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Formation of Modified Fatty Acids and Oxyphytosterols during Refining of Low Erucic Acid Rapeseed Oil

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Formation of *trans* fatty acids and cyclic fatty acid monomers was investigated during refining of low erucic acid rapeseed oil. The first steps of the refining process, that is, degumming, neutralization, and bleaching, hardly modified the fatty acid profile. In contrast, deodorization produced substantial quantities of *trans* fatty acids (>5% of total fatty acids) and small amounts of cyclic fatty acid monomers (650 mg of cyclic fatty acid monomers/kg of oil) when severe conditions (5−6 h at 250 °C) were used. α-Linolenic acid was the main precursor of cyclic fatty acid monomers. The influence of deodorization on the chemical composition of low erucic acid rapeseed oil was studied additionally. Whereas free fatty acids, peroxides, and tocopherols decreased, neither total polar compounds nor oxyphytosterols changed during deodorization. Oxyphytosterols were identified by GC-MS. Three oxyphytosterols not yet observed in oil were tentatively identified as 6β-hydroxycampestanol, 6β-hydroxysitostanol, and 6β-hydroxybrassicastanol. Brassicasterol oxides were the most abundant oxyphytosterols.

KEYWORDS: Canola oil; refining; *trans* fatty acids; cyclic fatty acid monomers; oxyphytosterols; deodorization

INTRODUCTION

Most vegetable fats and oils must be refined to render them consumable. Modifications that occur during oil refining are well documented (*1*). On the one hand, a number of components detrimental to quality, that is, phospholipids, free fatty acids, pigments, peroxides, traces of metals, insecticides, herbicides, traces of solvent, and volatile components responsible for off-odors and off-flavors, are removed during oil refining. On the other hand, a number of beneficial components, for example, essential fatty acids (*2*, *3*), long-chain polyunsaturated fatty acids (*4*), tocopherols (*5*, *6*), tocotrienols (*6*), sterols (*6*), and carotenoids, can be lost during oil refining. Furthermore, a number of potentially detrimental components, for example, *trans* fatty acids (*2*, *3*), cyclic fatty acid monomers (*7*), polar compounds (*8*), and polymers (*5*, *8*), can be produced.

Although *trans* fatty acid formation during oil refining has already been described (*2*, *3*, *8*, *9*), only limited data are available on cyclic fatty acid monomers (*7*). Moreover, no similar study related to oxyphytosterols has been reported in the literature. The aim of the present work was, therefore, to study modifications of fatty acids (i.e., formation of cyclic fatty acid monomers and *trans* fatty acids) and sterols (i.e., formation of oxyphytosterols) during the extraction and refining of vegetable oil. Low erucic acid rapeseed oil was chosen as substrate because it is an important commercial oil and also because it contains a significant amount of α-linolenic acid, which is very sensitive to heat treatment.

Most investigations related to modifications of oils during heat treatment have been carried out on a laboratory scale. Only a few have been performed under either pilot-scale or industrial-scale conditions. Herein, the formation of cyclic fatty acid monomers and *trans* fatty acids was investigated during the industrial refining of rapeseed oil. As deodorization was shown to be the key step, the influence of deodorization time and temperature on the formation of *trans* fatty acids, oxyphytosterols, and cyclic fatty acid monomers was studied additionally during batch pilot-scale deodorization of semirefined rapeseed oil.

MATERIALS AND METHODS

Materials. *Industrial Samples.* Low erucic acid rapeseed of Swiss origin was purchased locally. Crude low erucic acid rapeseed oil was obtained industrially by mechanical extraction. Semirefined low erucic acid rapeseed oil was produced by chemically refining of the crude oil, that is, degumming by addition of citric acid, neutralizing with caustic soda, bleaching, and winterization. Fully refined low erucic acid rapeseed oil was obtained by semicontinuous deodorization (cross-flow stripping, multitray design) of the winterized low erucic acid rapeseed oil. Current industrial conditions (total residence time of 3 h, maximum

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Table 1. Fatty Acid Composition of Industrially Fully Refined Low Erucic Acid Rapeseed Oil ($n = 2$)

fatty acid	% of total fatty acids	fatty acid	% of total fatty acids
$C_{11:0}$	0.03 ± 0	$C_{18:3}$ *trans*	0.47 ± 0.02
$C_{14:0}$	0.04 ± 0	$C_{18:3}$ *n*–3 *cis*	8.26 ± 0.02
$C_{15:0}$	0.01 ± 0.01	$C_{20:0}$	0.57 ± 0.01
$C_{16:0}$	4.71 ± 0	$C_{20:1}$ *n*–9 *cis*	1.26 ± 0.04
$C_{16:1}$ *n*–7 *cis*	0.19 ± 0	$C_{20:2}$ *n*–6 *cis*	0.06 ± 0
$C_{17:0}$	0.04 ± 0	$C_{22:0}$	0.38 ± 0.02
$C_{17:1}$ *n*–7 *cis*	0.05 ± 0	$C_{22:1}$ *n*–9 *cis*	0.11 ± 0.08
$C_{18:0}$	1.72 ± 0.01	$C_{24:1}$ *n*–9 *cis*	0.13 ± 0.01
$C_{18:1}$ *cis*	61.70 ± 0.05	others	0.06
$C_{18:2}$ *trans*	0.08 ± 0.01		
$C_{18:2}$ *n*–6 *cis*	20.00 ± 0.05		

temperature of 235 °C) were applied. The fatty acid composition of industrially fully refined low erucic acid rapeseed oil is reported in **Table 1**.

