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Sterols and sterol oxides in the potato products, and sterols in the vegetable oils used for industrial frying operations

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SUMMARY

Sterols and sterol oxides in the potato products, and sterols in the vegetable oils used for industrial frying operations

The objective of this study was to determine the composition of sterols in vegetable oils used in industrial frying operations, and sterols and sterol oxides in the fried potato products. The sterols and sterol oxides were enriched by saponification of oils and by solid phase extraction. Preparative thin layer chromatography, capillary gas chromatography, gas chromatography-mass spectrometry, and nuclear magnetic resonance spectroscopy, were used to give qualitative and quantitative data. The results revealed that the content of desmethylsterols in palm oil, sunflower oil, high oleic sunflower oil, and rapeseed oil/palm oil blend were, 790, 4501, 3550, and 4497 ppm, respectively. Sitosterol was the major desmethylsterol in all samples. Palm oil also contained the lowest levels of total unsaponifiables. The sterols and unsaponifiable contents in sunflower oil were, to some extent, higher than in high-oleic sunflower oil. The compositions of sterols after two days of frying were neither markedly different in the oils nor in the potato products fried in these oils compared with the original oils. Isomerised sterols were tentatively quantified to account for 10 ppm, 50 ppm and 20 ppm, in rapeseed oil/palm oil blend, sunflower oil, and high-oleic sunflower oils, respectively. Lipids extracted from French fries prepared in rapeseed oil/palm oil blend contained the highest levels of total sterol oxides, 191 ppm, and epoxides of both sitosterol and campesterol were the major contributors, together at a level of 172 ppm. On the other hand, lipids extracted from French fries prepared in sunflower oil and high-oleic sunflower oil contained 7α-hydroxy-, 7β-hydroxy-, 7-keto- and both epimers of epoxysitosterol, generally in equal amounts. All samples also contained small amounts of different oxidation products of campesterol and stigmasterol.

KEY-WORDS: Crisps – French fries – Industrial frying – Sterols (composition) – Sterol oxides – Vegetable oils.

1. INTRODUCTION

Different vegetable oils with altered fatty acid compositions generated by breeding programmes, are being used as an alternative to saturated fats and oils or hydrogenated vegetable oils in frying operations. Some of these vegetable oils are high-oleic safflower oils (Fuller et al., 1971), low-linolenic soybean oils (Mounts et al., 1994), high-oleic canola (Warner et al., 1994), and high-oleic sunflower oils (Eskin et al., 1989). The fatty acid composition is the main determining factor for the rate of lipid oxidation during frying operations. However, tocopherols and phytosterols, being minor components in the vegetable oils, are of importance for stability also. The phytosterols are mainly C-28 and C-29 carbon steroid alcohols (Kochhar, 1983). Phytosterols are known to be considerably less absorbed than cholesterol and it is known that the phytosterols have a cholesterol lowering effect in blood (Vanhanen et al., 1993). The composition of the phytosterols is of interest also since Δ5-avenasterol is known to act as an antioxidant and antipolymerisation agent during heating of vegetable oils (Gordon and Magos, 1983). However, very little effort has been made to increase the concentrations of these important minor components in the vegetable oils during development of new vegetable oils. Because of health concerns, most of the studies on oxidation products of sterol have been focused on cholesterol (Peng and Morin, 1992). Phytosterols, with structures similar to that of cholesterol, also undergo oxidation as cholesterol, but literature reports on phytosterol oxidation products are sparse (Bösinger et al., 1993). Occurrence of phytosterol oxides in vegetable oils and in potato products prepared in vegetable oils have been reviewed recently (Dutta et al., 1996).

As a part of the whole project, the task of this laboratory was to study the contents of phytosterols in the four vegetable oils/fat blends and subsequent changes in the content of sterols and the oxidation products of these sterols during frying operations. This paper deals with the content of sterols and their qualitative and quantitative changes in the vegetable oils during frying and in the fried products.

2. MATERIALS AND METHODS

The details in the description of the origin of the vegetable oils, their refining processes, and the production of different fried potato products are given elsewhere (Niemelä et al., 1996). The oils, before and

during frying operations, and the fried products were supplied by Raisio Margini Oy, Raisio, Finland, and INRA, Dijon, France. As soon as they were received, the oil samples and the fried potato products were stored at -20°C until further analysis.

Reagents

Standard sample of sitosterol was purchased from Research Plus Inc. (New Jersey, USA); campesterol, stigmasterol, 19-hydroxycholesterol, 5α-cholestane and stearic acid were purchased from Sigma Chemical Company (St. Louis, MO, USA). All chemicals and solvents used were of analytical grade and purchased from Merck (Dermstadt, Germany) unless otherwise stated.

