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ORIGINAL PAPER

Sterols and Oxidized Sterols in Feed Ingredients Obtained from Chemical and Physical Refining Processes of Fats and Oils

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Abstract The by-products obtained from conventional chemical and physical refining processes for edible fats and oils are important sources of valuable fatty components such as sterols, tocopherols, fatty acids, etc., and are also used as ingredients in animal feed formulations. Reports on sterol composition and content are limited, and the levels of oxidized sterols in these valuable by-products are unknown. This study analyzed by-product fractions from European refineries intended for use as ingredients in animal feeds for their content and composition of sterols and sterol oxidation products. The complex mixtures of sterol oxidation products were separated and quantified by multidimensional capillary columns, a medium polar DB-17MS and an apolar DB-5MS, in GC and GC–MS. Sterol content ranged from 0.1 to 3.4 and 0.03 to 5.0 g/100 g in the by-product fractions collected from chemical and physical refining processes, respectively, while the corresponding ranges for sterol oxidation products were 0.02–17 and 0.02–1.5 mg/100 g.

Keywords Acid oil fraction · Chemical refining · Distillate · Physical refining · Phytosterols · Phytosterol oxidation products · POPs · Soapstock · Sterols

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Introduction

Crude fats and oils contain free fatty acids, acylglycerols, phosphatides, pigments, sterols (cholesterol and phytosterols), sterol oxidation products (SOPs), tocopherols, fat-soluble vitamins, hydrocarbons, trace metals, and possible contaminants. Crude fats and oils require refining to be suitable for human consumption [1]. The conventional processes for refining crude oils are based on chemical and physical processes (Fig. 1), including degumming, neutralization, bleaching, and deodorization [1]. In the chemical refining process, the neutralization step removes free fatty acids as sodium soap (Reaction 1), and the soapstock formed is removed by centrifugation:

$$\mathrm{RCOOH} + \mathrm{NaOH} \longrightarrow \mathrm{RCOO^-Na^+(soap)} \quad \text{Reaction 1}$$

$$2\mathrm{RCOONa} + \mathrm{H_2SO_4} \longrightarrow 2\mathrm{RCOOH} + \mathrm{Na_2SO_4} \quad \text{Reaction 2}$$

The soap fraction is split with a mineral acid to release the fatty acids (Reaction 2), and this acid oil fraction is then restored from the water/oil emulsion [1, 2]. In this paper, this fraction is referred to as the acid oil fraction from chemical refining, designated AOCHE [3]. The acid oil fraction from the physical refining process together with some other volatile components generated from acid degumming followed by steam stripping under vacuum (steam distillation) is referred to as the distillate fraction from physical refining, designated AOPHY [3].

In the neutralization step during the chemical refining process, up to 21% of sterols from the crude oil can be transferred to soapstock. Sterols can comprise up to 70% of the unsaponifiable fraction in soapstock [4, 5]. The distillate fraction from the physical refining process can contain 2–20% sterols [4, 6]. The by-products from chemical and physical processes are rich in e.g. free fatty acids, sterols

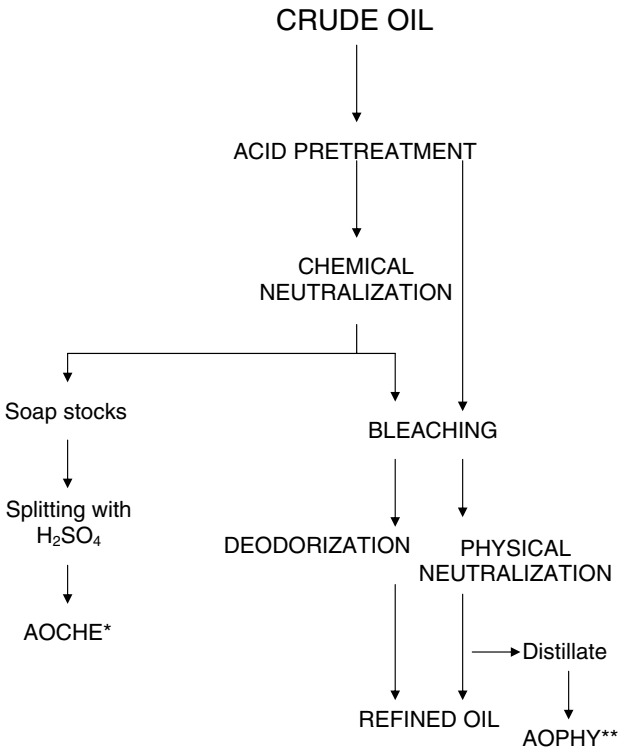

Fig. 1 Flow chart showing the major steps in chemical and physical refining processes for edible fats and oils *AOCHE* by-products fraction after splitting of soapstock from chemical refining of edible fats and oils, **AOPHY* by-product distillate fraction from physical refining of edible fats and oils

and tocopherols, which are useful in a wide range of industries, e.g. animal feed manufacturing, pharmaceuticals, and bio-diesel production [1, 7].