Pilot-Plant Deodorization of Industrial Semirefined Low Erucic Acid Rapeseed Oil. Trials were performed on a batch pilot-scale Andreotti Impianti S.p.A. (Sesto Fiorentino/Florence, Italy) deodorizer (steam injection in a gas lift tube, batch size of 50−70 kg). About 1.25 h was necessary to heat the oil to the deodorization temperature. A homemade device allowed samples to be taken during processing without the introduction of air into the deodorizer.

About 55 kg of semirefined low erucic acid rapeseed oil was deodorized for 6 h at either 200, 225, or 250 °C under a pressure of 1−3 mbar and with 1%/h (based on oil) direct steam injection. Steam was continuously injected from the beginning of the heating phase until the oil was cooled to 100 °C. A sample was collected when the oil reached the deodorization temperature (t_0), after the first 30 min of deodorization (except for deodorization at 200 °C), then at 1 h intervals until a total of 6 h, and finally after the oil had cooled to ambient temperature.

Methods. *Fatty acid methyl esters* (FAMEs) were prepared by reacting the oil in *n*-hexane with a 2 N methanolic solution of KOH at room temperature (IUPAC standard method 2.301) and centrifuging at 2000 rpm for 5 min. The upper layer, containing the FAMEs, was then diluted with *n*-hexane and injected into a GC 8160 chromatograph (CE Instruments, Rodano/MI, Italy) equipped with an AS800 automatic sampler and a flame ionization detector. Injection was on-column. Separation was achieved on a 100 m × 0.25 mm i.d., 0.20 μm, WCOT fused silica capillary column coated with CP-Sil 88, type FAMEs, with a 2.5 m × 0.53 mm i.d. precolumn fused silica retention gap (both from Chrompack, Middelburg, The Netherlands). The carrier gas was hydrogen (purity > 99.999 vol %) at 150 kPa. The temperature program was as follows: 60 °C, 5 min isothermal, 15 °C/min to 165 °C, 1 min isothermal, 2 °C/min to 225 °C, 5 min isothermal. Data acquisition and peak integration were done using Chrom-Card software version 1.20 (CE Instruments). Peaks were identified by comparison of retention times with a mix of 37 methyl esters of the most common fatty acids (standard GLC-Nestlé 37 supplied by Nu-Chek Prep, Elysian, MN). Response factors were determined using the same GLC-Nestlé 37 standard. Identification of geometrical isomers of $C_{18:2}$ and $C_{18:3}$ was based on retention times of standards consisting of a mix of isomers of linoleic and linolenic methyl esters (Supelco Inc., Bellefonte, PA) and on literature data (*2*, *10*−*12*). All geometrical isomers of $C_{18:2}$ could be well separated. However, complete separation of geometric isomers of $C_{18:3}$ could not be achieved under the present conditions. Thus, the *cis*-9,*trans*-12,*trans*-15 $C_{18:3}$ (*ctt*) and the *trans*-9,*cis*-12,*trans*-15 $C_{18:3}$ (*tct*) isomers coeluted as did the *cis*-9,*cis*-12,*trans*-15 $C_{18:3}$ (*cct*) and the *trans*-9,*trans*-12,*cis*-15 $C_{18:3}$ (*ttc*) isomers. Isomerization degree (ID) was defined as percentage of *trans* isomers relative to the parent fatty acid plus *trans* isomers.

Cyclic fatty acids were quantified according to the method of Sébédio et al. (*13*). Briefly, total FAMEs spiked with an internal standard (16:0 ethyl ester) were fully hydrogenated, and the fraction containing the cyclic fatty acids and the internal standard was isolated by HPLC on an 250 × 10 mm Ultrabase-C18 column (Shandon, Cheshire, U.K.), using a mixture of acetonitrile/acetone (90:10, v/v) at a flow rate of 4 mL/min. The fraction was further analyzed by GLC (Hewlett-Packard, 5980 series 2, Palo Alto, CA) on a 120 m × 0.25 mm BPX70 column (Interchim, Montluçon, France), using hydrogen as carrier gas. The column was programmed from 60 to 175 °C at 20 °C/min and held at this temperature until completion of analysis. The injector (splitless) was at 250 °C, and the FID was maintained at 280 °C.