Analytical

Lipid extraction. In brief, a 2.5 g sample of crisps was homogenised twice by an Ultra-Turrax T25 homogeniser (Jankel & Kunkel GmbH, Staufen, Germany) each time with 50 ml of hexane: 2- propanol (HIP) (Hara and Radin, 1978) at maximum speed for 30 sec. The homogenates were filtered and the filtrates were collected in a separatory funnel. The pooled filtrate was mixed with 50 ml of 6.67 % anhydrous Na_2SO_4 in water and the upper phase was collected and evaporated to dryness under vacuum at 30° C by a rotary evaporator. In the case of French fries, a 40 g sample was extracted twice with 100 ml of HIP and 100 ml of 6.67 % Na_2SO_4 was used. The comparison of the methods of extraction was done only with the crisps sample. The other solvent systems compared were chloroform : methanol (C:M) (Folch et al., 1957), and hexane. However in the case of hexane extraction, no washing with aqueous salt solution was used.

Saponification for sterol and sterol oxide analyses For sterols, ca 20 mg of total lipids and 20 mg of 5α-cholestane as internal standard were mixed well with 2 ml 2 M KOH in 95 % ethanol in ground glass stoppered tubes. The tubes were kept for 45 min at 60°C in a glycerol bath under shaking. The reaction was stopped by cooling the tubes under running cold water and 1 ml water, 2 ml hexane and 0.1 ml ethanol were added. The tubes were shaken vigorously and then centrifuged briefly in a Hettich centrifuge EBA 12 (Hettich, Tuttlingen, Germany). The hexane layer was transferred to small glass tubes and dried under nitrogen, and the lipids were dissolved in 0.1 ml chloroform and stored at -20 °C until further analyses. For sterol oxides, ca 0.5 g of lipids with ca 10µg of 19-hydroxycholesterol as internal standard were mixed well with 5 ml of 2 M potassium hydroxide in ethanol in a glass tube and left overnight (about 18 hr) in the dark at room temperature (Park & Addis,1986). After addition of 10 ml of dichloromethane and 10 ml of 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 evaporated under nitrogen and the unsaponifiables were dissolved in chloroform for enrichment of sterol oxides, by solid phase extraction (SPE) as described below.

Synthesis of phytosterol oxides. For the preparation of different phytosterol oxides different methods were used (Nourooz-Zadeh and Appelqvist, 1992; Fieser and Fieser,1967; Chicoye et al., 1968). The oxidised sterols were purified by preparative thin layer chromatography as described below.

Thin layer chromatography (TLC). For both analytical and preparative purposes, precoated TLC plates, silica gel 60, 20 X 20 cm, 0.25 mm thicknesses were used (Merck, Darmstadt, Germany). For preparative purposes to separate Δ5-sterols from Δ7-sterols, total unsaponifiables from 0.5 g oils were 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 the same sample was also applied. The plate was developed in hexane : diethyl ether (50 : 50, v/v) up to the top of the plate. After a brief drying of the plate, that part of the plate containing the 0.5 cm band was cut off. This part of the plate was sprayed with phosphomolybdic acid, 10% in ethanol : diethyl ether (50 : 50, v/v) and was heated in an oven at 120°C for 15 min. The Δ5-sterols and Δ7-sterols zones were scraped off and the sterols were eluted from the gel consecutively with 5 ml and 2.5 ml of chloroform: methanol (2:1,v/v). The solvents were evaporated under nitrogen and the sterols were dissolved in 0.5 ml chloroform and stored at -20°C until further analysis. For the purpose of purifying the oxidised sterols, ca. 20 mg of the sample was applied as a 16 cm band on the TLC plate by an automatic applicator along the side of the sample band. A 0.5 cm band of a standard cholesterol oxide samples was also applied. The plate was developed in diethyl ether: cyclohexane (9:1, v/v) and different sterol oxides were extracted from silica gels as described by Aringer and Nordström, (1981).

Enrichment of sterol oxides from total unsaponifiables by solid phase extraction (SPE). In brief, a 0.5 g silica cartridge (International Sorbent Technology Ltd, Mid Glamorgan, U. K.) was used for this purpose. The total unsaponifiables prepared earlier by cold saponification were dissolved in 1 ml hexane: diethyl ether (75:25, v/v) and were loaded on to the column. The tube was washed with an additional 2 ml hexane : diethyl ether (75:25, v/v) and eluted through the columns at a rate of ca. 4 ml per min. Thereafter, the column was eluted with 6 ml hexane : diethyl ether (60 : 40, v/v) and the eluates were discarded. The sterol oxides and the remaining sterols were eluted with 5 ml acetone (De Vore, 1988). The acetone was evaporated under nitrogen and the residue was again dissolved in 1 ml hexane : ether (75 : 25). This SPE extraction was repeated as described above except that the column

was eluted with 3 ml hexane : ether (60:40, v/v). Finally the column was eluted with 5 ml acetone and the acetone was evaporated under nitrogen. The residue was derivatised to TMS-ethers as described below for subsequent analyses by capillary column gas chromatography (GC) and GC-mass spectrometry (MS).

Preparation of TMS-ether derivatives of sterols and sterol oxides. Total unsaponifiables and the enriched sterol oxides were derivatised to TMS-ethers by adding 100 µl of Tri-Sil reagent (Pierce, Illinois, USA) and incubating the tubes 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 centrifuged for 3 min. The hexane layer was transferred to another tube, avoiding any solid particles, and evaporated to dryness, dissolved in 0.5 ml hexane and analysed by GC and GC-MS.

