Minor Constituents of Vegetable Oils During Industrial Processing[1]

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ABSTRACT: We report the effects of individual steps of industrial refining, carried out in Brazil, on the alteration of selected minor constituents of oils, such as corn, soybean, and rapeseed oils. Total sterols, determined by capillary gas chromatography (GC), decreased by 18–36% in the fully refined oils, compared with the crude oils. The total steradienes, dehydration products of sterols, were determined *via* a simple clean-up on a short silica gel column, followed by high-performance liquid chromatography (HPLC) with ultraviolet detection. The level of steradienes, normally not present in crude oils, increased after each refining step, especially after deodorization. Thus, the content of steradienes increased after deodorization by about 15- to 20-fold in corn and soybean oils, and by about 2-fold in rapeseed oil. The total steryl esters were also determined *via* clean-up on a short silica gel column, followed by HPLC with evaporative light scattering mass detection. A minor decrease in the level of steryl esters was observed after complete refining. The individual tocopherols and tocotrienols were determined by HPLC with a fluorescence detector. The level of total tocopherols and tocotrienols decreased by about 2-fold after complete refining of corn oil and by about 1.5-fold in soybean and rapeseed oils. In all three cases, maximum reduction of tocopherols was observed after the deodorization step. The level of polymeric glycerides, determined *via* clean-up on a short silica gel column followed by size-exclusion HPLC, increased to some extent (0.4–1%) during refining. The level of *trans* fatty acids, determined by capillary GC, also increased to a substantial extent (1–4%) after refining.

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Vegetable oils are constituted predominantly of triglycerides; however, the presence of minor proportions of other constituents in such oils is of great importance. Crude oils are generally processed by degumming, alkali refining, bleaching, and deodorizing to obtain an odorless, bland, and oxidatively stable oil that is acceptable to consumers. Each processing step has specific functions for removing certain minor components, which can act as prooxidants or antioxidants (1).

The objective of refining an edible oil is to remove unacceptable materials with the least possible effect on desirable components and with the least possible loss of oil. However, chemical or physical refining subjects oils to high temperatures, alkali, and metal processing equipment that can cause alterations in their chemical composition (2). Transformations of minor components in vegetable oils during definite steps of technological processing are of great interest from analytical and nutritional viewpoints (3). The composition of some minor components provides highly specific information about the identity of an oil, particulary when the analysis is performed directly without derivatization (4). Moreover, the composition of minor components of oils is also used to either characterize the oil or the processing steps through which it has been obtained.

A significant proportion of the edible oil requirements of the Brazilian population is supplied through vegetable sources, such as corn, soybean and, more recently, rapeseed. These oils are utilized as salad and cooking oils, and in production of margarine and shortenings. The aim of this study was to investigate the effects of chemical refining techniques that are carried out industrially on the composition of some minor compounds, such as sterols, steradienes (5–7), steryl esters, tocopherols, polymeric glycerides (8,9), and *trans* fatty acids in corn, soybean, and rapeseed oils refined in Brazil. Chromatographic methods were used for these analyses.

EXPERIMENTAL PROCEDURES

Samples. Three different types of oil were used: corn, soybean, and rapeseed. The oils were obtained from processing lines in commercial factories in Brazil. Samples were taken before and after each stage of chemical refining and stored under nitrogen in a refrigerator until used.

Determination of sterols. The total sterol content and composition of sterols were determined according to Arens *et al.* (10) by capillary gas chromatography (GC) of the trimethylsilyl derivatives of the sterols. Separations were carried out in

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a Fractovap 2900 series chromatograph (Carlo Erba Strumentazione, Milano, Italy) with an Ultra 2 capillary column, 25 m × 0.25 mm, 0.25 μm film thickness. Column temperature was programmed as follows: 265°C (30 min), followed by a temperature increase at the rate of 15°C/min to 285°C. Injector and detector temperature was 300°C. The integrator used was SP 4290 (Spectra-Physics, Darmstadt, Germany).

Determination of steradienes. Steradienes were determined by high-performance liquid chromatography (HPLC) with an RP-18 column (E. Merck, Darmstadt, Germany), with UV-detection at 235 nm according to Schulte (11).