Tocopherols were measured by HPLC using a LaChrom acquisition system (degasser L-7612, pump L-7100, autosampler L-7200, fluorometric detector L-7480, interface module D-7000) from Merck-Hitachi (E. Merck, Darmstadt, Germany; Hitachi Ltd., Tokyo, Japan) controlled by a D-7000 HPLC system manager software from Merck-Hitachi. Conditions for HPLC measurements were as follows: column, 250 mm × 4 mm i.d., 5 μm Lichrospher Si 60 (direct phase); precolumn, 4 × 4 mm, 5 μm Si 60 (both columns from E. Merck); mobile phase, mixture of *n*-hexane and dioxane; program, *n*-hexane/dioxane 95:5 v/v for 23 min, *n*-hexane/dioxane 50:50 v/v for 5 min, and finally *n*-hexane/dioxane 95:5 v/v for 10 min; flow rate, 1.6 mL/min; fluorescence excitation, 295 nm; fluorescence emission, 330 nm. Peaks were identified by comparison with a standard (each tocopherol homologue diluted to 0.005 mg/mL in *n*-hexane). Analyses were achieved in duplicate, and the mean value is reported.

Oxyphytosterols were analyzed according to methods commonly used for oxycholesterols: 500 mg of oil was saponified at room temperature for 16 h, in the dark, under nitrogen, using 10 mL of 1 M ethanolic potassium hydroxide. After the addition of purified water, the nonsaponifiable matter was extracted two times with dichloromethane. The two fractions were collected and washed several times with water until neutral pH was reached. This fraction was then redissolved in 500 μL of *n*-hexane/*tert*-butylmethyl ether (TBME) (90:10 v/v) and purified by solid-phase extraction on 3 mL, 500 mg of LC-Si silica cartridges (Supelco, L'Isle d'Abeau, France) using successively 35 mL of *n*-hexane/TBME (90:10 v/v) and 15 mL of *n*-hexane/TBME (80:20 v/v). As recommended by Lai et al. (*14*), a vacuum manifold (Supelco) was used to ensure a regular solvent flow rate of 0.6 mL/min through the cartridge. The oxyphytosterols were then eluted with 10 mL of acetone, and 5α-cholestane was added as internal standard. After evaporation of solvents, the samples were redissolved in 200 μL of anhydrous pyridine and 200 μL of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane. The TMS ether derivatives were obtained by heating for 30 min at 60 °C. The reagents were evaporated under nitrogen, and the residue was dissolved in hexane for gas chromatographic analysis. The TMS derivatives were analyzed on a 30 m × 0.25 mm i.d., 0.25 μm, DB-5 capillary column (J&W Scientific, Folsom, CA). The column was placed in a 5890 series 2 Hewlett-Packard gas chromatograph with a needle-falling injector (temperature = 290 °C) and a flame ionization detector (temperature = 300 °C). The carrier gas was helium. The oven temperature was programmed at 20 °C/min from 250 to 275 °C and then reached 290 °C at 1 °C/min. The analysis was completed at 290 °C during 35 min. Peaks were quantified using Diamir software (JMBS, Fontaine, France). GC separation of oxyphytosterols will be detailed elsewhere (Joffre et al., in preparation).

GC-MS was carried out on a 5890 Hewlett-Packard gas chromatograph coupled to a 5970 mass selective detector. The injection was made in splitless mode. A 30 m × 0.25 mm i.d. HP-5 column (Hewlett-Packard) was used. After 1 min at 50 °C, the oven temperature was programmed at 20 °C/min to 270 °C and then reached 290 °C at 1 °C/min. The oven temperature remained at 290 °C until the end of the analysis. The transfer line was operated at 290 °C. The other chromatographic conditions were the same as for flame ionization detection GC. The mass spectrometer was operated at an ionization energy of 70 eV. Mass spectra were recorded from *m*/*z* 100 to 700. Oxyphytosterols were identified by comparison of retention times and mass spectra with those of standards (Joffre et al., in preparation). Uncommon oxyphytosterols were identified as follows: a large amount of unsaponifiables obtained as previously described (5 × 500 mg of oil) was separated by TLC on three silica plates (Merck, Fontenay sous bois, France), using *n*-hexane/TBME/acetic acid (70:30:1 v/v) as solvent. Two successive migrations were operated, and the compounds were revealed using dichlorofluorescein. The band having an R_f between

0 and 0.03 was removed and extracted using dichloromethane and acetone successively. The corresponding fractions of the three plates were pooled. This fraction, which contained the three unknowns, was then hydrogenated in chloroform/methanol (2:1 v/v) for 5 h using PtO_2 as catalyst. The compounds were then analyzed using GC-MS, as described before.

Cholesterol, which represents <0.1% of total sterols in low erucic acid rapeseed oil, was used to check that the detected oxyphytosterols were not artifacts formed during analysis. Cholesterol oxides were removed on silica as previously described. It was then added to the oil in an amount equivalent to sitosterol. The entire analysis of oxysterols was then achieved as previously described, and significant *m*/*z* ions of oxycholesterols were searched as signs of artifact formation. This checking was effected two times.