Capillary column gas chromatography (GC). The TMS-ether derivatives of sterols and sterol oxides were analysed by a Varian 3700 gas chromatograph (Varian, Palo Alto, U.S.A.) equipped with a flame-ionisation detector and a falling needle injector. A WCOT fused silica capillary column CP-Sil-5 CB (Chrompack, Middelburg, The Netherlands), 30 m X 0.25 mm, 0.25 µm film thickness was used. Helium was used as carrier gas at an inlet pressure of 17 psi and as make-up gas at a flow rate of 30 ml per min. The GC oven was run with a temperature programme: At 258°C for 31 min and then raised to 270°C at a rate of 3°C/min and left for an additional 15 min. Detector temperature was at 300°C. The peaks were computed by an HP 3396A integrator (Hewlett -Packard, Avondale, U.S.A). Identification of the four common desmethyl sterols was done by comparing the relative retention times of 5α-cholestane with those of standard cholesterol, campesterol, stigmasterol and sitosterol. Δ7-Stigmastenol and Δ5-avenasterol were identified by comparing the elution pattern of the peaks in the GC with those from samples known to be rich in these sterols viz. safflower oil (Kochhar, 1983) and oat lipids (Dutta and Appelqvist, 1996 a). The confirmation of structure of the sterols was done by GC-MS as described below. The amount of sterols was determined from the weight of 5α-cholestane as internal standard. No response factors were used. All the samples were analysed in duplicate and the mean results are reported. The identification of sterol oxides was done by comparing the retention times and mass spectra of samples with those of reference compounds by GC and GC-MS as described below.

Gas chromatography-Mass spectrometry (GC-MS) for identification of sterols and sterol oxides. GC-MS analyses were performed on a HP5890 series II gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A) 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 sterols were separated on a WCOT fused silica capillary column CP-Sil-5 CB (Chrompack, Middelburg, The Netherlands), 30 m X 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 purge delay time was 0.8 min. A programmed oven temperature was used at 60°C for 2 min, raised to 265°C at a rate of 20°C/min, and then 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 at 200°C.

Nuclear magnetic resonance (NMR) spectroscopy. Standard sitosterol and separated fractions of desmethylsterols were analysed by ^{1}HNMR (400 MHz) at 30°C on a Varian VXR-400 instrument. Deuteriochloroform ($CDCl_3$) was used as solvent and the chemical shifts were correlated to $CHCl_3$ (δ 7.26).

RESULTS

Hexane, the typical solvent for neutral lipid extraction from dry high fat products was compared with CM (Folch et al, 1957) and HIP (Hara and Radin, 1978). The method of lipid extraction with HIP was shown to extract the most polar lipids from rat brain, comparable to CM. In addition, HIP is less toxic than CM (Hara & Radin, 1978). The results on total lipids extracted by different solvents are presented in table I. Since there were no marked differences in total lipids extracted by the HIP and CM methods, HIP was used for subsequent extraction of lipids from potato products. Lipids extracted by hexane may contain some non-lipid components which probably contributed to slightly higher amounts of lipids compared with CM/HIP methods, since no purification by aqueous salt solution is used with hexane extraction.

Table I
Content of total lipids as extracted by different solvents from a sample of crisps fried in sunflower oil

Solvent	Total lipids, %	Mean
Hexane, !00 %	33.7	33.0
	32.2	
Chloroform : Methanol*)	32.0	31.7
	31.5	
Hexane : 2-Propanol **)	34.2	33.6
	33.0	

*) Folch et al , 1957, **) Hara & Radin, 1978.

Table II
Content of major desmethylsterols and total unsaponifiables (ppm) in different vegetable oils used for industrial preparation of crisps

Sample	Campesterol	Stigmasterol	Sitosterol	Δ5-Avenasterol	Δ7-Avenasterol	Δ7-Stigmastenol	Others	Total unsaponifiables
ICO2PO-0	227 (29)	59 (7)	438 (55)	43 (5)	-	22 (3)	291	1081
ICO2PO-2	249 (26)	78 (8)	544 (56)	39 (4)	13 (1)	51 (5)	492	1466
ICO1SO-0	452 (10)	342 (8)	2402 (53)	188 (4)	241 (5)	875 (19)	2588	7089
ICO2SO-2	420 (9)	331 (7)	2413 (54)	188 (4)	250 (6)	875 (19)	2185	6664
ICO1HOSO-0	257 (7)	303 (8)	1919 (54)	139 (4)	183 (5)	749 (21)	1934	5484
ICO3HOSO-2	241 (7)	272 (8)	1746 (54)	130 (4)	160 (5)	681 (21)	1825	5055

All values are mean of duplicate analyses. (Figures in parenthesis denote corresponding percentage composition).
ICO2PO-0 = palm oil from group 2 before frying, ICO2PO-2 =palm oil from group 2 after two days of frying, ICO1SO-0 = sunflower from group 2 before frying, ICO2SO-2 = sunflower oil from group 2 after two days of frying, ICO1HOSO-0 = high oleic sunflower oil from group 1 before frying, ICO3HOSO-2 = high oleic sunflower oil from group 3 after two days of frying, Others = unidentified sterols and other unsaponifiables.

