and the standard was cut off. This part of the plate was sprayed with a reagent prepared by dissolving 1 g each of phosphomolybdic acid and cerium sulfate in 5.4 mL of concentrated sulfuric acid. The volume was made up to 100 mL with water and the plate was warmed at 120°C for 15 min. Sterol oxide zones were scraped off, and the gel was eluted with 5 mL + 2.5 mL of chloroform/methanol (2:1, vol/vol). For dihydroxy sterols, the TLC plate was developed once more in diethyl ether, raising the solvent front to 10 cm to raise the triols fraction above the origin. Thereafter, the dihydroxysterols were extracted as described above. The individual sterol oxides purified by this procedure were further purified at least once more by preparative TLC as described earlier. This was done because we found, after checking by analytical HPTLC after the same method of development as for preparative TLC, that a sterol oxide fraction was still not pure. At least two preparative TLC plates were required to collect a reasonable amount of a sterol oxide fraction for further analysis by GC and identification by GC–MS.

Completeness of saponification was also checked regularly by analytical TLC. The unsaponifiables, along with TLC-reference standard 18-4A (Nu-Chek-Prep Inc., Elysian, MN), were spotted on a TLC plate and developed in the solvent system hexane/diethyl ether/acetic acid (85:15:1, vol/vol/vol). After the solvent front had reached the top, the plate was dried in air for a short time and then sprayed with 10% phosphomolybdic acid in diethyl ether/ethanol (50:50, vol/vol). The plate was kept in an oven at 120°C for 15 min for color development.

Enrichment of sterol oxides from total unsaponifiables by SPE. As an alternative approach to preparative TLC, a new method of enrichment of the sterol oxides by two-fold SPE was developed. By this alternative, exposure of the sterol oxides to light and air, as during preparative TLC work, is minimized. For this purpose, a 0.5-g silica cartridge (International Sorbent Technology Ltd., Mid Glamorgan, England) was solvated by 3 mL hexane. The total unsaponifiables were dissolved in 1 mL hexane/diethyl ether (75:25, vol/vol) and were charged to the column. The tube was washed with an additional 2 mL hexane/diethyl ether (75:25, vol/vol) and eluted through the column at a rate of *ca*. 4 mL/min. Thereafter, the columns were eluted with 6 mL hexane/diethyl ether (60:40, vol/vol), and the eluates were discarded. The sterol oxides and the remaining sterols were then eluted with 5 mL acetone. The acetone was dried under nitrogen, and the residue was again dissolved in 1 mL hexane/diethyl ether (75:25). Another 0.5-g silica column was prepared as before, and the sample was charged again and was eluted with 3 mL hexane/diethyl ether (60:40, vol/vol), and the eluate was discarded. Finally, the column was eluted with 5 mL acetone, the acetone was dried under nitrogen, and the sterol oxides were derivatized to trimethylsilyl (TMS)-ethers as described below for subsequent analysis by GC and GC–MS.

Preparation of TMS-ether derivatives of sterol oxides . Ten μg of 5α-cholestane was added as internal standard in the ground-stoppered glass tubes that contained sterol oxides. After drying the solvent, 75 μL of Tri-Sil reagent (Pierce, Rockford, IL) was added, and the tubes were kept at 60°C for 45 min. Thereafter, the solvent was evaporated under a stream of nitrogen, and the TMS-ether derivatives were dissolved in 1 mL hexane. The tubes were sonicated in an ultrasonic bath for 1 min and were centrifuged for 3 min. The hexane layer was transferred to another tube, avoiding any solid particles, and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 1 mL hexane, and the tubes were stored at −20°C for subsequent analyses by GC and GC–MS within 2 d after derivatization.

Capillary column GC analysis of sterol oxides. The TMS-ether derivatives were analyzed in a Varian 3700 gas chromatograph (Varian, Palo Alto, CA), equipped with a flame–ionization detector and a falling needle injector. A fused-silica capillary column CP-Sil-5 CB (Chrompack, Middelburg, The Netherlands), 30 m × 0.25 mm, 0.25-μm film thickness was used. Helium was used as carrier gas at an inlet pressure of 11 psi and as makeup gas at a flow rate of 30

mL/min. The GC was run at an oven temperature of 265°C and a detector temperature of 290°C. The peak areas were computed by an HP 3396A integrator (Hewlett-Packard, Avondale, PA). Sterol oxides were quantitated from the weight of 5α-cholestane. No response factors were used for sterol oxides. All samples were analyzed in duplicate, and the mean results were calculated.

GC–MS for identification of sterol oxides. GC–MS analyses were performed on a HP5890 series II gas chromatograph (Hewlett-Packard) coupled to a TRIO-1000 mass spectrometer with an LAB-BASE™ data system, version R2.10 (Fisons Instruments, VG MASSLAB, Manchester, England). The TMS derivatives of the sterol oxides were separated on a WCOT fused-silica capillary column CP-Sil-5 CB (Chrompack), 30 m × 0.25 mm, 0.25-μm film thickness. Helium was used as carrier gas at an inlet pressure of 20 psi. The injector temperature was 230°C, the samples were injected in a splitless mode, and the purge delay time was 0.8 min. The oven temperature was kept at 60°C for 2 min, then raised to 265°C at a rate of 20°C/min and held at this temperature for 30 min. The mass spectra were recorded at an electron energy of 70 eV, and the ion source temperature was 200°C.