Peroxide value (PV), expressed as milliequivalents of O_2 per kilogram of oil, was measured according to the official AOCS acetic acid−isooctane method (Cd 8b-90).

Free Fatty Acids (FFA). The amount of FFA was determined according to IUPAC standard method 2.201. Results are expressed as milligrams of oleic acid per 100 mg of oil.

Polar Compounds. Total polar content was determined by column chromatography according to AOCS standard method Cd 20-91. Both polar and nonpolar compounds were quantified. Analyses were performed in duplicate.

RESULTS AND DISCUSSION

Modification of Fatty Acids during Industrial Refining. The fatty acid profile of crude hexane-extracted low erucic acid rapeseed oil was characterized by a large amount of $C_{18:1}$ *cis* isomers (~60%, principally oleic acid) and linoleic acid (~20%) and a smaller amount of α-linolenic acid (~8%) and palmitic acid (~5%). Levels of other fatty acids were below 2%. Only *trans* isomers of $C_{18:3}$ could be detected (detection limit of 0.01%) in the crude oil and at a very low level (0.03% of total fatty acids). Similar fatty acid profiles were recorded in semirefined and fully refined low erucic acid rapeseed oils. In both oils the levels of *trans* fatty acids were low: below the detection limit for $C_{18:2}$ and 0.03% $C_{18:3}$ *trans* isomers in the semirefined low erucic acid rapeseed oil and 0.1% $C_{18:2}$ and 0.5% $C_{18:3}$ *trans* isomers in the fully refined low erucic acid rapeseed oil (**Table 1**).

Cyclic fatty acid monomers could not be detected in crude low erucic acid rapeseed oil (detection limit of 40 mg/kg) and were found at a very low amount (48 mg/kg) in the semirefined low erucic acid rapeseed oil. A slightly higher amount of cyclic fatty acid monomers was, however, detected in the fully refined low erucic acid rapeseed oil: 103 mg/kg.

Modifications during Pilot-Scale Deodorization. *Peroxides and Free Fatty Acids.* Whereas peroxides were present in semirefined low erucic acid rapeseed oil (PV = 2.6 mequiv of O_2/kg of oil), no peroxides could be detected in deodorized samples. For all three temperatures, as soon as the deodorization process was started, that is, at time t_0, PV fell below the detection limit (<0.2 mequiv of O_2/kg of oil).

Whatever the temperature between 200 and 250 °C, the amount of FFA decreased steadily during deodorization from 0.18% in the semirefined oil to 0.02% in the deodorized oils. The higher the temperature, the sharper was the decrease. At 200 °C it took ~5 h to reach an FFA content lower than 0.05%, whereas 3 and 2 h were sufficient when the deodorization was achieved at 225 and 250 °C, respectively.

Tocopherols. Whereas no loss of tocopherols was observed during deodorization at 200 °C, a small decrease was recorded at 225 °C (from 227 ± 2 to 171 ± 1 mg/100 g of oil for the α homologue and from 315 ± 3 to 228 ± 4 mg/100 g of oil for the γ homologue). A large reduction of tocopherol content (up to ~80%) was recorded during deodorization at 250 °C (**Figure 1**).

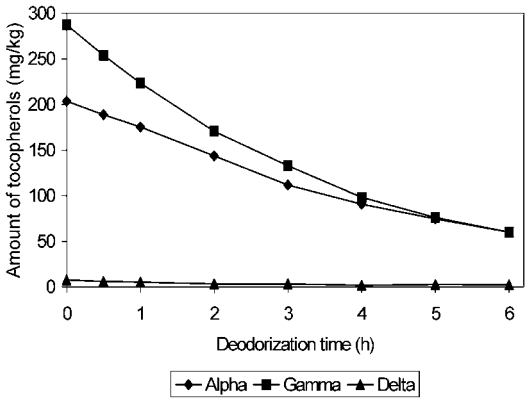

Figure 1. Loss of tocopherols as a function of deodorization time at 250 °C.

Oxyphytosterols. Interestingly, neither the 5,6-epoxide- nor the 5α,6β-dihydroxysterols (phytotriols), but rather compounds oxidized at position 7 (7α-hydroxy-, 7β-hydroxy- or 7-keto-), were detected in semirefined and fully refined low erucic acid rapeseed oil (**Table 2**). Three uncommon compounds were also observed. Two of them showed mass spectrometric fragmentation patterns (**Table 3**) similar to those of 6β-hydroxycampestanol and 6β-hydroxysitostanol (*15*). Purification and hydrogenation of these two compounds did not change their mass spectra. These observations confirm that they do not have ethylenic bonds and strengthen their tentative identification as 6β-hydroxycampestanol and 6β-hydroxysitostanol. The third unknown compound contains two hydroxy groups as shown by the M − 90 and M − 180 peaks of its mass spectrum, which correspond to the loss of one and two TMSOH moieties, respectively. The intense peaks at *m*/*z* 345 and 255 probably correspond to the loss of a lateral chain having an ethylenic bond at position 22 (*16*). In addition, this compound disappeared during hydrogenation, whereas the amount of 6β-hydroxycampestanol increased. All of these facts led us to think that this compound may be the derivative of a brassicasterol analogue to either 6β-hydroxycampestanol or 6β-hydroxysitostanol, that is, the (24*R*)-24-methyl-3β,6β-dihydroxycholest-22-ene (abbreviated here as 6β-hydroxybrassicastanol).