Contents of the major desmethylsterols in the oils used for the preparation of crisps at day 0 and after two days of frying operation are presented in table II. In all these oils, sitosterol is the major component. The other identified desmethyl sterols were campesterol, stigmasterol, Δ7-stigmastenol, Δ5-avenasterol, and Δ7-avenasterol. In addition palm oil contained small amounts of cholesterol at a level of ca 10 ppm which is included in the column «Others». A small amount of Δ7-avenasterol was detected in palm oil after two days of frying. The concentration of stigmasterol was comparatively lower in palm oil compared with the other two oils. Palm oil contained much lower amounts of total unsaponifiables, ca 0.1 % of oils compared with sunflower oil, which had ca 0.7 %, and high-oleic sunflower oil, which had ca 0.5 % of total unsaponifiables in the oils.

The components presented under the column «Others» are 4-mono- and 4, 4´-dimethylsterols in addition to other components, e.g. tocopherols. The

Table III
Content of major desmethylsterols and total unsaponifiables (ppm) in the lipids extracted from Crisps fried in different vegetable oils

Sample	Campesterol	Stigmasterol	Sitosterol	Δ5-Avenasterol	Δ7-Avenasterol	Δ7-Stigmastenol	Others	Total unsaponifiables
IC1PO3-0	250 (24)	84 (8)	547 (53)	62 (6)	17 (2)	65 (6)	401	1425
IC1SO2-0	349 (9)	277 (7)	2055 (54)	186 (5)	222 (6)	748 (19)	2124	5961
IC1HOSO3-0	249 (8)	258 (8)	1613 (52)	137 (4)	181 (6)	649 (21)	1891	4977

All values are mean of duplicate analyses. (Figures in parenthesis denote corresponding percentage composition).
IC1PO3-0 = crisps prepared in palm oil from group 3 at the day of preparation, IC1SO2-0 = crisps prepared in sunflower oil from group 2 at the day of preparation, IC1HOSO3-0 = crisps prepared in high oleic sunflower oil from group 3 at the day of preparation, Others = unidentified sterols and other unsaponifiables.

high-oleic sunflower oil contained slightly lower amounts of total unsaponifiables, as well as the major desmethylsterols, compared with sunflower oil. There was a slight decrease in the contents of sterols in both sunflower and high-oleic sunflower oil after two days of frying, but palm oil had a small increase. This may be due to real effects of processing or cross-contamination at interchange of oils in the frying vats.

The content of desmethylsterols in the lipids extracted from samples of crisps prepared in these oils is presented in table III. The content of total unsaponifiables and the major desmethylsterols were slightly lower in the lipids extracted from crisps fried in sunflower oil and high-oleic sunflower oil compared with the frying oils. However, the lipids extracted from crisps fried in palm oil were similar to those of day 2 frying oil. The content of sitosterol was considerably less, 2055 ppm in the lipids extracted from crisps, compared with 2400 ppm in the sunflower oil at day 0.

The content of sterols in the oils used in the preparation of French fries is presented in table IV. In the preparation of French fries, a blend of partially hydrogenated rapeseed oil and palm oil was used as a reference oil. The content of total unsaponifiables was slightly higher in the rapeseed oil / palm oil blend, 0.53 %, compared with 0.48% and 0.44 % in sunflower oil and high-oleic sunflower oils, respectively. In the blend of rapeseed oil / palm oil, the major components of the desmethylsterols were sitosterol, campesterol, brassicasterol and Δ5-avenasterol. Also Δ7-avenasterol and Δ7-stigmastenol were present in this blend in quantifiable amounts. Since no great differences in the content of sterols were observed in the frying oils used for crisps after two days of frying operations (table II), no analyses were performed from the oils used in the preparation of French fries other than at day O.

As part of studies in this project, total unsaponifiables were separated by preparative TLC to yield fractions of unoxidised and oxidised sterols. On preparative TLC plates, a spot was located with R_f only slightly less than that of «unoxidised» sterols. This fraction was first assumed to contain «oxidised» sterols based on the R_fs reported previously (Pie et al.,1990). However, that method was developed to separate cholesterol from oxidised cholesterol. Later analyses by GC, GC-MS and NMR revealed that this fraction mainly contained Δ7-sterols e.g., Δ7-stigmastenol and Δ7-avenasterol. However, several other peaks on GC and GC-MS were tentatively identified from this fraction. Two NMR spectra from standard sitosterol and from the Δ7-sterols fraction are presented in figures 1a and 1b. A few mass spectra of tentatively identified components found in all the oils except in palm oil, are presented in Figures 2 a, b, c, and d. A peak eluted before campesterol in GC and GC-MS (fig. 2a) having M^+ at 484, is probably an isomer of stigmasterol or it could also be α-spinasterol. However, the identity as α-spinasterol was not confirmed by comparing GC and GC-MS data for this sample with those of standard α-spinasterol. Another component eluting after stigmasterol, having M^+ at 472, might be Δ7-campesterol. Two other components were observed, one eluting before sitosterol, having M^+ at 486, and the other one eluting after Δ7-stigmastenol, having M^+ at 486 (fig. 2c and 2d). Preliminary quantitative results showed that the amounts of these isomerised sterols are in the range of 10 ppm in rapeseed/palm oil blend, 50 ppm in the sunflower oil and 20 ppm in high oleic sunflower oil at day 0. For more accurate results, further studies are required.