RESULTS AND DISCUSSION

The analysis of sterol oxides in food can be divided into the following steps: (i) lipid extraction, (ii) enrichment of sterols and sterol oxides from total lipids with or without saponification, (iii) enrichment of sterol oxides from total unsaponifiables by different chromatographic methods, (iv-a) identification of the oxysterols by capillary column GC–MS or HPLC–MS, (iv-b) quantitative analysis by GC or by HPLC, eventually in combination with MS (1). Oxidation products of cholesterol in various food products have been extensively researched with various methods during recent decades. However, no standard methods have yet been elaborated. As a consequence, considerable variations in the levels of cholesterol oxides in similar kinds of foods, reported from different laboratories, are observed (1,4). Foods of animal origin are known to have cholesterol as the main sterol, but plant-based food products also contain a large number of sterols. Among these sterols, sitosterol, campesterol, and stigmasterol generally dominate, except for the Cruciferae, which contain a considerable amount of brassicasterol (14). Owing to structural similarity with cholesterol, all these phytosterols have the potential to be oxidized and can generate a large number of oxidation products. As a consequence, the analysis of phytosterol oxides becomes more cumbersome compared with cholesterol oxidation products.

One of the crucial steps in sterol oxide analysis is the enrichment of oxides from unoxidized sterols. Many approaches are taken by different laboratories, and a mild but quick method is preferable. Preparative TLC is a rather simple method, but owing to long exposure of the sample to air during workup, it has considerable drawbacks. Different column chromatographic and preparative HPLC methods are also used (1), but these methods are quite tedious and time-consuming. A simple and quick alternative is the SPE method. A double elution with the SPE technique to enrich the sterol oxides fraction was developed. Validation of the analytical methods in the determination of sterol oxides is important (15). No recovery test was done on this new SPE method of enrichment because of the lack of sufficient amounts of extremely pure standard phytosterol oxides. However, the completeness of the sterol oxide enrichment was checked regularly by analytical HPTLC and visual examination of the plates after color development from the eluted fractions during SPE extraction, and no particular losses of the polar oxidation products were revealed.

Separations of different phytosterol oxides under the capillary column and GC conditions used were similar to those of cholesterol oxides. The elution order of the sterol oxides analyzed in the column used in this study is 7α-hydroxy-, 7β-hydroxy-, 5β,6β-epoxy-, 5α,6α-epoxy-, triols and 7-ketosterols. The quantitation of different sterol oxides by GC analyses was at the 0.1 ppm level in the lipids. Less than this level is reported as not detected. We observed that any of the unoxidized phytosterols can overlap with some oxidized component in the GC separation, and therefore it is necessary that removal of the unoxidized sterols from the unsaponifiables be complete.

Some common polar phytosterol oxides were synthesized in this laboratory because no standard samples of any phytosterol oxides are available commercially. Most methods of synthesis are developed for synthesis of cholesterol oxides. However, owing to structural resemblance, the methods are also applied with phytosterols (16). Different oxides of campesterol and sitosterol were co-products from the commercial sample of mixed sitosterol and campesterol.

There are several reports of the mass spectral data of a number of phytosterol oxides. Often, mass spectra from underivatized sterol oxides or data on the intensity of ion fragmentation from derivatized or underivatized sterol oxides are presented in tables (10,16–23). To our knowledge, only the full-scan mass spectra of 7-ketositosterol and dihydroxysitosterol as their TMS-ether derivatives have already been reported (24,25). Therefore, it was of interest to present full-scan mass spectra of oxidation products of sitosterol and campesterol (Fig. 1A and B) because the abundance of ion fragments may vary considerably owing to the use of different instruments and analytical conditions (26).

In an extensive study on a large number of sterol oxides, including campesterol and sitosterol oxides as their TMS-ether derivatives, where identification was achieved by GC–MS, the data on mass spectrometric fragmentation patterns were tabulated (23). Discussion on the MS fragmentation of authentic samples of different oxidation products of sitosterol and campesterol in this study will be focused mainly on the major and typical fragments, and comparisons are made basically with that report. In addition, a few other reports on sitosterol and cholesterol oxides are cited whenever applicable (1,25,27,28).