This identification is further supported by a similar investigation carried out with soybean oil, that is, an oil rich in stigmasterol, the homologue of brassicasterol having an ethyl instead of a methyl group at position 24. During this study traces of a compound showing the same type of mass spectrometric fragmentation and with a mass atomic 14 units higher than that of 6β-hydroxybrassicastanol were observed (A. Grandgirard, unpublished results); accordingly, this compound may be the derivative of the stigmasterol analogue to 6β-hydroxybrassicastanol. Provided enough material can be obtained, for example, through synthesis, structures of these oxyphytosterols should be further confirmed by NMR.

The utilization of cholesterol to check the method showed that 6β-hydroxycholestanol was not formed, suggesting that the observed hydroxystanols are not artifacts. Very low amounts (just above the detection limit) of 7α-hydroxycholesterol, 7-β-hydroxycholesterol, and 7-ketocholesterol were observed, demonstrating that very little 7-hydroxy- and 7-ketophytosterols could be formed during analysis. In all cases, this artifact

Table 2. Oxyphytosterols in Low Erucic Acid Rapeseed Oils ($n = 2$)

	amount (μg/g) in semirefined oil	amount (μg/g) in fully refined oils after deodorization at		
		200 °C	225 °C	250 °C
7α-hydroxybrassicasterol	0.8 ± 0.1	0.9 ± 0.1	1 ± 0.1	1.9 ± 0.1
7α-hydroxycampesterol	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
7α-hydroxysitosterol	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
7β-hydroxybrassicasterol	1.5 ± 0.2	1.5 ± 0.1	1.9 ± 0.7	1.6 ± 0.7
7β-hydroxycampesterol	0.8 ± 0.3	0.5 ± 0.1	0.7 ± 0.1	0.4 ± 0.1
7β-hydroxysitosterol	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
7-ketobrassicasterol	27.2 ± 3.3	26.3 ± 3.2	26.8 ± 0.9	22.2 ± 0.9
7-ketocampesterol	1.4 ± 0.3	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.1
7-ketositosterol	1.7 ± 0.3	1.6 ± 0.2	1.7 ± 0.1	1.8 ± 0.1
6β-hydroxybrassicastanol[a]	8.2 ± 0.8	8.9 ± 0.5	9.5 ± 0.2	9.9 ± 0.6
6β-hydroxycampestanol[b]	14.3 ± 1.1	14.2 ± 0.9	15.6 ± 0.1	17 ± 0.2
6β-hydroxysitostanol[b]	12.8 ± 0.9	12 ± 0.5	13.8 ± 0.1	14.6 ± 0.3
total oxyphytosterols	70.1	68.7	74	72.5
total brassicasterol oxides	37.7	37.6	39.2	35.6
total campesterol oxides	16.8	16.3	18	19.3
total sitosterol oxides	15.6	14.8	16.8	17.6
total 7-keto derivatives	30.3	29.1	29.8	25.4
total 7α-hydroxy derivatives	1.6	1.9	2	3
total 7β-hydroxy derivatives	2.9	2.6	3.3	2.6
total 6β-hydroxystanols	35.3	35.1	38.9	41.5

[a] Hypothetical. [b] Tentative identification.

Table 3. Mass Spectrometric Data for the Three Uncommon Oxyphytosterols (as Trimethylsilyl Ethers) Present in Low Erucic Acid Rapeseed Oil[a]

possible structure	M	major significant ions (*m*/*z*)									
6β-hydroxybrassicastanol	560 (49)	545 (6)	470 (9)	455 (8)	380 (3)	365 (4)	427 (46)	433 (18)	343 (26)	345 (100)	255 (40)
6β-hydroxycampestanol	562 (35)	547 (36)	472 (79)	457 (29)	382 (59)	367 (23)	417 (31)	383 (66)	343 (37)	327 (13)	204 (78)
6β-hydroxysitostanol	576 (34)	561 (36)	486 (81)	471 (26)	396 (60)	381 (22)	431 (32)	397 (67)	357 (33)	341 (13)	204 (81)

[a] Relative abundances are reported in parentheses.

formation was <5% of the measured oxyphytosterols. This was included in the quantitative data of phytosterols oxidized at position 7.