Determination of steryl esters. Steryl esters were determined according to unpublished data (R.Ap. Ferrari, W. Esteves, K.D. Mukherjee, and E. Schulte) after separation of this fraction *via* clean-up on a short silica gel column. The total steryl esters were measured by HPLC on an RP-18 column with acetonitrile/dichloromethane/methanol (1:1:1, vol/vol/vol) 1 mL/min for elution and evaporative light scattering detection, for quantitation. The detector was calibrated with β-sitosteryl oleate.

Determination of tocopherols and tocotrienols. The composition of tocopherols and tocotrienols and their content were determined by HPLC according to Balz *et al.* (12) as follows. An HPLC pump L-6200 (Merck), injector with 20 μL sample loop (type 7125; Rheodyne, Berkeley, CA), HPLC fluorescence detectors (F-1050 Fluorescence Spectrophotometer; Merck-Hitachi), and integrator (Chromato-Integrator D-2500; Merck-Hitachi), were used. An HPLC steel columm, 4 mm i.d., filled with LiChrospher 100 Diol, particle size 5 μm (Merck no. 50836), was connected to a steel guard column, 4-mm long, 4-mm i.d., filled with LiChrospher 100 Diol with particle size 5 μm (Merck no. 50960). Elution was carried out with *n*-hexane/*t*-butylmethylether (TBME) (92:8, vol/vol) for canola and soybean oils and 94:6 vol/vol for corn oil at a flow rate of 1.3 mL/min. All solvents were degassed (vacuum and ultrasonic bath) before and after every 4–5 h of operation. Detector wavelengths chosen were 295 nm for excitation and 330 nm for emission. Solutions (0.2 % wt/vol) of oil prepared in *n*-hexane were used for analysis in the HPLC system.

Tocopherols were quantitated from a standard curve of area ratios vs. α-tocopherol concentration. For calibration, standard solutions of 0.5, 1, 5, and 10 μg/mL α-tocopherol in *n*-hexane were used, which were prepared fresh from a stock solution containing 1 mg/mL of α-tocopherol in *n*-hexane; the stock solution, stored at 0–4°C, was stable for at least 7 d. Mixtures of α-, β-, γ-, and δ-tocopherol isomers (Merck no. 15496), dissolved in *n*-hexane, were prepared fresh from a stock solution containing 1 mg/mL of each tocopherol in *n*-hexane. The solutions stored at 0–4°C were stable for at least 7 d.

Determination of polymeric glycerides. Polymeric glycerides were determined with or without clean-up on a short silica gel column by size-exclusion HPLC (Phenogel 5 100A column, 300 × 7.8 mm; Phenomeuex, Torrance, CA) with refractive index detector according to Beljaars *et al.* (9). Elution was carried out with dichloromethane at a flow rate of 0.5 mL/min.

Determination of trans *fatty acids.* The lipids were hydrolyzed to fatty acids, that were derivatized to methyl esters (FAME) with diazomethane. FAME were analyzed by capillary GC in a Sato-10 column, 50 m × 0.32 mm i.d. with 0.25 μm film thickness (Sato, Mönchengladbach, Germany). Column temperature was programmed from 100 to 220°C at the rate of 1.5°C/min. Injector and detector temperatures were 200 and 240°C, respectively. Helium was used as the carrier gas at a head pressure of 15 psi. Authentic standards of FAME were used for peak identification.

RESULTS AND DISCUSSION

All data reported here were obtained by reliable and established methods with known coefficients of variation. The results presented are arithmetic means of duplicate determinations.

Sterols comprise a major portion of unsaponifiable matter of most vegetable oils. The total sterol content of the three oils investigated after each step of refining is given in Table 1. The total sterols decreased by 36% in fully refined corn oil, compared with the crude oil. Refining of soybean oil led to an 18% reduction and of rapeseed oil to a 24% reduction of total sterols. The relative proportion of the individual sterols was essentially not altered during the refining process (R.Ap. Ferrari, W. Esteves, K.D. Mukherjee, and E. Schulte, in preparation).