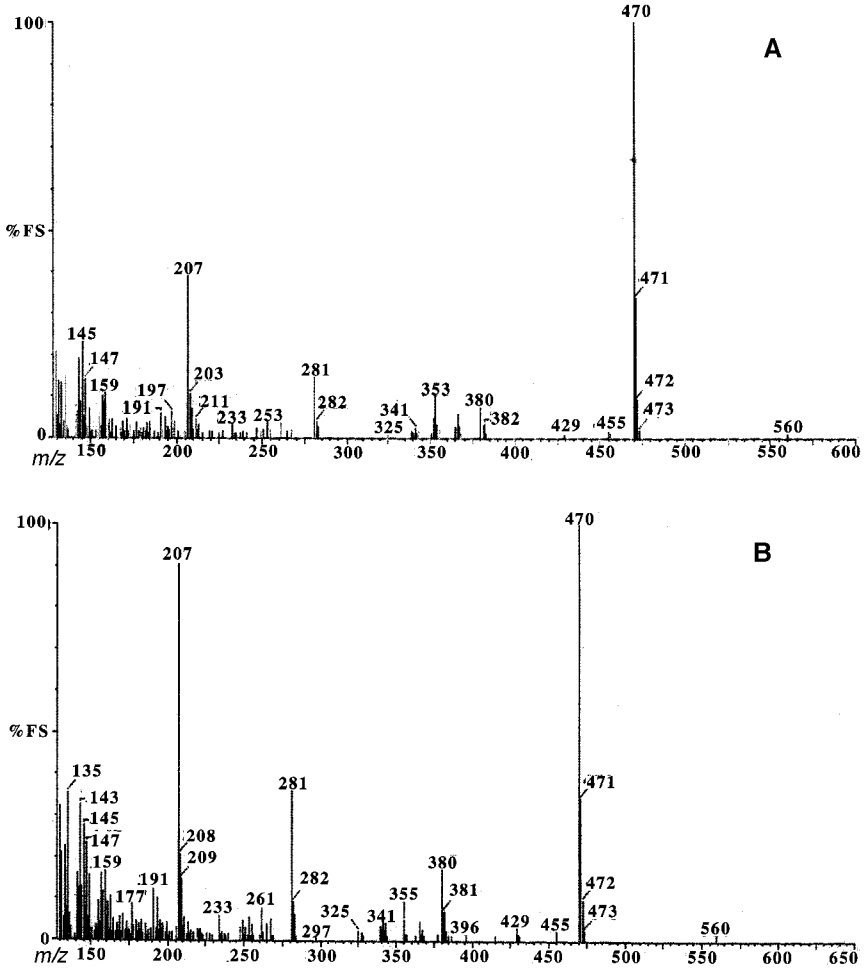

FIG. 1. (A) Mass spectrum of an authentic sample of trimethylsilyl (TMS)-ether derivative of 7α-hydroxycampesterol shows the molecular ion at 560 and a base peak at 470 ($M^+ - 90$). (B) Mass spectrum of an authentic sample of TMS-ether derivative of 7β-hydroxycampesterol shows the molecular ion at 560 and a base peak at 470 ($M^+ - 90$). % FS = percentage full scale.

Mass spectra of 7α-hydroxy- and 7β-hydroxycampesterol as di-TMS ether derivatives are shown in Figure 1A and 1B. The fragmentation patterns are generally similar for both epimers. The intensity of the molecular ion (M^+) at *m/z* 560 is low at 1.3 and 1.6% for 7α-hydroxy- and 7β-hydroxycampesterol, respectively. The base peak was observed at *m/z* 470 ($M^+ - 90$) for both epimers. The other typical fragments for both epimers (the respective intensity presented in parentheses) were at *m/z* 455 ($M^+ - 90 - 15$, 2.1–2.2%), 380 ($M^+ - 180$, 7.3–17.7%), 365 ($M^+ - 180 - 15$, 2.8–4.8%), 343 ($M^+ - 90$ – side chain, 1.7–4.4%), 253 ($M^+ - 180$ – side chain, 4.4–5.5%), and 129, which is a typical fragment of Δ5-sterols (20.8–32.3%). A few other unspecified peaks at *m/z* 233 (3.7–6.1%) and 159 (11.1–16.8%) were also observed, as reported previously (23). Mass spectra of 7α-hydroxy- and 7β-hydroxysitosterol as di-TMS derivatives are presented in Figure 2A and 2B. Similar to epimers of 7-hydroxycampesterol, the intensity of molecular ions at *m/z* 574 (M^+) was low for both 7α-hydroxysitosterol (1.3%) and 7β-hydroxysitosterol (1.5%). Likewise, the base peak was observed for both epimers at *m/z* 484 ($M^+ - 90$), as was observed for epimers of 7-hydroxycampesterol. The other peaks described for 7-hydroxycampesterol were also present in 7-hydroxysitosterols at 14 mass units higher than those of 7-hydroxycampesterol, although small differences were observed in their intensity (see Figs. 1A, 1B, 2A and 2B). This observation agrees with published data (23,25).

Mass spectra of TMS-ether derivatives of 7-ketocampesterol and 7-ketositosterol are presented in Figures 3 and 4. The intensity of the molecular ion for 7-ketocampesterol was 87.7%, and that of 7-ketositosterol at *m/z* 500 was 56.6%. For both 7-ketosterols the molecular ions we found were not the base peaks reported previously (23,25). Instead, an unspecified peak at *m/z* 174 (100%) was the base peak for both 7-ketosterols, which was reported previously for 7-ketocholesterol (28). This peak at 174 and another unspecified peak at *m/z* 142 at 25.7% for 7-ketocampesterol and at 17.5% for 7-ketositosterol seem to be unspecified and typical fragments for TMS-ether derivatives of 7-ketosterols (1,23,27,28). The other typical peaks for 7-ketocampesterol (the corresponding