The contents of different sterol oxides in the lipids of French fries produced in vegetable oils and prepared by heating at 250°C for 15 min, are presented in table V. All

Table IV
Content of major desmethylsterols and total unsaponifiables (ppm) in different vegetable oils used for industrial preparation of French fries

Sample	Brassicasterol	Campesterol	Stigmasterol	Sitosterol	Δ5-Avenasterol	Δ7-Avenasterol	Δ7-Stigmastenol	Others	Total unsaponifiables
IFORP-0	270 (6)	1627 (35)	61 (1)	2308 (50)	217 (5)	27 (<1)	117 (2)	781	5278
IFOSO-0	-	340 (10)	280 (9)	1741 (54)	163 (5)	169 (5)	533 (16)	1596	4822
IFOHOSO-0	-	302 (9)	274 (9)	1765 (55)	131	134 (4)	571 (18)	1252	4429

All values are mean of duplicate analyses (figures in parenthesis denote corresponding percentage composition).
IFORP-0 = Rapeseed oil/palm oil blend before preparation of French fries, IFOSO-0 = sunflower oil before preparation of French fries, IFOHOSO-0 = high oleic sunflower oil before preparation of French fries,- = not detected, < = less than, Others = other unidentified sterols and other unsaponifiables.

the oxidation products of sitosterol, campesterol, and stigmasterol were identified based on the relative retention times and mass spectra of TMS ether derivatives of samples by comparing with those of authentic samples. Mass spectra of the samples were almost identical with those of authentic samples analysed under the same analytical conditions. Mass spectral data of different phytosterol oxides will be reported elsewhere (Dutta, 1996).

French fries prepared in the palm/rapeseed oil blend had the highest levels of sterol oxides (191 ppm) in the lipids, compared with sunflower oil (39 ppm) and high-oleic sunflower oil (69 ppm). The major contribution of the oxysterols in palm/rapeseed oil blend is from the epoxysterols of both campesterol and sitosterol, at 111 and 62 ppm, respectively. The epimeric epoxysterols are presented together in the table. However, the analyses showed that the 5α,6α-epoxysterol dominated in both campesterol and sitosterol epoxides. On the other hand, the major sterol oxides in both sunflower and in high-oleic sunflower oil are 7α-, 7β- hydroxysitosterol, and 7-ketosterols. In high oleic sunflower oil, these sterol oxides were present in slightly higher amounts than in sunflower oil. The epimeric epoxysterols are also major components in these oils, but in lower amounts than in rapeseed/palm oil blend, at a level of 6 and 13 ppm, respectively (table V). In the lipids of samples of French fries prepared in rapeseed oil/palm oil blend, sunflower oil, and high-oleic sunflower oil, 7α-hydroxystigmasterol was present at levels of 1.7, 0.8 and 0.5 ppm, respectively.

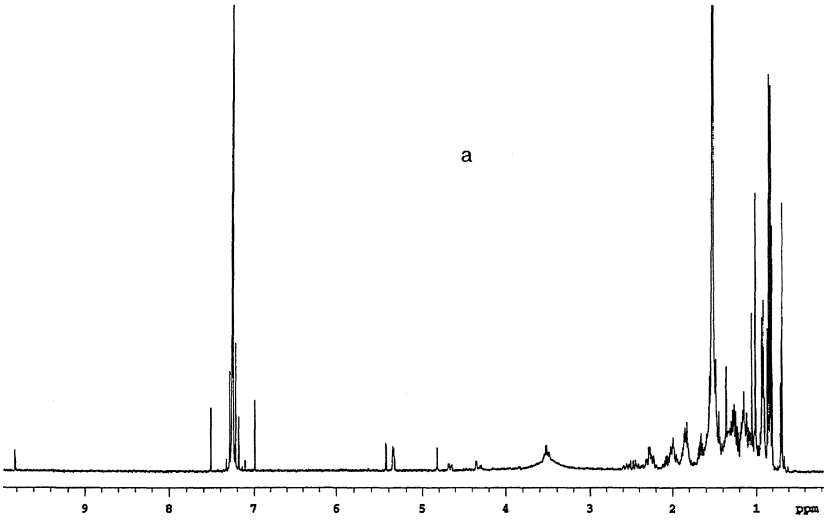

Figure 1a
5.3-5.4 ppm regions of 400 MHz ^{1}H NMR spectra of standard sitosterol