During refining of crude oils and fats, the amount of sterols gradually decreases in each stage of the process, keeping their relative proportions rather constant [4]. A number of factors may contribute to loss of sterols, including adsorption, partitioning, hydration, and oxidation. In general, complete refining of vegetable oils results in a variable phytosterol loss in the refined oil, ranging from 10 to 70% [4, 5]. Some of the sterols can be oxidized during refining steps such as heating, degumming, neutralization, bleaching, and deodorization, and during storage and handling [5]. However, limited information is available on the levels of sterols in the by-product fractions collected from chemical and physical refining processes [5–11]. To our knowledge, no published data are available on the levels of SOPs in these by-product fractions.

Cholesterol, the main sterol in animals, can generate numerous cholesterol oxidation products (COPs). Possible adverse biological effects of COPs have been extensively studied [4]. Phytosterols, plant sterols with a structure similar to that of cholesterol, can also be oxidized, generating numerous phytosterol oxidation products (POPs). A study on absorption of POPs in animals showed that very small amounts of POPs were absorbed by rats [12]. In another study, the levels of POPs in the plasma of 13 healthy human volunteers was found to range between 5 and 57 ng/mL. It was suggested that these POPs originated either from the diet or from in vivo oxidation of plasma phytosterols [13]. At present, only limited information is available on the biological effects of POPs [4, 14].

Vegetable oils and animal fats are essential in animal feed formulations. Some animal feed ingredients are by-products of chemical and physical refining of edible fats and oils [7]. Since these kinds of by-products from edible fat and oil refining form part of the human food chain as ingredients in animal feeds, it is very important to document their quality in terms of oxidative status. The health implications of cholesterol, phytosterols, and COPs are well documented. However, the biological effects of POPs on animal and human health need more investigation, as feed quality is crucial for animal health and welfare, and ultimately human health. The lack of information on the levels of sterols and particularly on SOPs (COPs + POPs) in these valuable by-products provides significant grounds for the work described in this paper. The main objective of this part of a multinational EU research project (FOOD-CT2004-007020) was to carry out qualitative and quantitative assessment of sterols and SOPs in samples of by-products from chemical and physical refining of edible fats and oils collected from various locations in Europe.

Experimental Procedures

Materials

As defined by Gasperini et al. [3], acid oil from chemical refining process (AOCHE), is a by-product of the oil and fat refining process produced after the splitting of soapstock with mineral acid. Acid oil from physical refining process (AOPHY), is a by-product of the oil and fat refining process produced after vacuum/steam distillation carried out by physical technology. Both type of samples were collected from different locations in Europe, the samples were of vegetable, animal, and of mixed origins. Details on sampling have been previously described [3]. Standard samples of cholesterol, brassicasterol and standard samples of COPs were obtained from Steraloids (Newport, RI, USA); a standard mixture phytosterols and 5α-cholestane from Sigma-Aldrich (Stockholm, Sweden); and Tri-Sil reagent from Pierce (Rockford, IL, USA). Ethanol was obtained from Kemetyl (Haning, Sweden). All other chemicals and solvents were of analytical grade and

were obtained from Merck Eurolab AB (Stockholm, Sweden), unless otherwise stated.

Methods

Analytical Thin-Layer Chromatography

Prior to hot saponification, analytical thin layer chromatography (TLC) was carried out on all samples in order to roughly estimate the required amount of internal standard to be added for quantification of sterols as described below. The solvent system for TLC was hexane:diethyl ether:acetic acid (85:15:1, v/v/v). The plate was sprayed with 10% phosphomolybdic acid in diethyl ether:ethanol (50:50, v/v) and heated at 120 °C for 15 min for color development [15].

Hot Saponification for Total Sterol Analysis

Approximately 0.02 g sample (duplicates) and the required amount of 5α-cholestane (40–60 μg) as an internal standard were saponified with 1 mL 2 M KOH in 95% ethanol in a glass tube at 100 °C for 10 min in a water bath. The reaction was stopped by cooling the tubes and then 1 mL water, 2 mL hexane, and 0.2 mL absolute ethanol were added. The tubes were shaken vigorously and centrifuged at 3000 rpm for 3 min. The upper hexane layer was transferred to a smaller glass tube. The solvent was evaporated under a nitrogen stream and derivatized to trimethylsilyl (TMS)-ether, as described below, before analysis by GC. All samples were analyzed in duplicates and mean values are presented. A few samples were also analyzed by GC–MS to confirm the identities of the sterols [15].

Preparation of TMS-Ether Derivatives for Sterols

The TMS-ether derivatives of sterols were prepared by adding 100 μL Tri-Sil reagent to the saponified samples [15]. The tubes were incubated at 60 °C for 45 min. The solvent was evaporated under a nitrogen stream, and the TMS-ether derivatives were dissolved in 200 μL hexane. The tubes were centrifuged at 3000 rpm for 3 min and kept at −20 °C before being analyzed by GC and GC–MS as described below.