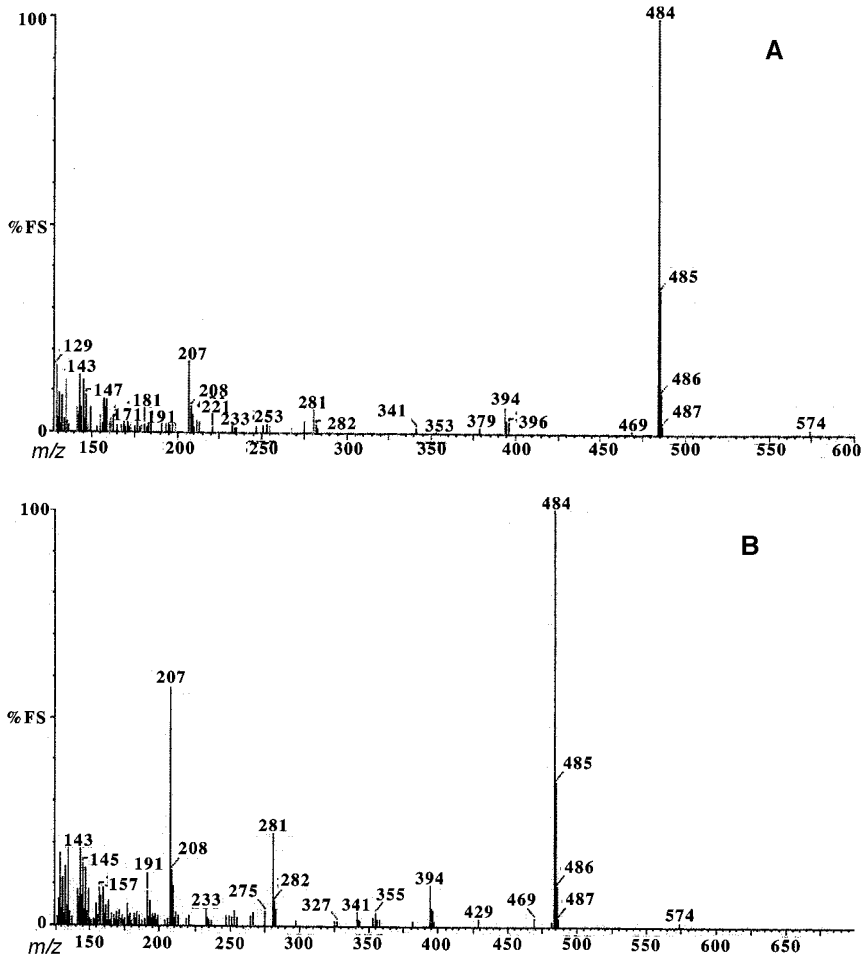

FIG. 2. (A) Mass spectrum of an authentic sample of trimethylsilyl (TMS)-ether derivative of 7α-hydroxysitosterol shows the molecular ion at 574 and a base peak at 484 ($M^+ - 90$). (B) Mass spectrum of an authentic sample of TMS-ether derivative of 7β-hydroxysitosterol shows the molecular ion at 574 and a base peak at 484 ($M^+ - 90$). See Figure 1 for other abbreviation.

ion at 14 mass units higher for 7-ketositosterol; the relative intensity in percentage for both are given in parentheses) are at *m/z* 471 ($M^+ - 15$, 10.9–6.4%) and at 396 ($M^+ - 90$, 46.9–42. 8%); peaks at *m/z* 187, 161, and 159 for both 7-ketocampesterol and 7-ketositosterol were also observed as reported previously (23).

Mass spectra of 5α,6α-epoxy- and 5β,6β-epoxycampesterol are presented in Figure 5A and 5B. The characteristic ion fragments for both epimers (relative intensity in parentheses for the respective epimers) were observed at *m/z* 488 (M^+, 50.0–36.6%), 473 ($M^+ - 15$, 28.2–22.3%), 470 ($M^+ - 18$, 11.1–100%), 398 ($M^+ - 90$, 63.3–38.8%), 383 ($M^+ - 90 - 15$, 27.3–17.4%), 380 ($M^+ - 90 - 18$, 43.6–24.6%), 365 ($M^+ - 90 - 15 - 18$, 17.8–13.2%), and 271 (M^+ – side chain – 90, 19.1–9.5%). Additional unspecified fragments at *m/z* 459 (22.7–26.7%), 370 (19.1–61.7%), and 343 (9.1–10.2%) were also observed and agree with a previous report (23). The base peak was at *m/z* 129 (100%) for 5α,6α-epoxycampesterol, whereas for 5β,6β-epoxycampesterol it was at *m/z* 470 ($M^+ - 18$, 100%).

The mass spectra of 5α,6α-epoxysitosterol and 5β,5β-epoxysitosterol are presented in Figure 6A and 6B. The molecular ion at *m/z* 502 was prominent for both epimers (56.1– 45.3%). Generally, the ion fragmentation pattern is similar to those of epimeric epoxycampesterol, apart from some differences in the intensity of the peaks (see Figs. 5A, 5B, 6A, and 6B). The base peak of 5β,5β-epoxysitosterol was an unspecified peak at *m/z* 135, and this peak was present at various intensities in campesterolepoxides and in 5α,6α-epoxysitosterol. Similar ion fragmentation patterns for both epimers of epoxycampesterol and epoxysitosterol were observed, with the exception of their relative intensity because the base peaks were different (23).

The mass spectra of dihydroxycampesterol (24α-methylcholestane-3β,5,6β-triol) and dihydroxysitosterol (24α-ethylcholestane-3β,5,6β-triol) are presented in Figures 7 and 8. These sterol oxides have three hydroxyl groups. It has been reported for cholestanetriol that TMS-ether derivatization during a longer period (60°C, 60 h) produces tris-TMS-ether derivatives, whereas the bis-TMS-ether derivative was pro-

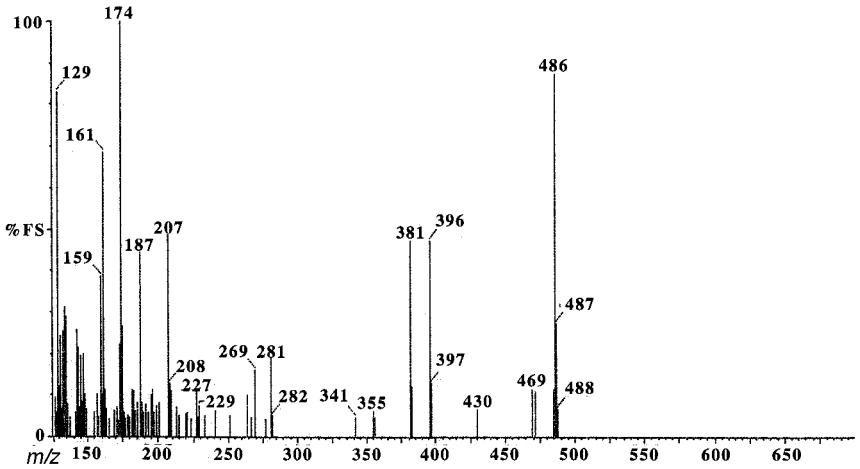

FIG. 3. Mass spectrum of an authentic sample of trimethylsilyl-ether derivative of 7-ketocampesterol shows the molecular ion at 486.