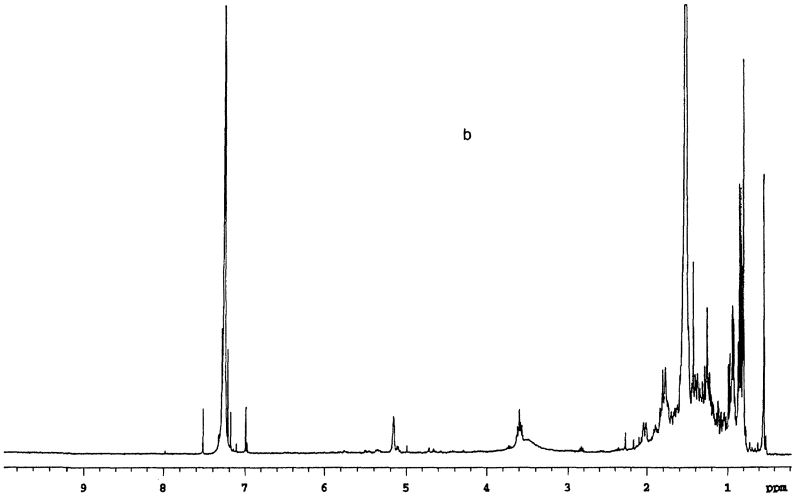

Figure 1b
5.16 ppm regions of 400 MHz ^{1}H NMR spectra of Δ7-sterols

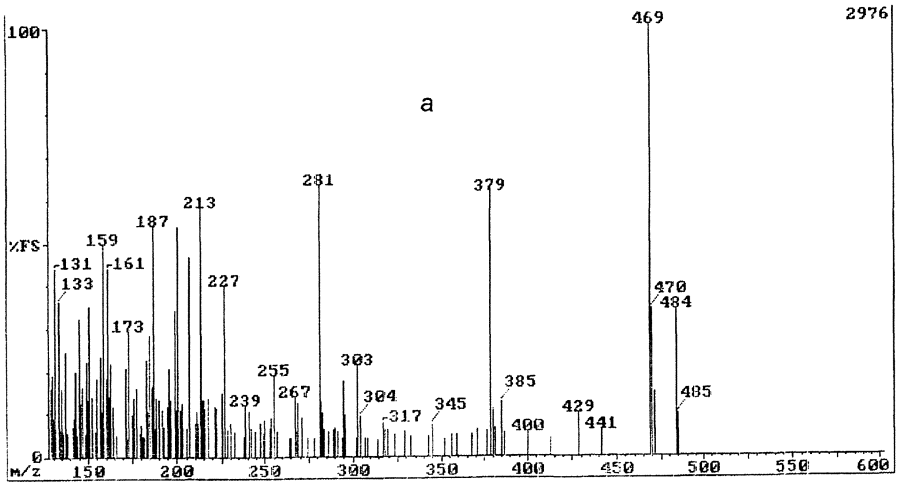

Figure 2a
Tentatively identified mass spectrum of Δ7-stigmasterol

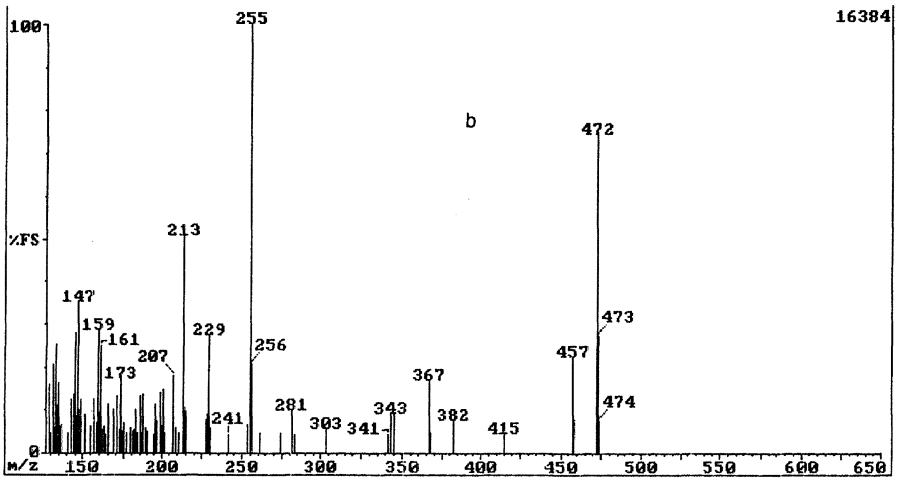

Figure 2b
Mass spectrum of Δ7-campesterol

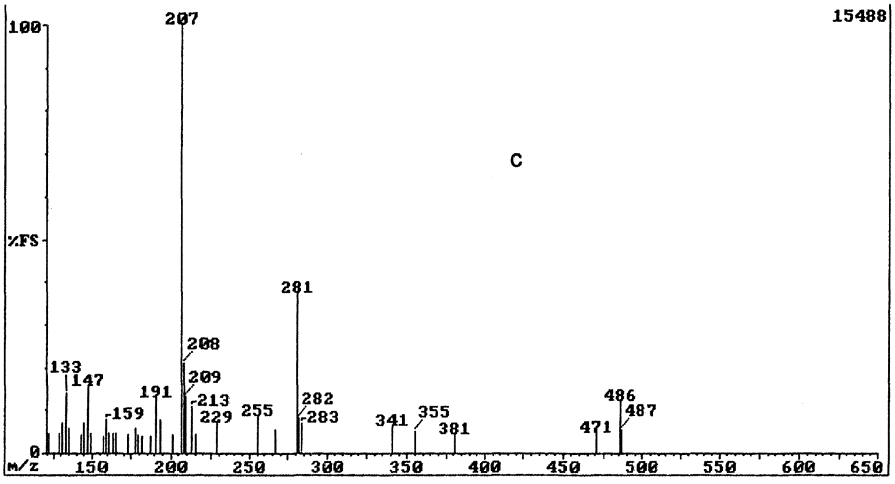

Figure 2c
Mass spectrum of an unidentified component eluting between stigmasterol and sitosterol