Analysis of Sterols by Gas Chromatography (GC)

Analysis of the sterols was performed on a Chromapack CP 9001 gas chromatograph (Chromapack, Middelburg, The Netherlands) equipped with a flame ionization detector and split/splitless injector. Separation of sterols was performed on a non-polar capillary column, DB-5MS (J&W Scientific, Folsom, CA, USA), 30 m × 0.25 mm × 0.50 μm film thickness. The samples were injected using an auto sampler CP 9050 at split mode of injection, and split ratio was 1:30. The oven temperature was programmed to 60 °C for 1 min, followed by gradual increments of 40 °C/min until it reached 310 °C, where it was held for 27 min. Detector and injector temperatures were 310 °C and 260 °C, respectively. Helium was used as the carrier gas at 80 kPa and nitrogen (N_2) as the make-up gas at a flow rate of 30 mL/min. Sterols were identified by comparing the retention times with those of sterol standards and 5α-cholestane was used as internal standard for quantification. The peak areas were integrated using software Maestro version 2.4 (Chromapack, Middelburg, The Netherlands).

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis of Sterols

The sterols in the unsaponifiable fraction were identified by the mass spectra of standard samples of sterols using a gas chromatograph (ThermoQuest Italia S. p. A., Rodano, Italy) coupled to a Voyager mass spectrometer with MassLab data system version 1.4 V (Finnigan, Manchester, England). The sterols were separated on the same column as was used for the GC analysis. Helium was used as the carrier gas at an inlet pressure of 80 kPa. The injector temperature was 250 °C and the samples were injected in splitless mode. Oven temperature was 60 °C for 0.5 min, then increased to 290 °C at a rate of 50 °C/min, and finally increased to 300 °C at a rate of 0.5 °C/min. The mass spectra were recorded at electron energy of 70 eV and the ion source temperature was 200 °C. The derivatized samples were generally analyzed within one week.

Synthesis of POPs

Since standard samples of phytosterol oxidation products (POPs) are not commercially available, several common POPs were synthesized using a mixture of phytosterols following methods described elsewhere [16, 17].

Cold Saponification for SOPs Analysis

Approximately 0.2 g of fat/oil was dissolved in 3 mL dichloromethane, mixed well with 10 mL of 1 M KOH in 95% ethanol in a glass tube and left overnight (about 18 h) in the dark at room temperature according to a previously published method [18]. After addition of 7 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 removed

under nitrogen and the unsaponifiables were dissolved in 1 mL hexane:diethyl ether (75:25, v/v) for enrichment of sterol oxides by solid phase extraction (SPE) as described below.

Purification of SOPs by Two-Fold Solid Phase Extraction

A 0.5 g silica cartridge was solvated by 3 mL hexane. The total unsaponifiables were dissolved in 1 mL hexane:diethyl ether (75:25, v/v) and charged onto the column. The tube was washed with an additional 3 mL hexane:diethyl ether (75:25, v/v) and eluted through the column at a rate of approximately 4 mL/min. Thereafter, the column was eluted with 3 mL hexane:diethyl ether (60:40, v/v) and the elute discarded. The SOPs and the remaining unoxidized sterols were eluted with 4 mL acetone:methanol (60:40, v/v). The acetone/methanol phase was dried under nitrogen and the residue was again dissolved in 1 mL hexane:diethyl ether (75:25, v/v). In the case of vegetable oils or mixed fats and oils, 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, v/v) and the elute discarded. Finally, the column was eluted with 4 mL acetone:methanol (60:40, v/v), 2–4 μg 5α-cholestane in hexane were added, dried under nitrogen and the SOPs were derivatized to TMS-ethers for subsequent analysis by GC and GC–MS [16].

Analysis of SOPs by TLC

After enrichment of SOPs by SPE, qualitative analysis and visual checking of the SOPs fractions were conducted by analytical TLC (silica gel 60, Merck, Darmstadt, Germany). Along with the sample, a mixture of standards was spotted to obtain a band of standard cholesterol oxides. The compounds were separated using diethyl ether:cyclohexane (9:1, v/v). After brief air-drying, the plate was sprayed with the color-developing reagent, prepared by dissolving 1 g each of phosphomolybdic acid and cerium sulfate (1 g) in conc. sulfuric acid (5.4 mL) and diluting up to 100 mL. The plates were heated for 15 min at 120 °C for color development.

Gas Chromatography for Analysis of SOPs

Two fused silica capillary columns, a DB-17MS 10 m × 0.18 μm × 0.18 μm and a DB-5MS 10 m × 0.18 μm × 0.18 μm (J & W Scientific, Folsom, CA, USA) were connected by a universal press-fit connector and fitted in a Varian GC 3400 gas chromatograph (Varian, Palo Alto, CA, USA). The DB-17MS capillary column was connected to the injector side and the DB-5MS capillary column to the detector side. The GC conditions were: oven temperature at 60 °C for 1 min, then increased to 260 °C at a rate of 50 °C/min, maintained at this temperature for 5 min, then increased to 280 °C at 1.0 °C/min and maintained for 10 min. Helium was used as the carrier gas at a pressure of 124 kPa and N_2 as the make-up gas at a flow rate of 30 mL/min. Sample was injected at splitless mode and injector temperature was 260 °C. Detector temperature was 310 °C. The SOPs were identified by comparing retention times obtained from the standard COPs and authentic POPs samples. Quantification of the SOPs were done by using internal standard (5α-cholestane) and mean of duplicate analysis were presented.