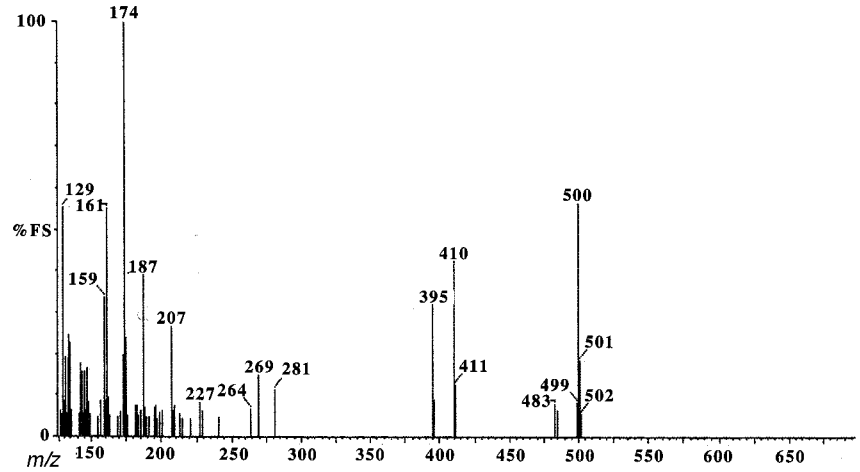

FIG. 4. Mass spectrum of an authentic sample of trimethylsilyl-ether derivative of 7-ketositosterol shows the molecular ion at 500. See Figure 1 for abbreviation.

duced at room temperature for 30 min, because derivatization at the 5-hydroxyl group is sterically hindered. Also, some of the major ion fragments are common for bis- and tris-TMS-ether derivatives, apart from their relative intensity (1). However, derivatization at 60°C for 60 min with TMCS (trimethylchlorosilane) and hexamethyldisilazane reagents could generate the tris-TMS-ether derivative of cholestanetriol (28). It was demonstrated in another report that, for the production of tris-TMS-ether derivative of cholestanetriol and dihydroxysitosterol, trimethylbromosilane was required instead of TMCS (24). Those published results and results from this study indicate that the bis- and tris-TMS-ether derivatives are co-products, and their ratio may vary depending on the reagents and reaction time. Also, the instrumental and analytical conditions, particularly the energy of electron impact ionization, will influence the presence of major and typical ion fragments because the base peak for cholestanetriol at *m/z* 403 (M^+ – Ring A) was reported (28), whereas for dihydroxysitosterol, a base peak at *m/z* 484 (M^+ – 180), and for cholestanetriol, a base peak at m/z *456* (M^+ – 180) were observed (24). It may be mentioned here that the ionization energy was at 70 eV (28) and at 22.5 eV (24), respectively. The results in this study on ion fragmentation from dihydroxycampesterol and dihydroxysitosterol reveal that the major fragments arise from tris-TMS-ether derivatives, along with some fragments that could arise from bis-TMS-ether derivatives. No molecular ion for tris-TMS-ether derivatives, either for dihydroxycampesterol at *m/z* 650 (M^+) or for dihydroxysitosterol at *m/z* 664 (M^+), were observed. The base peaks for both dihydroxycampesterol and dihydroxysitosterol were observed at *m/z* 417 (M^+ – Ring A), and at 14 mass units higher at *m/z* 431 (M^+ – Ring A), respectively; this was also observed for cholestanetriol (28). The other major fragments from the tris-TMS-ether derivative of dihydroxycampesterol were at *m/z* 560 (M^+ – 90, 31.3%), 545 (M^+ – 90 – 15, 13.1%), 470 (M^+ – 180, 73.1%), 455 (M^+ – 180 – 15, 17.0%),