The content of the steryl ester fraction varies in edible oils (13). Because steryl esters degrade during GC analysis (14,15), HPLC was used for their determination. The total steryl esters were determined *via* clean-up on a short silica gel column, followed by HPLC with evaporative light scattering detection (results are given in Table 2). Among the samples analyzed, crude corn oil had the highest level of steryl esters (about 1100 mg/100 g), followed by rapeseed oil (about 760 mg/100 g), and soybean oil (about 120 mg/100 g). A minor decrease in the level of steryl esters was observed after complete refining. The composition of the constituent sterols and fatty acids of steryl esters was altered to some extent during the refining stages (R.Ap. Ferrari, W. Esteves, K.D. Mukherjee, and E. Schulte, in preparation).

TABLE 1
Concentration of Total Sterols in Corn, Soybean, and Rapeseed Oils After Different Processing Steps

	Total sterols (mg/100 g)		
Sample	Corn oil	Soybean oil	Rapeseed oil
Crude	1113.9	359.5	820.6
Degummed	—[a]	321.5	772.0
Neutralized	859.2	313.9	797.8
Bleached	848.8	288.8	650.4
Winterized	818.3	—[a]	—[a]
Deodorized	715.3	295.4	393.0

[a]Not part of this refining process.

TABLE 2
Concentration of Total Steryl Esters in Corn, Soybean, and Rapeseed Oils After Different Processing Steps

Sample	Total steryl esters (mg/100 g)		
	Corn oil	Soybean oil	Rapeseed oil
Crude	1090	121	759
Degummed	—[a]	166	785
Neutralized	1100	132	786
Bleached	1040	126	755
Winterized	808	—[a]	—[a]
Deodorized	911	114	735

[a]Not part of this refining process.

TABLE 3
Concentration of Total Steradienes in Corn, Soybean, and Rapeseed Oils After Different Processing Steps

Sample	Total steradienes (mg/kg)		
	Corn oil	Soybean oil	Rapeseed oil
Crude	ND[a]	ND	0.6
Degummed	—[b]	0.5	9.0
Neutralized	1.5	0.6	0.5
Bleached	10.3	1.2	225
Winterized	7.7	—[b]	—[b]
Deodorized	126	28.4	393

[a]ND, not detected.
[b]Not part of this refining process.

Industrial processing of edible fats and oils leads to several artifactual lipid derivatives, such as dehydrated sterols (5). For example, sterols of edible plant oils are dehydrated to unsaturated hydrocarbons, steradienes, by thermal stress, e.g., during refining of an oil (4). The analysis of dehydrated sterols has gained some importance as a method to detect bleaching of fats and oils (16,17). The analysis of steradienes may be useful as proof that fats and oils have undergone a refining process. Moreover, the occurrence of these compounds can be used for the detection of processed fats or their blending with fats and oils that are declared genuine and unrefined. Deleterious effects of dehydrated sterols on human health have not been detected to date (5).

Concentrations of steradienes during refining of corn, soybean, and rapeseed oils are given in Table 3. The level of steradienes, normally not present in crude oils, increased after refining. Thus, the content of steradienes of the bleached oils increased after deodorization by about 15- to 20-fold in corn and soybean oils, and by about 2-fold in rapeseed oil. Of the three samples analyzed, rapeseed oil had the highest level of steradienes after complete refining. Deodorization was the principal step that increased the level of these compounds; this observation agrees with Mennie *et al.* (7). The steradienes, present in small quantities in crude oils (Table 4), are probably formed by heat treatment of rapeseed before or during the extraction process.

Another group of minor components of vegetable oils are tocopherols, which are important because of their vitamin activity and their antioxidant properties to protect vitamin A, β-carotene, and essential fatty acids against oxidation. Tocopherols are sensitive to light, heat, alkali, and metal contaminants; therefore, they are easily oxidized to tocoquinones, which no longer have antioxidant properties. The tocopherol content and pattern of oils are characteristic and depend on

TABLE 4
Changes in the Composition of Tocopherols (T), Tocotrienols (T3), and Plastochromanol-8 (p-8) During Refining of Corn, Soybean, and Rapeseed Oils Determined by High-Performance Liquid Chromatography with Fluorescence Detection