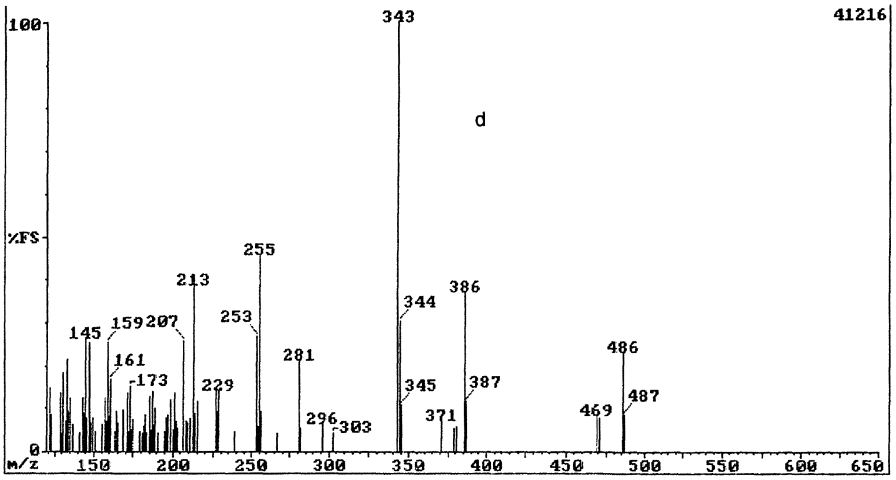

Figure 2d
Mass spectrum of an unidentified component eluting after Δ7-stigmastenol

Table V
Levels of sterol oxides (ppm) in the lipids of French fries samples fried in palm/rapeseed oil blend, sunflower oil and high-oleic sunflower oil, prepared for consumption by heating at 250°C for 15 minutes

Sample	7α-OH	7ß-OH	7-Keto	Epoxy	Triol	Total
IFFRP2						
Sitosterol	3.9	3.3	3.8	110.6	3.5	190.7
Campesterol	2.0	nd	nd	61.9	nd	
Stigmasterol	1.7	nd	nd	nd	nd	
IFFSO2						
Sitosterol	8.1	9.8	11.8	5.2	1.0	38.7
Campesterol	1.2	0.3	nd	0.4	nd	
Stigmasterol	0.8	nd	nd	nd	nd	
IFFHOSO2						
Sitosterol	12.1	13.0	18.4	9.2	10.9	69.0
Campesterol	0.9	nd	nd	4.0	nd	
Stigmasterol	0.5	nd	nd	nd	nd	

All values are mean of duplicate analyses.
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 fryied in high-oleic sunflower oil. nd = not detected, Epoxy = include both 5α, 6α-epoxy- and 5ß, 6ß-epoxysterol

DISCUSSION

The sterols in vegetable oils are grouped in three classes, viz 4-desmethylsterols, 4-monomethylsterols and 4, 4´-dimethylsterols (Kochhar, 1983), the 4-desmethylsterols being the dominating ones. The analysis of these three groups of sterols requires separation by chromatographic methods, generally by preparative TLC. In the present study, no such class separation was done, and identification of sterols was only done on desmethylsterols.

The composition of the desmethylsterols and the amounts of total unsaponifiables in the three vegetable oils are within the range of the published results (Itoh et al., 1973; Weihrauch and Gardner, 1978; Sciancalepore and Dorbessan, 1981; Kochhar, 1983; Zullo et al., 1989; Serani and Piacenti, 1992). However after two days of frying, palm oil contained small amounts of Δ7-avenasterol. Also a small increase in the total amounts of unsaponifiables as well as individual sterols was observed in this oil, and in the lipids extracted from crisps (see table II & III). This may be due to exchange of lipids from the potatoes during frying. Although potato contains very small amounts of lipids ca. 0.5% in dry weight (Lepage, 1968), the proportion of sterols in the potato lipids is quite high, ca. 16 % of the total lipids (Pun and Hadziyev, 1978).

Sterols and total unsaponifiables in the oil blend used for preparation of French fries will reflect that of rapeseed oil, because the proportion of rapeseed oil / palm oil was 80:20. The results essentially concur with published results for rapeseed oil (Itoh et al., 1973; Weihrauch and Gardner, 1978; Kochhar, 1983). Brassicasterol was detected only in this blend at a level of 270 ppm or 6 % in the desmethylsterols. It also had a higher proportion of campesterol, 35 % compared with ca 10 % in sunflower and high-oleic sunflower oil. From the literature reports it is also known that rapeseed oil contains relatively higher amounts of sterols as well as total unsaponifiables (Kochhar, 1983).