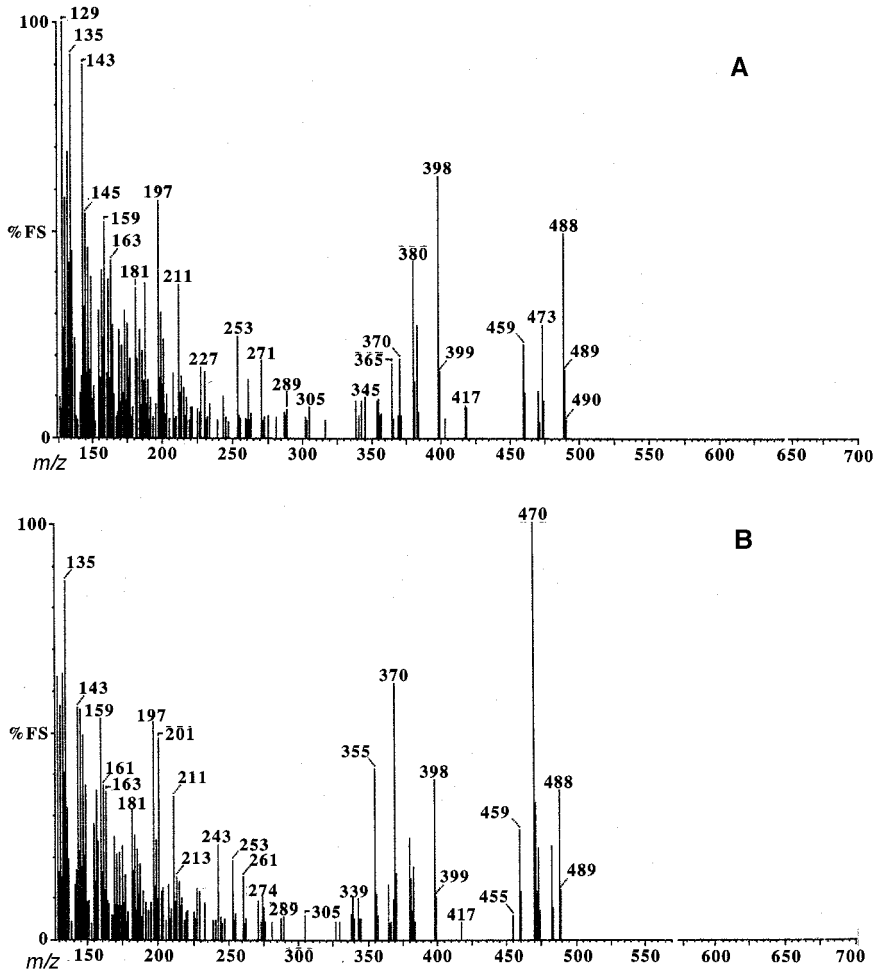

FIG. 5. (A) Mass spectrum of an authentic sample of trimethylsilyl (TMS)-ether derivative of 5α,6α-epoxycampesterol shows the molecular ion at 488. (B) Mass spectrum of an authentic sample of TMS-ether derivative of 5β,6β-epoxycampesterol shows the molecular ion at 488. See Figure 1 for other abbreviation.

380 (M^+ – 270, 9.1%), 365 (M^+ – 270 – 15, 4.6%), and 253 (M^+ – side chain – 270, 6.7%). All these peaks, at almost similar intensities, were observed at 14 mass units higher for dihydroxysitosterol. As mentioned earlier, some of these fragments may also generate from the bis-TMS-ether derivative because small peaks at *m/z* 578 (M^+, 0.7%) and at 592 (M^+, 0.6%), equivalent to the molecular ion of the bis-TMS-ether derivative of dihydroxycampesterol and dihydroxysitosterol, were also present. The present data are comparable to those presented for cholestanetriol (28) and for dihydroxysitosterol and cholestanetriol (24), except that the molecular ions for both dihydroxycampesterol and dihydroxysitosterol as tri-TMS-ether derivatives were absent. The full-scan mass spectrum of dihydroxycampesterol has not been reported until now. To increase the intensity of the molecular ions for the above-mentioned phytosterol oxides as TMS-ether derivatives, different instrumental conditions, e.g., lower source temperature and a voltage lower than 70 eV, were tested in this study (results are not presented here). However, no considerable differences were observed. In addition, chemical ionization with methane as reagent gas was also tested and no considerable improvements were observed (results not presented here). Reagent gases other than methane, particularly NH_3, may be of interest to investigate in this respect (29).

The sterol oxides were quantitated by capillary column GC by comparing the relative retention times with those of the authentic samples in this study. Identifications were accomplished by comparing with the mass spectra of authentic samples. The abundances of different ion fragments in the samples were, however, not exactly the same as those of authentic samples because the amounts of sterol oxides present in the samples were generally small. Apart from those sterol oxides that were present in larger amounts, the identification was accomplished by examination of the mass spectrum and selective ion monitoring of a few typical peaks.

The levels of total sterol oxides in the lipids of chips fried in palm oil were 5 ppm at 0 time, 6 ppm after 10 wk, and *ca.* 9 ppm after 25 wk of storage (Table 1). The major contributions from different sitosterol oxides were almost at equal levels from 0.6 to 0.9 ppm, except for the dihydroxysitosterol, which was at a considerably lower level, 0.2 ppm in the lipids. The contents of sterol oxides in chips fried in sunflower oil