Oil	Concentration (mg/100 g)							
	α-T	α-T3	β-T	γ-T	p-8	γ-T3	δ-T	Total
Corn								
Crude	23.5	2.5	5.5	157.7	7.1	5.4	7.9	209.7
Neutralized	25.0	3.6	3.7	166.9	8.4	6.6	8.2	222.5
Bleached	23.1	3.5	3.8	165.8	8.3	6.5	9.2	220.1
Winterized	22.6	3.5	3.6	159.5	8.4	6.3	7.8	211.7
Deodorized	10.6	2.1	1.7	61.5	7.5	4.0	2.9	90.3
Soybean								
Crude	19.5	ND[a]	8.3	117.1	8.2	ND	77.4	230.4
Degummed	21.3	ND	7.9	188.3	4.0	ND	74.4	295.9
Neutralized	19.9	ND	7.6	168.0	3.9	ND	72.2	271.6
Bleached	21.0	ND	7.8	185.2	4.2	ND	70.0	288.2
Deodorized	14.8	ND	4.9	126.7	5.1	ND	47.8	199.2
Rapeseed								
Crude (solvent extracted)	37.3	ND	1.8	91.7	29.2	ND	5.2	165.2
Crude (pressed)	30.2	ND	ND	83.4	15.6	ND	4.3	133.4
Degummed	28.5	ND	1.2	79.7	18.0	ND	5.3	132.7
Neutralized	33.9	ND	1.3	89.3	27.6	ND	4.2	156.4
Bleached	27.9	ND	1.7	82.5	18.1	ND	5.7	136.0
Deodorized	22.5	ND	1.5	59.2	18.3	ND	4.1	105.5

[a]ND, not detected.

plant genotype, climatic conditions of growth and harvest, polyunsaturated fatty acid content of oil, and processing and storage conditions (3). Vegetable oils contain four methyl-substituted derivatives of tocol (α-, β-, γ-, and δ-tocopherol) and four corresponding tocotrienols (α-, β-, γ-, and δ-tocotrienol). These eight compounds differ distinctly in their biological activities and hence in their importance for overall vitamin E supply; α-tocopherol is the most active form, but the other compounds may also make major contributions (12).

In recent years, numerous authors have reported methods for the analysis of tocopherols in oils. Several of these methods involve HPLC, but other techniques, such as spectrophotometry and GC, have also been employed (18). In some of these methods, a major problem is associated with the oxidation of tocopherols during their extraction when insufficient precautions are taken to prevent oxidation. This problem does not occur in a recent method involving HPLC with a diol-column (12); moreover, this method is simple because it does not require any initial purification.

In this study, the individual tocopherols and tocotrienols were determined by HPLC with a fluorescence detector. The results of tocopherol analysis are given in Table 4. The level of total tocopherols and tocotrienols decreased in corn oil by about 2-fold after complete refining (Table 4). Similarly, in soybean and rapeseed oils, the level of total tocopherols decreased by about 1.2- to 1.5-fold (Table 4). In all three cases, maximum reduction was observed after the deodorization step (Table 4).

Even though total tocopherol content decreased during processing, the relative proportions of individual tocopherols in oils were relatively constant during refining (Table 4). Gutfinger and Letan (19) also reported that no significant differences were observed between the composition of tocopherol fractions in crude and deodorized soybean oils. γ-Tocopherol was the major component, which constitued more than 68% in refined corn oil, 64% in refined soybean oil, and 55% in refined rapeseed oil; only corn oil contained tocotrienols (Table 4). The loss of total tocopherols by refining was 57, 14, and 36% in corn, soybean, and rapeseed oils, respectively.

Technological processing and oxidation during storage might affect the levels of tocopherols in oils. The tocopherol content decreased during each step of processing and was markedly reduced during deodorization (Table 4) because the tocopherols are volatile under these conditions. Because the tocopherols are powerful natural antioxidants, optimal processing conditions are those that retain these compounds in their active form. Abuse of an oil by exposure to air or heat will reduce the content of tocopherols by oxidation and polymerization.

Among the three crude oils, crude soybean oil from industry was the richest in tocopherols (230.4 mg/100 g). δ-Tocopherol was detected in high levels in soybean oil, whereas rapeseed oil was the richest in α-tocopherol. There seems to be a relationship between the fatty acid composition of the triglycerides of the oils and the type of tocopherol in the oils. Oils that were rich in unsaturated acids (Tables 5–7) also contained considerable amounts of tocopherols (Table 4).