GC–MS Analysis of SOPs

Both COPs and POPs were identified by comparing the mass spectra obtained against those of standard COPs, synthesized authentic samples of POPs and published data [19]. The TMS derivatives of SOPs were separated on the same column combination as was used in GC analysis of SOPs, using helium as the carrier gas at 180 kPa. The injector temperature was 260 °C and the samples were injected in splitless mode. The purge delay time was 1 min. The oven temperature was programmed at 60 °C for 1 min, increased to 260 °C at a rate of 50 °C/min and then held at this temperature for 10 min before being finally increased to 280 °C at 1 °C/min. The mass spectra were recorded at electron energy of 70 eV and the ion source temperature was 200 °C. Spectra were scanned in the range 50–600 *m/z*.

Statistical Analysis

Pearson's correlation between total sterols and major individual sterols with sterol oxidation products were calculated using the Minitab statistical software (version 15 Minitab Inc., PA, USA).

Results and Discussion

The 25 by-product samples from chemical refining (AOCHE) analyzed for total sterols and SOPs were mainly of plant origin, with the exception of samples AOCHE13 and AOCHE14. The levels of total sterols in these samples are shown in Fig. 2 whereas the individual sterols with their descriptive statistics are in Table 1.

Among the 25 samples in this category, seven samples contained a considerable amount of brassicasterol, and two samples contained only cholesterol. The contents of SOPs in AOCHE samples showed wide variation, ranging from

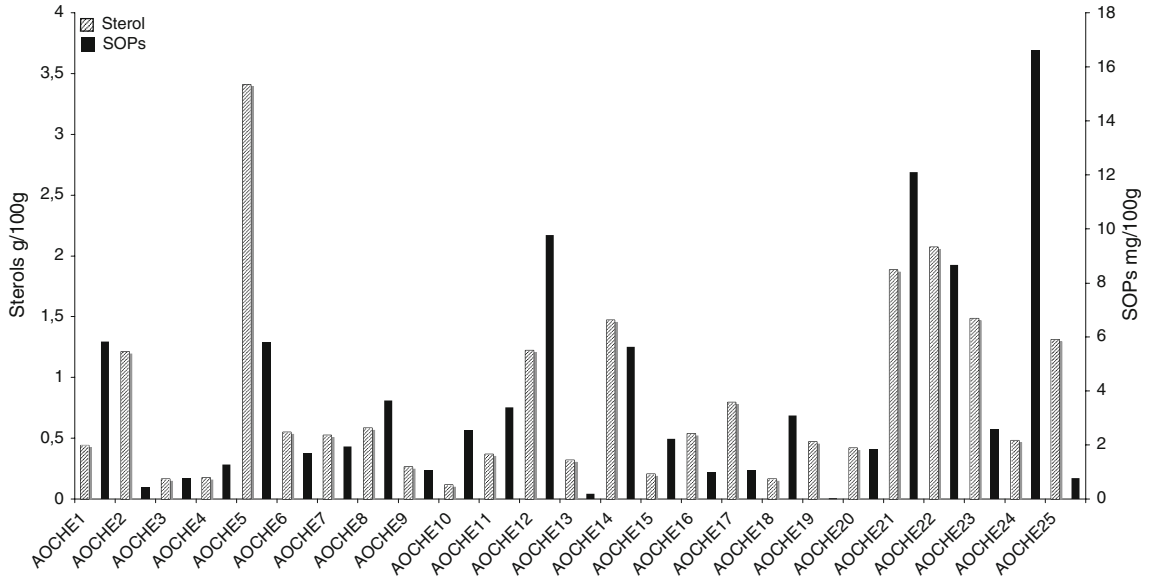

Fig. 2 Content of sterols and sterol oxidation products (SOPs) in the acid oil fraction of samples from the chemical refining process (AOCHE)

Table 1 Content of individual sterols (g/100 g) in the acid oil fraction of samples from the chemical refining process (AOCHE) collected from various locations in Europe