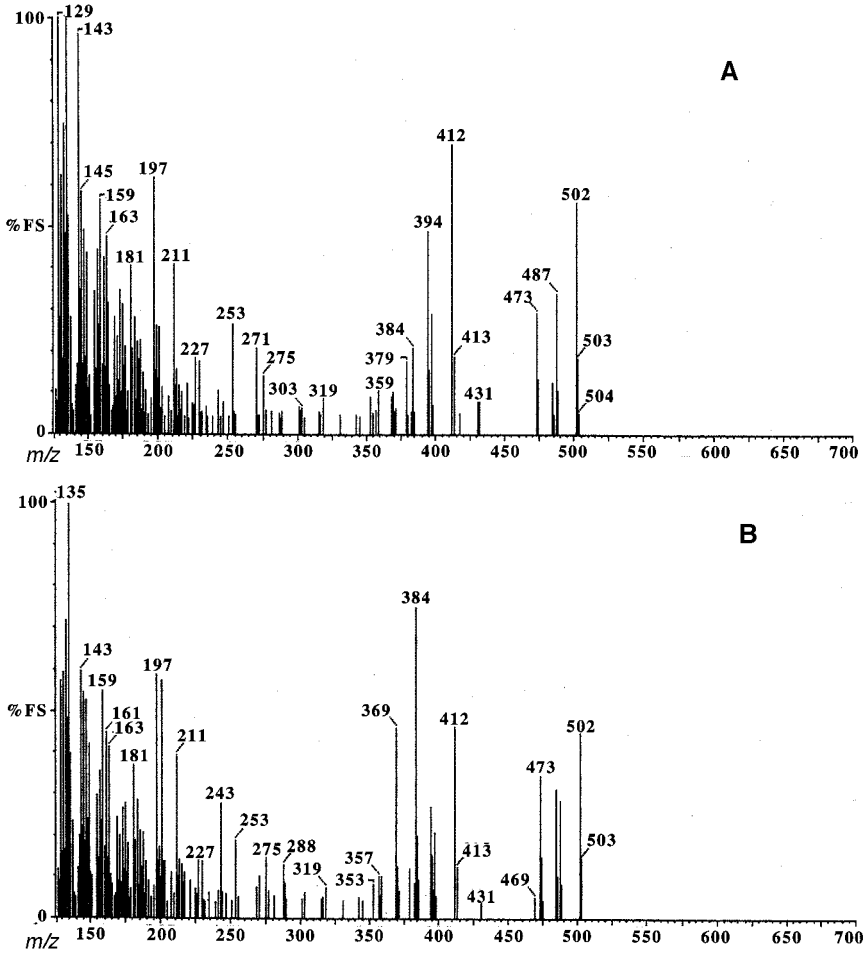

FIG. 6. (A) Mass spectrum of an authentic sample of trimethylsilyl (TMS)-ether derivative of 5α,6α-epoxysitosterol shows the molecular ion at 502. (B) Mass spectrum of an authentic sample of TMS-ether derivative of 5β,6β-epoxysitosterol shows the molecular ion at 502. See Figure 1 for other abbreviation.

ranged from 46 to 49 ppm at 0 to 25 wk of storage, respectively (Table 2). 7-Ketositosterol was present in the highest amount, *ca*. 16 ppm, and remained almost unchanged during storage. The content of sterol oxides in the chips, fried in high-oleic sunflower oil, was 35 ppm at 0 time, and this amount increased to *ca*. 59 ppm after 25 wk of storage (Table 3). The proportions of different oxides of campesterol and sitosterol in the chips prepared in high-oleic sunflower oil were rather similar and were also dominated by 7-ketocampesterol and 7-ketositosterol.

Few reports on sterol oxides in chips that have been fried in vegetable oils have been published. Potato chips fried in cottonseed oil and packaged in foil, stored at 40°C for 95 d and at 23°C for 150 d, were investigated for the content of ox-

TABLE 1
Levels of Sterol Oxides (ppm)[a] at 0, 10, and 25 wk of Storage in Lipids of Potato Chips Fried in Palm Oil[b]

Sample	Weeks	7α-OH	7β-OH	7-Keto	Epoxy[c]	Dihydroxy	Total
Sitosterol	0	0.6	0.8	0.9	0.9	0.2	5.0
Campesterol		0.3	0.2	0.7	0.3	0.1	
Sitosterol	10	0.5	1.0	1.3	0.8	0.2	6.1
Campesterol		0.3	0.3	1.0	0.6	0.1	
Sitosterol	25	1.2	1.4	2.0	1.9	0.9	8.6
Campesterol		0.1	0.2	0.7	0.2	nd[d]	

[a]Means of duplicate analyses. [b]Chips fried in palm oil from group 3 (Ref. 7). [c]Includes both 5α,6α-epoxy- and 5β,6β-epoxysterol. [d]n.d., not detected, that is, <0.1 ppm.

TABLE 2
Levels of Sterol Oxides (ppm)[a] at 0, 10, and 25 wk of Storage in Lipids of Potato Chips Fried in Sunflower Oil[b]

Sample	Weeks	7α-OH	7β-OH	7-Keto	Epoxy[c]	Dihydroxy	Total
Sitosterol	0	4.4	9.9	16.1	3.5	1.2	45.8
Campesterol		0.9	1.5	4.7	2.4	1.2	
Sitosterol	10	4.9	10.4	16.5	4.4	1.2	49.4
Campesterol		1.1	1.9	6.0	2.1	0.9	
Sitosterol	25	6.6	8.8	15.7	4.6	0.8	47.1
Campesterol		0.6	1.5	5.3	2.6	0.6	

[a]Means of duplicate analyses. [b]Chips fried in sunflower oil from group 2 (Ref. 7). [c]Includes both 5α,6α-epoxy- and 5β,6β-epoxysterol.

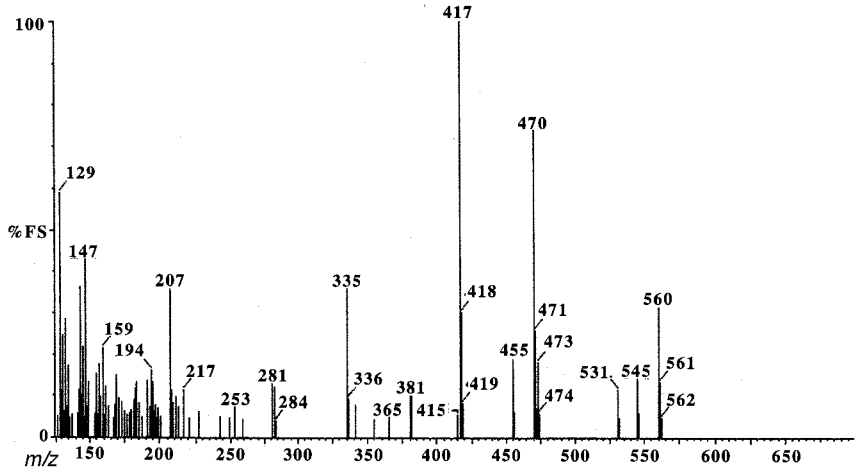

FIG. 7. Mass spectrum of an authentic sample of the trimethylsilyl (TMS)-ether derivative of dihydroxycampesterol; the base peak for tris-TMS ether derivative is at *m/z* 417 (M^+ – Ring A). See Figure 1 for other abbreviation.

