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Quality Changes and Tocopherols and γ-Orizanol Concentrations in Rice Bran Oil During the Refining Process

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Abstract The quality changes and the concentrations of tocopherols and γ-oryzanol, during successive steps of rice bran oil refining (RBO), were studied. For this purpose, samples of crude, degummed, neutralized, bleached, dewaxed and deodorized RBO were taken from an industrial plant and analyzed. The moisture, pH, acidity, peroxide value and unsaponifiable matter, were determined. The fatty acid composition was evaluated by GC, and the concentrations of tocopherols and γ-oryzanol were determined using HPLC with fluorescence and UV–Vis detection, respectively. To identify γ-oryzanol components, fractions of the HPLC eluant were collected and analyzed using mass spectrometry. Oil refining reduced the peroxide value and acidity to 1 and 3% of the values obtained in crude RBO, respectively. The fatty acid composition were not significantly altered during refining. The concentrations of the tocopherols in RBO followed the order $\alpha > (\beta + \gamma) > \delta$. The total concentration of tocopherols was 26 mg/100 g, and remained practically unaltered during refining. Up to nine components were distinguished in γ-oryzanol. After collecting the elution fractions, up to six components were identified by electrospray mass spectrometry. Refining reduced the total concentration of γ-oryzanol to 2% of its initial value.

Keywords Fatty acids · γ-Oryzanol · Oil quality · Oil refining · Rice bran oil · Tocopherols

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Introduction

Rice bran, a by-product of rice processing, contains 16–22% lipids, which makes the extraction of rice bran oil (RBO) profitable [1]. When compared to other vegetable oils, RBO contains low concentrations of linoleic acid and high levels of natural antioxidants, which is important from the viewpoint of its oxidative stability. RBO is very stable at high temperature, the smoke point is 254 °C, which is high due to the relatively high content of saturated fatty acid and waxes, making it suitable for frying. It has a mild flavor, and is rich in tocopherols (vitamin E), γ-oryzanol and phytosterols. Due to its stability under storage and cooking conditions, and to potential health benefits, RBO is very popular in several Asian countries, ca. 1.3 million tons were produced in 2006. India, China, Myanmar and Japan are the most important world producers of RBO [2]. It is also appreciated in several specialty oil niche markets. It is used extensively in Japan, Korea, China, Taiwan and Thailand as q premium edible oil.

Several tocopherols (α-, γ- and δ-, and much lower concentrations of β-tocopherol), are found in RBO. Tocopherols possess antioxidant properties and play a protective role against some forms of cancer. γ-Oryzanol is a complex mixture of esters of ferulic acid with sterols and triterpenic alcohols [3]. The activity of γ-oryzanol as a cardio-protective agent, and its ability to reduce the

absorption of hepatic cholesterol and to prevent arteriosclerosis, have been studied [4]. Due to their positive health benefits, the possible losses of tocopherols and γ-oryzanol during oil refining is of interest.

Before sale for human consumption, undesirable matter which interferes with the physical, chemical and sensory characteristics of the oil is removed by refining [5]. The conventional stages of the RBO refining process include degumming, neutralization, bleaching, dewaxing and deodorizing (Fig. 1).

During degumming, hot water is used to remove hydratable phospholipids. Approximately 90% of the total phospholipids in crude oil absorb water and agglomerate into a gum phase. A NaOH solution is then used to remove free acids. Neutralization produces soaps that are removed by centrifugation. The free fatty acid contents of RBO are relatively high; therefore, this is a difficult step, generally entailing high oil losses. During bleaching, the natural pigments present in the oil are removed by adsorption on bleaching earth (bentonite) and carbon [6].

High molecular weight compounds, including hydrocarbons and waxes, are removed by dewaxing. The oil is then maintained at low temperatures, and the crystallized material is removed by filtration or centrifugation. Finally, any residual component which could have any undesirable effect on the color and odor of the oil is removed. Deodorizing is carried out with steam at high temperature, followed by volatilization at low pressures [6].

The objective of this work was to evaluate the global quality changes of RBO, as estimated by the moisture, pH, peroxide value and acidity, and the amount of unsaponifiable matter, as well as the possible changes of the concentrations of fatty acids, tocopherols and γ-oryzanol, during the successive stages of refining.

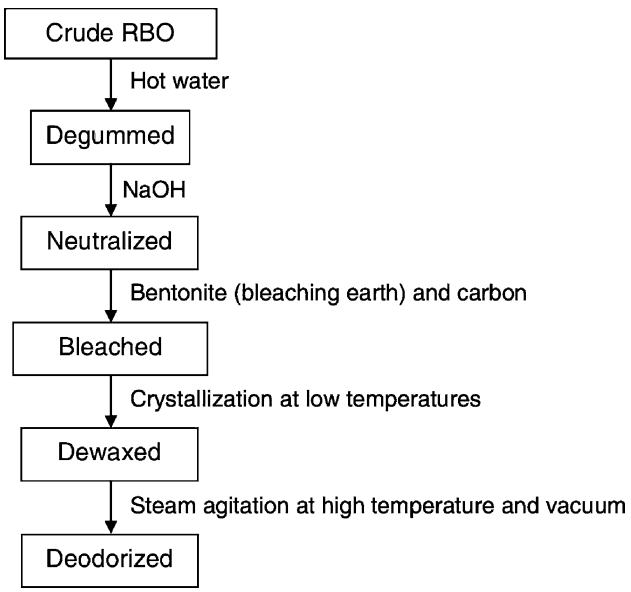

Fig. 1 Stages of RBO refining

Materials and Methods

Reagents and Samples

Analytical grade diethyl ether, phenolphthalein, sodium hydroxide, sodium thiosulphate, hydrochloric acid, isooctane, isopropanol, acetonitrile (Vetec, Rio de Janeiro, Brazil), absolute ethanol, glacial acetic acid, chloroform, starch, potassium iodine, petroleum ether and methanol (Synth, Diadema, Brazil), were used.