To our knowledge, very few studies have reported on the possible presence of oxyphytosterols in refined oils (*17*−*19*). In their pioneering work, Nourooz-Zadeh and Appelqvist (*17*) did not observe oxyphytosterols at the detection limit of 0.2 ppm. However, they considered only the free sterol fraction and did not analyze the 7-keto compounds. Some years later, Dutta et al. (*18*) found 39.9−46.7 ppm of oxyphytosterols in sunflower oil, high oleic sunflower oil, and a rapeseed oil/palm oil blend. The detected compounds were the 7-hydroxy, 7-keto, epoxy, and triol derivatives of sitosterol, campesterol, and stigmasterol. In agreement with Nourooz-Zadeh and Appelqvist (*17*), we could not detect any epoxy compounds and triols in deodorized low erucic acid rapeseed oil. However, we found 0.3−0.8 ppm of 7-hydroxy derivatives of sitosterol and campesterol, whereas Nourooz-Zadeh and Appelqvist did not detect these compounds. All of these apparent discrepancies may come from (i) differences in storage conditions (time, temperature) of the sample before analysis; (ii) differences in the analytical method, analysis of either total oxyphytosterols or those contained only in the free sterol fraction; or (iii) differences in the amount of sample used for analyses [because we used a much larger amount (5 times larger) than Nourooz-Zadeh and Appelqvist, higher amounts of oxysterols are measured, which makes their identification easier].

In addition, we observed three 6β-hydroxystanols in low erucic acid rapeseed oil, among them probably 6β-hydroxysitostanol and 6β-hydroxycampestanol. These compounds have not been precisely detected in oils. However, an unknown compound was present in soybean oil (*17*), and the indications given by these authors (e.g., retention time, probable mass at 576) lead us to think that this unknown compound could be 6β-hydroxysitostanol.

Another point of interest is the fragility of brassicasterol. In semirefined and fully refined low erucic acid rapeseed oil brassicasterol oxides indeed represent most of the oxyphytosterols (49−54.7%, **Table 2**), whereas brassicasterol represents only 9.5% of total sterols (39.7% for campesterol and 45.3% for sitosterol). This is because 7-ketobrassicasterol is the most abundant oxyphytosterol in all low erucic acid rapeseed oils. That brassicasterol is more easily oxidized was already mentioned by Przybylski et al. (*20*) and has to be further documented.

Polar Compounds. There were no changes in the concentration of polar compounds during deodorization. Whatever the deodorization temperature and duration, the total polar content was always between 2.6 ± 0.1 and 3.0 ± 0.1 mg/100 mg of oil ($n = 2$).

Trans Fatty Acids. Trans isomers of both $C_{18:2}$ and $C_{18:3}$ could be clearly identified after pilot-scale deodorization of low erucic acid rapeseed oil, that is, after the initial heating period, 6 h at the processing temperature and the cooling period (**Figure 2** and **Table 4**). Higher levels of geometrical isomers of $C_{18:3}$

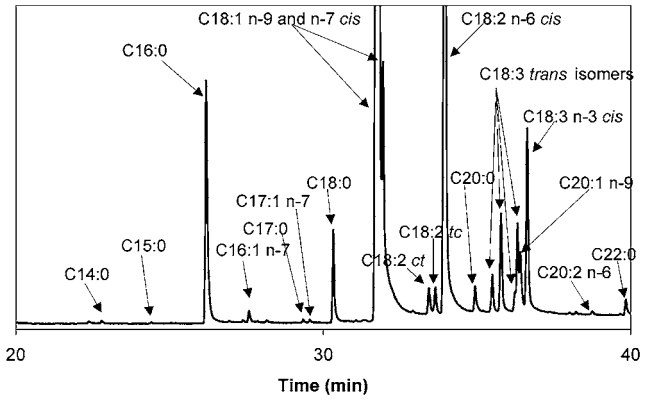

Figure 2. Typical GC chromatogram (only major peaks are shown) of low erucic acid rapeseed oil deodorized for 6 h at 250 °C.

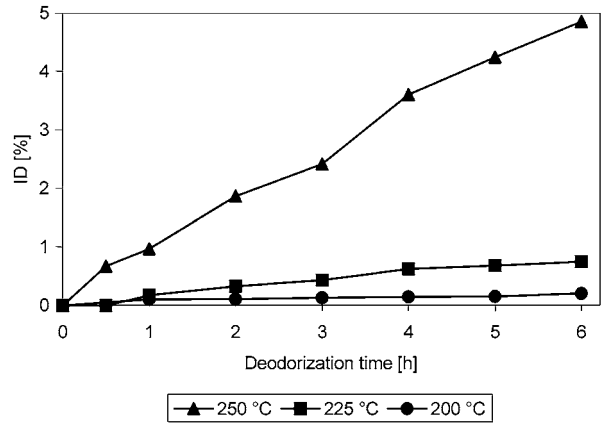

Figure 3. Geometrical isomerization degrees (ID) of linoleic acid as a function of deodorization time and temperature.