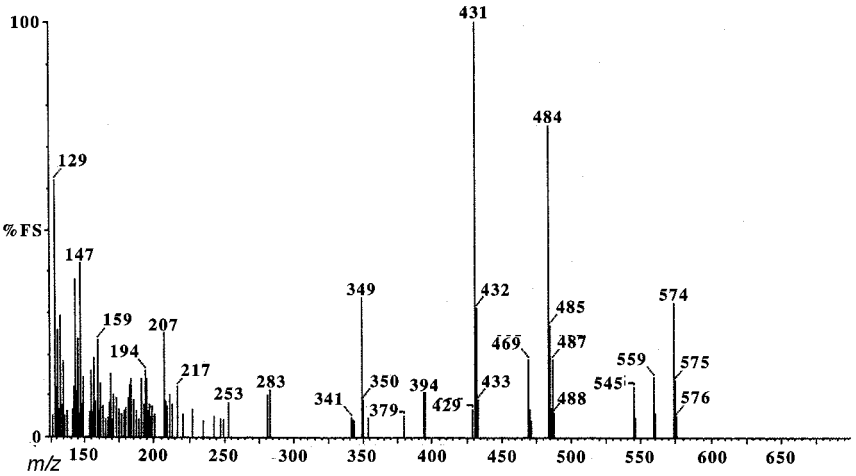

FIG. 8. Mass spectrum of an authentic sample of the trimethylsilyl (TMS)-ether derivative of dihydroxysitosterol. The base peak for tris-TMS ether derivative is at *m/z* 431 (M^+ – Ring A). See Figure 1 for other abbreviation.

idized phytosterols (5). No "detectable" amounts of sterol oxides were found in the chips during storage at 23°C. However, the detection limit of the HPLC method used in that study was not specified. Chips stored at 40°C for 95 d had β-epoxysitosterol, 6 ppm; 7α-hydroxysitosterol, 13 ppm; and 7β-hydroxysitosterol, 9 ppm in the lipids. Only three sitosterol oxides were quantitated, and the total amount was 28 ppm in the lipids. The authors suggested that the oxidation products were a result of elevated temperature because the sample stored at 25°C did not produce any detectable oxidation products. Results from the present study show that storage at 25°C up to 25 wk did not increase the amounts of oxidized sterols to any great extent, except for chips prepared in high-oleic sunflower oil, where the content of sterol oxides tended to increase after 10 wk of storage. In the present study, the total level of six oxides generated from campesterol and sitosterol were at maximum levels of 9, 49, and 59 ppm in the lipids of chips fried in palm oil, sunflower oil, and high-oleic sunflower oil, respectively, after storage for 25 wk. The lower level of total sterol oxides in the lipids of chips fried in palm oil may be due to considerably lower levels of total sterols in palm oil (30).

TABLE 3
Levels of Sterol Oxides (ppm)[a] at 0, 10, and 25 wk of Storage in Lipids of Potato Chips Fried in High-Oleic Sunflower Oil[b]

Sample	Weeks	7α-OH	7β-OH	7-Keto	Epoxy[c]	Dihydroxy	Total
Sitosterol	0	2.8	7.8	10.9	3.1	1.7	35.1
Campesterol		1.0	1.7	3.3	2.0	0.8	
Sitosterol	10	2.7	11.3	14.2	4.2	2.3	54.8
Campesterol		1.3	2.4	12.2	2.9	1.3	
Sitosterol	25	5.3	11.5	18.1	6.3	3.6	58.5
Campesterol		0.7	1.5	6.2	3.5	1.8	

[a]Means of duplicate analyses. [b]Chips fried in high-oleic sunflower oil from group 3 (Ref. 7). [c]Includes both 5α,6α-epoxy- and 5β,6β-epoxy-sterol.

The content of sterol oxides in potato chips fried in an unspecified oil was reported (6). It was demonstrated in that

study that potato chips contained α-epoxycholesterol at 4 ppm and 25-hydroxycholesterol at 7 ppm in the samples. The same sample contained β-epoxysitosterol, 4 ppm; dihydroxysitosterol, 3 ppm; 7α-hydroxysitosterol, 5 ppm; 7β-hydroxysitosterol, 7 ppm; and 7-ketositosterol, 2 ppm. In addition, the chip samples contained α-epoxycampesterol, 3 ppm; β-epoxycampesterol, 8 ppm; dihydroxycampesterol, 5ppm; and a trace amount of 7α-hydroxycampesterol. The total amount of the oxidation products from cholesterol, sitosterol, and campesterol in that chip sample was rather high, being 48 ppm. The level of unoxidized cholesterol was 12 ppm, whereas a level of total oxidized cholesterol of 12 ppm in the samples was demonstrated in that report (6).

When the levels of total sterol oxides are converted from ppm in lipids to ppm in the chips at an average lipid content at 33% (30), as in this study, one finds that the levels are *ca.* 3, 16, and 19 ppm in samples fried in palm oil, sunflower oil, and high-oleic sunflower oil, respectively, after 25 wk of storage. These levels may be considered quite low compared with the levels of cholesterol oxidation products in some foods of animal origin (4). From the results presented here, it can be concluded that the sterol oxides are qualitatively and quantitatively quite similar in potato chips prepared in regular sunflower oil and high-oleic sunflower oil. More studies are required on the assessment and on the kinetics of the formation of phytosterol oxides during industrial frying operations with vegetable oils, as well as during storage conditions of the food products, all of which are important in identifying factors that contribute to the generation of these oxidation products.

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