TABLE 5
Fatty Acid Composition of Corn Oil After Different Processing Stages

Fatty acid[a]	Composition (%)				
	Crude	Neutralized	Bleached	Winterized	Deodorized
16:0	13.1	12.4	12.8	12.0	12.5
16:1	0.2	0.2	0.2	0.2	0.2
18:0	2.1	2.2	2.3	2.3	2.3
Σ 18:1	34.1	34.6	35.5	34.9	34.8
Σ 18:2 *trans*[b]	0.1	0.1	0.1	0.1	1.2
18:2	47.9	47.9	46.5	47.8	46.4
20:0	0.6	0.6	0.6	0.6	0.6
Σ 18:3 *trans*[c]	<0.1	<0.1	<0.1	<0.1	0.2
18:3	0.9	0.9	0.8	0.9	0.6
Σ 20:1	0.5	0.5	0.5	0.6	0.5
22:0	0.2	0.2	0.2	0.2	0.2
24:0	0.2	0.2	0.2	0.2	0.2
Others	0.1	0.2	0.3	0.2	0.3
Σ *trans*	0.1	0.2	0.1	0.1	1.5

[a]Fatty acids are designated by number of carbon atoms:number of double bonds.
[b]Constituted mainly of 18:2 Δ9 *trans*, 12 *trans*, 18:2 Δ9 *cis*, 12 *trans*, and 18:2 Δ9 *trans*, 12 *cis*.
[c]Constituted mainly of 18:3 Δ6 *trans*, 9 *cis*, 12 *trans*, 18:3 Δ6 *cis*, 9 *cis*, 12 *trans*, 18:3 Δ6 *cis*, 9 *trans*, 12 *cis* and 18:3 Δ6 *trans*, 9 *cis*, 12 *cis*.

Prolonged heating or overheating of fats and oils can produce degradation products, including polymerized triglycerides. During heating, a wide variety of chemical reactions

TABLE 6
Fatty Acid Composition of Soybean Oil After Different Processing Stages

Fatty acid[a]	Composition (%)				
	Crude	Degummed	Neutralized	Bleached	Deodorized
16:0	11.5	10.8	10.7	10.8	11.2
17:0	0.1	0.1	0.1	0.1	0.1
17:1	0.1	0.1	0.1	0.1	0.1
18:0	3.3	3.5	3.6	3.5	3.5
Σ 18:1 *trans*[b]	<0.1	<0.1	<0.1	0.1	0.1
Σ 18:1	24.2	24.7	25.0	24.8	24.9
Σ 18:2 *trans*[c]	0.1	0.1	0.1	0.6	2.0
18:2	52.6	52.5	52.2	52.2	50.2
20:0	0.4	0.4	0.4	0.4	0.4
Σ 18:3 *trans*[d]	<0.1	<0.1	<0.1	0.1	2.7
18:3	6.4	6.3	6.5	6.2	3.5
Σ 20:1	0.4	0.5	0.5	0.4	0.4
22:0	0.5	0.5	0.5	0.5	0.5
24:0	0.2	0.2	0.2	0.2	0.2
Others	0.2	0.3	0.1	<0.1	0.2
Σ *trans*	0.1	0.2	0.2	0.7	4.6

[a]Fatty acids are designated by number of carbon atoms:number of double bonds.
[b]Constituted mainly of 18:1 Δ9 *trans*.
[c]Constituted mainly of 18:2 Δ9 *trans*, 12 *trans*, 18:2 Δ9 *cis*, 12 *trans*, and 18:2 Δ9 *trans*, 12 *cis*.
[d]Constituted mainly of 18:3 Δ6 *trans*, 9 *cis*, 12 *trans*, 18:3 Δ6 *cis*, 9 *cis*, 12 *trans*, 18:3 Δ6 *cis*, 9 *trans*, 12 *cis*, and 18:3 Δ6 *trans*, 9 *cis*, 12 *cis*.