Sample	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	Sitosterol	Δ^5-avenasterol
AOCHE1	tr	0.05	0.15	0.03	0.21	0.01
AOCHE2	tr	nd	0.63	0.35	0.21	0.03
AOCHE3	tr	nd	0.04	0.02	0.09	0.01
AOCHE4	tr	nd	0.03	0.03	0.09	0.03
AOCHE5	tr	nd	0.71	0.48	2.02	0.20
AOCHE6	tr	nd	0.02	0.01	0.48	0.04
AOCHE7	tr	nd	0.08	0.05	0.38	0.02
AOCHE8	tr	nd	0.02	0.02	0.50	0.04
AOCHE9	tr	nd	0.01	0.01	0.21	0.03
AOCHE10	tr	0.01	0.03	0.01	0.05	0.01
AOCHE11	tr	0.04	0.12	0.02	0.17	0.01
AOCHE12	0.01	0.16	0.44	tr	0.55	0.06
AOCHE13	0.32	nd	nd	nd	nd	nd
AOCHE14	1.47	nd	nd	nd	nd	nd
AOCHE15	tr	nd	0.03	0.03	0.14	0.01
AOCHE16	tr	nd	0.12	0.10	0.30	0.02
AOCHE17	tr	nd	0.08	0.20	0.47	0.04
AOCHE18	tr	nd	0.01	tr	0.14	0.01
AOCHE19	tr	nd	0.02	0.02	0.37	0.06
AOCHE20	0.02	0.01	0.08	0.06	0.24	0.02
AOCHE21	tr	0.35	0.62	0.02	0.84	0.05
AOCHE22	tr	nd	0.30	0.28	1.41	0.09
AOCHE23	tr	0.22	0.52	nd	0.73	0.02
AOCHE24	tr	nd	0.05	0.06	0.35	0.02
AOCHE25	tr	nd	0.29	0.27	0.70	0.05
Mean	0.46	0.12	0.16	0.10	0.43	0.04
Median	0.17	0.05	0.05	0.03	0.24	0.03
Minimum	0.01	0.01	0.01	0.01	0.03	0.01
Maximum	1.47	0.35	0.71	0.48	2.02	0.2

tr below the quantification limit (<0.004 g/100 g), *nd* not detected

0.02 to 17 mg/100 g (Fig. 2). The levels of individual POPs in AOCHE samples from camepesterol, stigmasterol and sitosterol are shown in Tables 2 and 3, respectively. Samples AOCHE13 and AOCHE14 contained only COPs, namely 7α-hydroxycholesterol (0.2–1.4 mg/100 g), 7β-hydroxycholesterol (<0.1–1.7 mg/100 g), β-epoxycholesterol (<0.1–0.7 mg/100 g), cholesteroltriol (0.1 mg/100 g), 25-hydroxycholesterol (0.1 mg/100 g), and 7-ketocholesterol (<0.1–2.1 mg/100 g) (data not shown in Table). In addition, a few samples in this category also had 7α-hydroxybrassicasterol (0.1–1.8 mg/100 g), 7β-hydroxybrassicasterol (trace–0.5 mg/100 g), β-epoxybrassicasterol (0.1–0.8 mg/100 g), brassicasteroltriol (0.1–0.3 mg/100 g), 7-ketobrassicasterol (trace–6.5 mg/100 g) (data not shown in Table). Among the other POPs, 7-hydroxy-, epoxy-, and 7-keto derivatives were generally dominant in all samples (Tables 2 and 3).

Of the 16 distillate samples from the physical refining process (AOPHY) analyzed for their content and composition of sterols and SOPs, most were of plant origin, while two samples originated from a mixture of vegetable oils and animal fats (AOPHY8 and AOPHY12), and one sample contained only cholesterol (AOPHY14). The levels of total sterols in AOPHY samples are shown in Fig 3 whereas the individual sterols with their descriptive statistics are in Table 4. The contents of total SOPs in AOPHY samples varied from 0.01 to 1.5 mg/100 g (Fig. 3). The highest amount of POPs occurred in samples AOPHY2 (1.5 mg/100 g) and AOPHY16 (1.4 mg/100 g) (Fig. 3). None of the mixed origin samples and a sample of animal origin contained quantifiable amounts of COPs. The total oxybrassicasterols in AOPHY1 and AOPHY16, were 0.3 and 0.8 mg/100 g, respectively, dominated by 7α-hydroxybrassicasterol and 7β-hydroxybrassicasterols.

Table 2 Content of campesterol and stigmasterol oxidation products (mg/100 g) in the acid oil fraction of samples from the chemical refining process (AOCHE) collected from various locations in Europe