Table 4. *Trans* Isomers of $C_{18:2}$ and $C_{18:3}$ Recorded after Pilot-Scale Deodorization ($n = 2$)

	% of *trans* isomers		
deodorization temp (°C)	$C_{18:2}$ *trans*	$C_{18:3}$ *trans*	total *trans*
200	0.03 ± 0	0.29 ± 0.01	0.32 ± 0.01
225	0.17 ± 0.03	1.26 ± 0.04	1.43 ± 0.07
250	1.03 ± 0.02	4.19 ± 0.07	5.22 ± 009

compared to $C_{18:2}$ were formed at all investigated temperatures. Also, for both $C_{18:2}$ (**Figure 3**) and $C_{18:3}$ (**Figure 4**) the concentration of *trans* isomers increased when the deodorization temperature or time increased.

These results are in agreement with published data reporting that residence time and operating temperature have the most important effect on the extent of *trans* isomers formation during laboratory-scale (*2*, *3*, *10*) or pilot-scale deodorization (*2*, *9*). In the same way, the present results confirm the higher sensitivity toward geometrical isomerization of α-linolenic acid compared to linoleic acid (*2*, *3*, *11*, *21*).

Interestingly, for both linoleic and α-linolenic acids the ID values recorded during pilot-scale deodorization were very close to those predicted on the basis of a recently developed mathematical model (*3*). As described by this model, formation of both *trans*-linoleic and *trans*-linolenic acid isomers followed a first-order reaction having a kinetic constant that varied according to Arrhenius' law (data not shown).

Cyclic fatty acid monomers were formed in much lower amounts during pilot-scale deodorization than were *trans* fatty acids. The initial level of cyclic fatty acid monomers measured

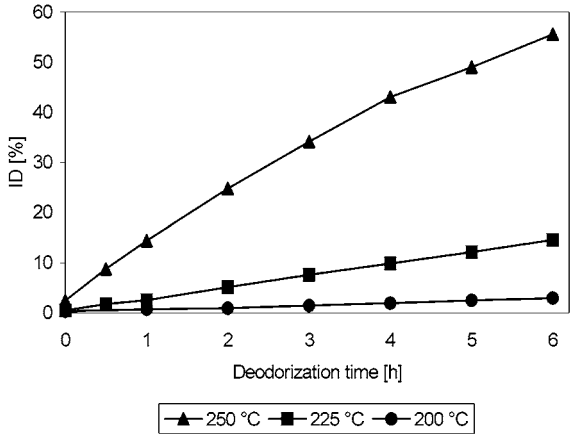

Figure 4. Geometrical isomerization degrees (ID) of α-linolenic acid as a function of deodorization time and temperature.

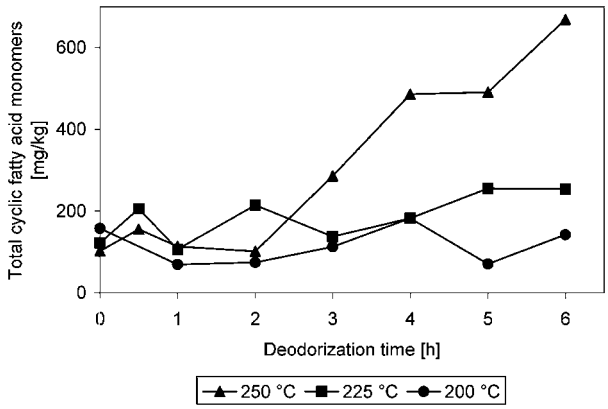

Figure 5. Formation of cyclic fatty acid monomers as a function of deodorization time and temperature.

in semirefined low erucic acid rapeseed oil (~50 mg/kg) increased only slightly during deodorization at 200 and 225 °C (**Figure 5**): 100−150 mg/kg after the initial heating period (t_0) and 150−250 mg/kg after 6 h of deodorization. A more important increase was recorded during deodorization at 250 °C, that is, ~650 mg/kg of cyclic fatty acid monomers was recorded after 6 h. Similar data were recently published by Hénon et al. (*7*). However, these authors did not report the structures of the cyclic fatty acid monomers found in the deodorized samples. GC-MS study of the hydrogenated cyclic fatty acid monomers fraction revealed the presence of six major cyclic fatty acid monomer structures as reported in **Table 5**. Whatever the temperature between 200 and 250 °C, higher amounts of all six major cyclic fatty acid monomers were recorded after 6 h of deodorization; the higher the process temperature and the longer the time, the higher the increase of cyclic fatty acid monomers. Among the different cyclic fatty acid monomers identified, those having a six-carbon-membered ring (components D and F) are typical of oils containing α-linolenic acid that have been heat treated. In fact, these cyclic fatty acid monomer profiles are very similar to those found for linseed oil heated at high temperature in the laboratory (*22*). Other small peaks having retention times similar to those of cyclic fatty acids previously recorded in frying oils were also detected (*23*). However, the quantity observed was too small to allow clean spectra. It would now be interesting to study the cyclic fatty acid monomers before hydrogenation to determine the degree of unsaturation as well as the geometry of the double bond(s). This study, which requires further enrichment and isolation of cyclic fatty acid monomers using $AgNO_3$−HPLC