Since the industrial frying operations for preparing crisps and French fries were done on two different occasions, it was of interest to analyse the sunflower oils and high-oleic sunflower oils used in the preparation of French fries as well. The total sterols and total unsaponifiables were to some extent lower in these oils than in oils used for preparation of crisps. This was mainly observed in sunflower oil (see tables II and IV). This difference may be due to variations from batch to batch in industrial processing, or also to environmental factors during seed development which may influence the content of sterols in vegetable oils (Kochhar, 1983). It may be mentioned here that the French fries samples contained much more water than the crisps samples. Also French fries samples contain less lipids than crisps. Because of the almost complete water exchange with frying oils in crisps, it may be assumed that the lipids in the crisps sample will be in equilibrium with the frying oils, in contrast to the case with French fries.

Vegetable oils during refining processes undergo qualitative and quantitative changes. Generally, the total amounts of sterols decrease during different refining processes. However, the proportion of the major sterols remain mainly unchanged (Kochhar, 1983). Different transformation products of sterols are also generated during the refining processes. The common transformation products are dehydrated or dehydrogenated products of sterols, commonly called sterenes, disteryl ethers generated from sterols, and transformation products from 24-methylenecycloartanol, this area has been reviewed recently (Dutta et al.,1996). In the present study, no attempts were made to analyse these transformed products of sterols. However, from the fractions separated by preparative TLC, several components were tentatively identified. Some of these are not known from the literature reports except for one of these components, Δ7-campesterol, which was reported to be present in sunflower oil (Sciancalepore and Dorbessan, 1981; Serani and Piacenti, 1992; Weihrauch and Gardner, 1978). In a study on soybean oils, it was reported that the content of Δ7-stigmastenol in crude oil was 28 ppm, but increased to 118 ppm following physical refining for 2 h at 300°C. It was suggested that increased amounts of Δ7-stigmastenol occurred as a result of isomerisation of sitosterol (Jawad et al., 1984). This isomerisation, in principle, can also occur to other Δ5-sterols. As mentioned earlier, some of the other Δ7-sterols are probably isomers of Δ5-sterols that are generated during the refining processes. In this context, a brief discussion is presented on the isomerisation of the sterols, supported by the ^{1}H NMR study. During oxidation of the sterols, the 7-allylic radicals may isomerise to the more stable tertiary radical, which in turn abstracts hydrogen from the media. In support of this assumption, the ^{1}H NMR spectrum of this sample shifted to a higher field for the olefinic proton. While the standard sample of sitosterol has its signal around 5.3-5.4 ppm, the Δ7-sterols fraction shows a signal at 5.16 ppm (Figures 1a and 1b). This assumption was further supported by MS data showing that not all these components were Δ5-sterols since these sterols generally have a base peak at m/z=129 (Rahier and Benveniste, 1989), which was not observed in the MS data presented in figures 2a, b, c, and d. The contributions of different refining processes to the isomerisation of sterols require further study.

Literature reports on the contents of different sterol oxides in potato products prepared in vegetable oils are scarce (Lee et al., 1985). In that report, French fries fried in mixed animal fat and vegetable oils were analysed by HPLC and the contents of cholesterol and phytosterol oxides were presented together. Only values of both epimers of epoxy- and 7-hydroxysterols were presented. The contents of these sterol oxides varied quite substantially. The highest levels of 7α- and 7β- hydroxysterols were 21 and 81 ppm respectively, and those for 5α,6α- and 5β,6β-epoxysterols were 19 and 27 ppm respectively. Lipids extracted from French fries samples, prepared in rapeseed oil/palm oil blend contained considerably higher amounts of sterol oxides (191 ppm) than sunflower oil (39 ppm) and high-oleic sunflower oil (69 ppm). The generation of the larger amounts of sterol oxides in IFFRP2 sample is probably a consequence of the amounts of sterols present in the oils (table IV) needs to be studied, since the rapeseed oil/palm oil blend contained higher levels of both campesterol (1627 ppm) and sitosterol (2308 ppm) than regular sunflower oil (campesterol, 340 ppm; sitosterol, 1741 ppm), and high- oleic sunflower oil (campesterol,302 ppm; sitosterol,1765 ppm). We have been unable to find any reports showing the presence of stigmasterol oxides in potato products fried in vegetable oils. French fries samples in this study, heated at 250°C for 15 min as instructed on the package, contained small amounts of 7α-hydroxystigmasterol. Further investigations were undertaken (Dutta, 1996) to study the effect of heating temperature on the generation of different sterol oxides.

From the results presented in this report, it may be concluded that qualitative and quantitative analyses of sterols in the high-oleic sunflower oil and in the French fries prepared in that oil were rather similar to those of regular sunflower oil. French fries prepared in the reference oil (rapeseed oil/palm oil blend) contained much higher levels of sterol oxides than levels found in the fries prepared in regular sunflower oil and high-oleic sunflower oil.

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