Samples	7α-HCa	7β-HCa	β-CaE	α-CaE	CaT	7-KCa	7α-HSt	7β-HSt	β-StE	α-StE	StT	7-KSt
AOCHE1	0.70	0.19	tr	nd	0.05	0.15	0.47	0.19	nd	nd	0.08	0.13
AOCHE2	0.10	tr	tr	nd	nd	tr	tr	tr	nd	nd	nd	tr
AOCHE3	0.11	tr	nd	0.02	0.03	0.05	0.03	0.05	nd	nd	nd	tr
AOCHE4	0.09	tr	nd	nd	nd	0.06	tr	tr	nd	nd	tr	tr
AOCHE5	0.07	0.09	nd	nd	nd	0.32	0.10	0.10	nd	nd	0.61	0.55
AOCHE6	0.05	0.07	nd	nd	nd	0.09	0.04	0.23	0.06	nd	tr	0.04
AOCHE7	0.06	0.11	nd	nd	nd	0.06	0.04	0.15	nd	nd	0.24	0.07
AOCHE8	0.04	0.24	0.03	tr	0.37	0.02	0.06	0.24	0.12	tr	0.31	0.17
AOCHE9	nd	nd	nd	nd	nd	nd	tr	tr	tr	nd	nd	tr
AOCHE10	0.11	0.05	nd	nd	0.09	0.14	0.11	0.31	0.06	nd	0.07	0.06
AOCHE11	0.06	0.02	nd	nd	nd	0.19	0.06	0.09	nd	nd	0.14	0.15
AOCHE12	0.20	0.17	0.13	nd	0.04	0.05	0.06	0.09	nd	nd	nd	0.06
AOCHE15	0.15	0.15	nd	nd	0.04	0.11	0.04	0.09	nd	nd	nd	0.16
AOCHE16	0.13	0.27	0.06	0.13	0.01	0.03	tr	tr	nd	nd	0.06	0.04
AOCHE17	0.06	0.07	nd	nd	0.27	0.18	0.03	0.02	nd	nd	0.06	tr
AOCHE18	0.04	0.10	nd	nd	0.03	0.10	0.04	0.08	nd	nd	0.69	0.08
AOCHE20	0.41	0.07	nd	nd	0.03	tr	nd	nd	nd	nd	nd	nd
AOCHE21	1.24	0.79	0.25	nd	nd	tr	1.11	0.09	nd	nd	nd	tr
AOCHE22	1.09	0.13	0.49	nd	0.73	0.05	0.79	0.21	nd	nd	0.09	0.47
AOCHE23	0.07	0.02	0.02	nd	0.06	0.15	0.04	0.02	nd	nd	0.01	0.07
AOCHE24	0.48	0.20	nd	0.08	0.42	0.70	0.55	0.01	0.02	0.06	0.74	2.01
AOCHE25	0.01	0.04	tr	nd	0.02	tr	0.01	0.04	0.03	tr	0.06	0.01
Mean	0.25	0.13	0.05	0.01	0.10	0.12	0.17	0.09	0.01	0.00	0.15	0.19
Median	0.1	0.09	0.00	0.00	0.03	0.06	0.04	0.09	0.00	0.00	0.06	0.06
Minimum	0.01	tr	0.00	0.00	0.00	tr	tr	tr	0.00	0.00	0.00	0.00
Maximum	1.24	0.79	0.49	0.13	0.73	0.7	1.11	0.31	0.12	0.06	0.74	2.01

tr below the quantification limit (<0.01 mg/100 g), *nd* not detected, *7α-HCa* 7α-hydroxycampesterol, *7β-HCa* 7β-hydroxycampesterol, *β-CaE* β-epoxycampesterol, *α-CaE* α-epoxycampesterol, *CaT* campesteroltriol, *7-KCa* 7-ketocampesterol, *7α-HSt* 7α-hydroxystigmasterol, *7β-HSt* 7β-hydroxystigmasterol, *β-StE* β-epoxystigmasterol, *α-StE* α-epoxystigmasterol; StT = stigmasteroltriol, *7-KSt* 7-ketostigmasterol

Table 3 Content of sitosterol oxidation products (mg/100 g) in the acid oil fraction of samples from the chemical refining process (AOCHE) collected from various locations in Europe

Samples	7α-HSi	7β-HSi	β-SiE	α-SiE	SiT	25-HSi	7-KSi
AOCHE1	0.43	0.30	tr	tr	0.10	0.07	0.30
AOCHE2	0.31	tr	nd	nd	nd	nd	tr
AOCHE3	0.29	0.06	tr	nd	0.03	nd	0.09
AOCHE4	1.09	tr	nd	nd	nd	nd	0.04
AOCHE5	0.25	1.27	nd	nd	0.71	0.10	1.69
AOCHE6	0.19	0.12	0.26	nd	nd	nd	0.54
AOCHE7	0.03	0.13	tr	nd	0.84	tr	0.26
AOCHE8	0.38	0.75	0.15	0.12	0.20	nd	0.44
AOCHE9	0.12	0.12	tr	nd	0.56	nd	0.26
AOCHE10	0.21	0.22	0.15	nd	tr	nd	0.34
AOCHE11	0.22	0.17	0.15	0.05	0.13	0.03	0.92
AOCHE12	0.18	0.13	0.15	nd	0.23	nd	0.34
AOCHE15	0.23	0.35	nd	nd	nd	nd	0.91
AOCHE16	0.03	nd	nd	nd	0.06	nd	0.17
AOCHE17	0.02	0.02	nd	nd	0.02	nd	0.31
AOCHE18	0.07	0.43	nd	nd	0.69	nd	0.74
AOCHE19	0.02	tr	nd	nd	nd	nd	tr
AOCHE20	0.05	0.13	nd	nd	0.16	nd	0.19
AOCHE21	0.07	0.23	0.13	nd	nd	0.05	0.25
AOCHE22	0.26	0.45	0.14	0.05	0.92	tr	2.77
AOCHE23	0.07	0.06	tr	tr	0.04	nd	0.14
AOCHE24	1.82	1.03	0.72	0.23	3.01	0.20	4.32
AOCHE25	0.10	0.24	0.05	0.02	0.10	0.01	0.03
Mean	0.28	0.27	0.08	0.02	0.34	0.02	0.65
Median	0.19	0.13	0.00	0.00	0.10	0.00	0.30
Minimum	0.02	tr	0.00	0.00	0.00	0.00	tr
Maximum	1.82	1.27	0.72	0.23	3.01	0.2	4.32

tr below the quantification limit (<0.01 mg/100 g), *nd* not detected, *7α-HSi* 7α-hydroxysitosterol, *7β-HSi* 7β-hydroxysitosterol, *β-SiE* β-epoxysitosterol, *α-SiE* α-epoxysitosterol, *SiT* sitosteroltriol, *25-HSi* 25-hydroxysitosterol, *7-KSi* 7-ketositosterol