Table 5. Major Cyclic Fatty Acid Monomers in Semirefined and Fully Refined Low Erucic Acid Rapeseed Oils (Identified by GC-MS after Total Hydrogenation of the Isolated Cyclic Fatty Acid Monomers Fraction) ($n = 1$)

	amount (μg/g) of cyclic fatty acid monomers					
	A[a]	B[a]	C[a]	D[a]	E[a]	F[a]
semirefined oil	9.1	13.0	8.4	6.6	4.3	6.6
after 6 h at 200 °C	12.3	13.0	8.7	11.7	6.9	15.4
after 6 h at 225 °C	48.0	58.2	30.0	46.5	19.3	52.0
after 6 h at 250 °C	130.4	159.9	68.2	134.5	66.9	109.1

[a] A = methyl 9-(2-butylcyclopentyl)nonanoate (*trans*); B = methyl 10-(2-propylcyclopentyl)decanoate (*trans*); C = methyl 9-(2-butylcyclopentyl)nonanoate (*cis*); D = methyl 9-(2-propylcyclohexyl)nonanoate (*trans*); E = methyl 10-(2-propylcyclopentyl)decanoate (*cis*); F = methyl 9-(2-proylcyclohexyl)nonanoate (*cis*).

techniques and GC-MS analyses (*24*), should allow the determination of the extent of cyclization of linoleic and oleic acids. This degree might be very small considering the cyclic fatty acid monomer profiles obtained after hydrogenation.

CONCLUSION

In conclusion, the present results clearly show that mechanical extraction of low erucic acid rapeseed oil produces only a very small, if any, transformation of fatty acids. Similarly, the first steps in chemical refining, that is, degumming, neutralization, bleaching, and winterization, also induce only a small alteration of the fatty acids. Although the oil is heated during neutralization (including the successive washings) and bleaching, its temperature does not exceed 100 °C during these processes. In contrast, oxyphytosterols are already present in semirefined low erucic acid rapeseed oil. It is not precisely known when these compounds are formed, nor is the mechanism of their formation clear. Further studies are necessary.

Deodorization, which is the final step in the refining of edible oils, is typically achieved at a temperature ≥200 °C. Its purpose is to reduce the content of undesirable volatile components (odorous components, peroxides, free fatty acids, pesticides) of the oil to comply with quality requirements of the end product. Thus, FFA and PV were reduced during deodorization of the semirefined low erucic acid rapeseed oil. Quantities of oxyphytosterols present in semirefined low erucic acid rapeseed oil were not highly modified during deodorization, whatever the temperature of the stripping operation between 200 and 250 °C. Consequently, oxyphytosterols do not seem to be a good tracer of oil deodorization.

However, the stripping operation takes place simultaneously with some thermal reactions that lead to, among other things, *trans* fatty acids and cyclic fatty acid monomers. Considering the known physiological effects of *trans* fatty acids on platelet aggregation (*25*) and retinal function (*26*) and of cyclic fatty acid monomers, shown to have detrimental effects during reproduction in rats (*25*, *27*), the amount of foreign fatty acids formed during deodorization is important from a nutritional point of view. Under the more drastic conditions, 6 h of deodorization at 250 °C, a large amount of *trans* fatty acids (5.3%) was formed (**Table 2**). Furthermore, >50% of the α-linolenic acid is lost as it is converted into geometric *trans* isomers under these conditions (**Figure 4**). It should be noted, however, that 6 h of deodorization at 250 °C is rather unusual in chemical refining. This operation is habitually carried out between 220 and 260 °C but during a shorter holding time, typically 30−60 min (*28*).

On the contrary only a small amount of cyclic fatty acid monomers was formed during deodorization. Even under the most severe conditions investigated (6 h at 250 °C) ∼650 mg/kg of cyclic fatty acid monomers was found in deodorized low erucic acid rapeseed oil. Although cyclic fatty acid monomers could not be detected in extra virgin olive oil (*29*), that is, in an oil which has not been heated, cyclic fatty acid monomers are commonly found in processed oils. Thus, 400−700 mg of cyclic fatty acid monomers/kg was measured in fully refined edible oils (*30*) and 30−600 mg of cyclic fatty acid monomers/kg was measured in fish oil concentrates (*31*). Much higher values (up to 6600 mg of cyclic fatty acid monomers/kg) were found in commercial frying oils (*32*).

During the past few years, refining technology has shifted toward physical refining, in which FFA are exclusively removed in the stripping step (*28*). Application of physical refining requires increasing stripping intensity. As *trans* fatty acids and cyclic fatty acid monomers were shown to be formed predominantly at high temperatures and at long residual times, the possibility of having these decomposition products in fully refined oils is increased.

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