TABLE 7
Fatty Acid Composition (%) of Rapeseed Oil After Different Processing Stages

Fatty acid[a]	Composition (%)				
	Crude	Degummed	Neutralized	Bleached	Deodorized
16:0	4.5	4.5	4.4	4.5	4.5
16:1	0.3	0.2	0.3	0.2	0.2
Σ 18:1 *trans*[b]	0.1	0.1	0.1	0.1	0.1
Σ 18:1	64.6	64.4	64.5	64.0	64.6
Σ 18:2 *trans*[c]	<0.1	<0.1	<0.1	<0.1	0.4
18:2	17.9	17.9	17.7	18.3	17.4
20:0	0.7	0.7	0.7	0.7	0.7
Σ 18:3 *trans*[d]	<0.1	0.1	<0.1	0.2	2.0
18:3	7.9	7.5	7.6	7.3	5.3
Σ 20:1	1.1	1.2	1.2	1.2	1.3
22:0	0.4	0.4	0.3	0.3	0.4
22:1	0.1	0.1	0.1	0.1	0.1
24:0	0.2	0.2	0.2	0.2	0.2
24:1	0.1	0.1	0.1	0.1	0.1
Others	2.8	2.6	2.8	2.8	2.7
Σ *trans*	0.1	0.2	0.2	0.3	2.4

[a]Fatty acids are designated by number of carbon atoms:number of double bonds.
[b]Constituted mainly of 18:1 Δ9 *trans*.
[c]Constituted mainly of 18:2 Δ9 *trans*, 12 *trans*, 18:2 Δ9 *cis*, 12 *trans*, and 18:2 Δ9 *trans*, 12 *cis*.
[d]Constituted mainly of 18:3 Δ6 *trans*, 9 *cis*, 12 *trans*, 18:3 Δ6 *cis*, 9 *cis*, 12 *trans*, 18:3 Δ6 *cis*, 9 *trans*, 12 *cis*, and 18:3 Δ6 *trans*, 9 *cis*, 12 *cis*.

result in the formation of compounds with high molecular weight and polarity (20). Recently, there has been increased interest in the polymeric triglycerides formed during the autoxidation of unsaturated fats and oils and their influence on the quality of fats (21). The oxidative and thermal degradations take place in the unsaturated acyl moieties of the triglycerides, which lead to modification of the nutritional properties of fats (22).

Lipid polymers can be present in refined oils, due mainly to the deodorization step, in which the oils are heated to high temperatures (8). The level of polymerized glycerides, determined *via* clean-up on a short silica gel column followed by size-exclusion HPLC, increased by 0.4–1% during refining (Table 8). Essentially similar levels of polymerized triglycerides were obtained irrespective of whether the analysis was carried out with or without precleaning (Table 8). The level of *trans* fatty acids, determined by capillary GC, also increased to a substantial extent (1–4%) after refining (Tables 5–7). All samples studied contained geometrical isomers of fatty acids, which were formed mainly during deodorization (Tables 5–7).

It is clear that the refining process induces geometrical isomerization of unsaturated fatty acids. Deodorization was the principal step that increased the level of these components. As demonstrated by some authors (23–25), the parameters that affect the formation of *trans* isomers are temperature and time of heating. Consequently, if the formation of geometrical isomers of fatty acids is to be avoided during the deodorization process, the temperature in the deodorizer should be relatively low and the heating time sufficiently short to minimize isomerization by heat (26).

TABLE 8
Concentration of Polymerized Triglycerides in Crude and Refined Oils

Sample	Concentration (%) of polymerized triglycerides determined	
	Precleaning	Without precleaning
Corn (crude)	<0.01	0.1
Corn (refined)	0.5	0.5
Soybean (crude)	0.1	0.1
Soybean (refined)	1.2	1.0
Rapeseed (crude solvent-extracted)	<0.01	<0.01
Rapeseed (crude pressed)	<0.01	<0.01
Rapeseed (refined)	0.3	0.4

Knowledge of the *trans* fatty acid content of fats and oils is important not only for the manufacturer as an aid in process optimization but also for health authorities, because unbalanced ingestion of *trans* fatty acid-containing fats and oils may have adverse effects on health (27). Based on the data reported here, it can be concluded that there was a gradual decrease throughout each consecutive stage of refining in the content of tocopherols and sterols, and slight changes in the relative distributions of individual tocopherols and sterols. There were no significant changes in content of total steryl esters. Deodorization was the principal stage of refining of oils that contributed to increases in the content of steradienes, polymerized glycerides, and *trans* fatty acids.

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