Samples AOPHY1, 2, 3, 7 and 16 contained considerable amounts of 7α-hydroxycampesterol (0.01–0.4 mg/100 g), 7β-hydroxycampesterol (trace–0.1 mg/100 g) and 7-ketocampesterol (trace–0.3 mg/100 g) while only the sample AOPHY16 contained campesteroltriol (0.02 mg/100 g). Only two samples AOPHY2 and AOPHY9 contained quite high amounts of 7α-hydroxystigmasterol and 7-ketostigmasterol 0.2 and 0.7 mg/100 g, respectively (data not shown in Table). The content of oxysitosterols, was generally dominated by 7-ketositosterol in all AOPHY samples ranging from trace to 0.5 mg/100 g, except AOPHY14. Sample AOPHY2 contained high amount of 7α-hydroxysitosterol (0.6 mg/100 g). In contrast to other samples, only AOPHY16 contained most of the oxysitosterols in detectable quantities (Table 5).

During refining of edible fats and oils, the content of total sterols decreases due to degradation and formation of products through isomerization (Δ^5 to Δ^7-sterol), dehydration, polymerization, and formation of hydrocarbons or sterenes and sterol oxidation products [4]. These qualitative and quantitative changes in sterols can be traced in the refined oil and in by-products such as soapstocks and distillate fractions collected after chemical and physical refining processes. The total sterols in soapstocks and in the distillate fractions from different vegetable oils have been reported to be in the range of 2–20 g/100 g [10]. In the present study, the levels of total sterols in AOCHE samples were generally less than those in AOPHY, are in agreement with previous studies [6–8, 10, 11].

To the best of our knowledge, this is the first report on the contents of oxidized sterols in soapstock and distillate fractions from edible oil refining processes. The levels of SOPs were higher in AOCHE samples than in AOPHY samples, with ranges 0.02–17.0 and 0.01–1.5 mg/100 g, respectively. The lower content of SOPs in AOPHY samples may be due to the high temperature applied during

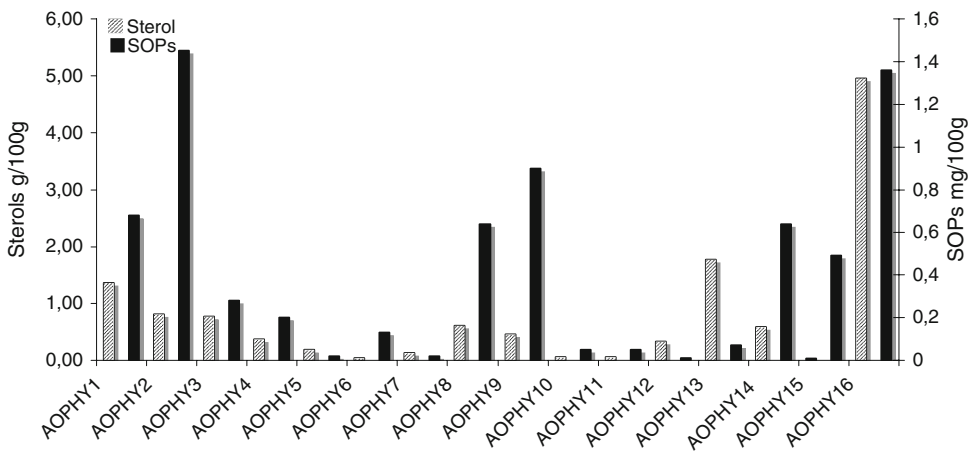

Fig. 3 Content of sterols and sterol oxidation products (SOPs) in the distillate fraction of samples from the physical refining process (AOPHY)

Table 4 Content of individual sterols (g/100 g) in the distillate fraction of samples from the physical refining process (AOPHY) collected from various locations in Europe

Samples	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	Sitosterol	Δ^5-avenasterol
AOPHY1	tr	0.03	0.36	0.19	0.76	0.02
AOPHY2	tr	nd	0.06	0.02	0.73	nd
AOPHY3	tr	nd	0.17	0.01	0.56	0.03
AOPHY4	tr	nd	0.02	0.01	0.31	0.02
AOPHY5	tr	nd	0.05	0.05	0.08	nd
AOPHY6	nd	nd	nd	nd	0.04	nd
AOPHY7	tr	nd	0.04	0.02	0.07	nd
AOPHY8	0.06	nd	0.03	0.01	0.48	0.03
AOPHY9	tr	nd	0.03	0.01	0.40	0.01
AOPHY10	tr	nd	0.01	0.01	0.03	nd
AOPHY11	tr	nd	0.01	0.01	0.04	nd
AOPHY12	0.01	0.01	0.08	0.05	0.18	tr
AOPHY13	tr	nd	0.24	0.23	1.21	0.07
AOPHY14	0.58	nd	nd	nd	nd	nd
AOPHY15	tr	nd	0.01	tr	0.02	nd
AOPHY16	tr	1.06	1.79	0.05	2.02	0.04
Mean	0.21	0.37	0.21	0.05	0.46	0.03
Median	0.06	0.03	0.05	0.05	0.31	0.03
Minimum	0.01	0.01	0.00	0.00	0.02	0.00
Maximum	0.58	1.06	1.79	0.23	2.02	0.22

tr below the quantification limit (<0.004 g/100 g), *nd* not detected

Table 5 Content of sitosterol oxidation products (mg/100 g) in the distillate fraction of samples from the physical refining process (AOPHY) collected from various locations in Europe

Samples	7α-HSi	7β-HSi	β-SiE	α-SiE	SiT	7-KSi
AOPHY1	0.14	tr	tr	tr	tr	0.08
AOPHY2	0.62	tr	nd	tr	nd	0.25
AOPHY3	tr	tr	tr	0.03	tr	0.22
AOPHY4	0.16	tr	tr	0.01	tr	0.03
AOPHY5	nd	nd	nd	nd	nd	0.02
AOPHY6	nd	nd	tr	0.03	tr	0.10
AOPHY7	nd	nd	tr	0.01	nd	0.01
AOPHY8	tr	nd	nd	nd	nd	0.52
AOPHY9	nd	nd	nd	nd	nd	0.05
AOPHY10	nd	nd	nd	nd	nd	0.05
AOPHY11	nd	nd	nd	nd	nd	0.05
AOPHY12	tr	tr	tr	0.01	tr	tr
AOPHY13	tr	tr	tr	0.02	tr	0.05
AOPHY15	tr	0.20	tr	tr	0.06	0.09
AOPHY16	0.14	0.14	0.05	nd	0.14	0.01
Mean	0.07	0.02	0.00	0.01	0.01	0.12
Median	0.00	0.00	0.00	0.00	0.00	0.05
Minimum	0.00	0.00	0.00	0.00	0.00	tr
Maximum	0.62	0.2	0.05	0.03	0.14	0.52

tr below the quantification limit (<0.01 mg/100 g), *nd* not detected, *7α-HSi* 7α-hydroxysitosterol, *7β-HSi* 7β-hydroxysitosterol, *β-SiE* β-epoxysitosterol, *α-SiE* α-epoxysitosterol, *SiT* sitosteroltriol, *7-KSi* 7-ketositosterol

vacuum distillation accelerating the breakdown and transformation of the SOPs into other unidentified degradation products. Further formation of SOPs has been prevented by the high amounts of natural antioxidants in AOPHY distillate [11]. Some sterols appeared to be more liable to breakdown than others, e.g. there was a higher content of oxybrassicasterols than the other SOPs in this study (data not shown in Table), although the content of brassicasterol in the sample was lower than other sterols. Similar results have been reported previously [4, 20]. This may be due to

Table 6 Correlation coefficient of total sterols and the major individual sterol with total oxyphytosterols (POPs) in the by-products from chemical and physical refining processes

By-products	Total sterols		Campesterol		Stigmasterol		Sitosterol	
	AOCHE	AOPHY	AOCHE	AOPHY	AOCHE	AOPHY	AOCHE	AOPHY
POPs	0.547*	0.597*	0.305	0.547*	−0.012	−0.044	0.339	0.635**

$0.01 < p \leq 0.05^{*}$; $p \leq 0.01^{**}$, *AOCHE* by-product fraction after splitting of soapstock from chemical refining of edible fats and oils; *AOPHY* by-product distillate fraction from physical refining of edible fats and oils

the structural arrangement in the brassicasterol molecule rendering it more easily oxidized than other sterols. However, systematic studies are required to clarify this phenomenon. Although stigmasterol has a double bond in the side-chain, similar to brassicasterol, the quantities of POPs observed in this study were quite different. Stigmasterol has an ethyl group at position C24 while brassicasterol has a methyl group, and this difference may affect in the relative rate of formation of their oxidation products [4]. Further studies are needed on this point.

It has been reported that the formation of SOPs is affected not only by the chemical nature of the sterols but also by their quantity [4]. In this study, by-products of plant or mixed origins contained higher amounts of sitosterol than other sterols, as well as higher amounts of oxysitosterols in the samples (Tables 1, 2, 3, 4, and 5).

There were positive correlations between total sterols ($0.01 < p \leq 0.05$) and total POPs in the by-products collected from both refining processes (Table 6). Concerning individual sterols there were significant positive correlations between campesterol ($0.01 < p \leq 0.05$), sitosterol ($p \leq 0.01$) and POPs in the samples collected from physical refining process. Stigmasterol had insignificant negative correlations in both the AOCHE and AOPHY samples.

The variations in the contents and composition of sterols and SOPs were due to the facts that the by-product samples from chemical and physical refining were collected from various production facilities and originated from different types of fats and oils, which were possibly subjected to different refining conditions.

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