To identify fatty acids, a mixture of standards containing the following acids was used: caproic (C6:0), caprylic (C8:0), caproleic (C8:1), lauric (C12:0), dodecenoic (C12:1), myristic (C14:0), miristoleic (C14:1), palmitic (C16:0), palmitoleic (C16:1), margaric (C17:0), heptadecenoic (C17:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), gadoleic (C20:1), eicosadienoic (C20:2), eicosatrienoic (C20:3), eicosatetraenoic (C20:4), eicosapentanoic (C20:5), behenic (C22:0), erucic (C22:1), docosadienoic (C22:2), docosatrienoic (C22:3), docosatetraenoic (C22:4), lignoceric (C24:0) and nervonic (C24:1) (Sigma, St. Louis, MO, USA). Standards of α-tocopherol (99%, Merck, Darmstadt, Germany), γ-tocopherol (>96%, Sigma), δ-tocopherol (>90%, Sigma), and γ-oryzanol (analytical grade, TCI, Tokyo, Japan), were also used.

Samples of RBO were obtained from Irgovel (Indústria Riograndense de Óleos Vegetais, Pelotas, Brazil). The samples were collected directly from the processing line, immediately after the extraction stage (crude oil), and after each of the processing steps, i.e. degumming (using water at 72 °C), neutralization (using NaOH solution at 80 °C), bleaching (using acid activated earth at 110 °C), dewaxing (at 12 °C) and deodorizing (residence time of 3 h at 230 °C). Three different lots of rice bran were sampled. The samples were kept frozen at −18 °C in translucent plastic containers until immediately before analysis. All samples were analyzed in duplicate.

Procedures for the Physical–Chemical Characterization of RBO

Measurements of pH were carried out at room temperature with a potentiometer. The AOCS official methods [7] were used to determine moisture, and peroxide value and acidity. Thus, moisture was established according to the loss of weight after treating the samples at 105 °C until constant weight in a porcelain capsule. To determine acidity, weighted oil samples of 2 g were diluted with 25 mL of a 2:1 (v/v) diethyl ether–ethanol mixture, and titrated with a KOH

solution in ethanol. To determine the peroxide value, weighed oil samples of 5 g were diluted with a 3:2 (v/v) acetic acid–chloroform mixture, and titrated with thiosulfate.

To quantify the unsaponifiable matter, the method described by Zambiazi [8] was applied. Briefly, 2.0–2.5 g oil was weighed and 25 mL 1.5 M KOH in methanol was added. During the first hour, the flask was shaken for about 1 min every 15 min. Then, the flask was allowed to rest for 18 h at room temperature in the absence of light. After this, the mixture was transferred to a separatory funnel, and 40 mL water and 40 mL petroleum ether were added. After shaking for 1 min, the aqueous lower phase was transferred to another separatory funnel. A volume of 20 mL petroleum ether was added to the second funnel, and 20 mL water was added to the first funnel. The two funnels were shaken, the aqueous phases were discarded, and the organic phases were joined. The solvent was evaporated at 60 °C in a double boiler, and the final residue was weighed.

Instrumentation and Procedures for Fatty Acids

A gas chromatograph (GC-14B, Shimadzu, Kyoto, Japan), with a flame ionization detector (FID), and a fused silica capillary column of 30 m × 0.25 mm × 0.25 μm DB-225 (50% cyanopropyl methyl and 50% methyl phenyl silicone, J&W Scientific, Folsom, CA, USA), was used. The injector and detector were both maintained at 250 °C. Nitrogen, at a rate of 1.0 mL min^{-1}, was used as carrier gas.

Derivatization of fatty acids was done according to Zambiazi et al. [9]; briefly, portions of 45 mg oil were weighed in test tubes with lids, to which 1 mL of petroleum ether and 12 mL of 0.5 M HCl in methanol were added. The tubes were vortex mixed and heated at 65 °C for 1 h. Then, 5 mL iso-octane and 6 mL distilled water were added, and the tubes were shaken. The upper layer was partially transferred to a 1.5 mL flask, from which 1.5 μL was taken and injected in the gas chromatograph with a 1:50 split. The initial column temperature of 100 °C was maintained for 0.5 min, then it was brought up to 150 °C with a linear ramp of 8 °C min^{-1}; after 0.5 more min, it was increased to 180 °C with a linear ramp of 1.5 °C min^{-1}; after 5 additional min it was finally increased up to 220 °C with a linear ramp of 2 °C min^{-1}. The temperature was maintained for 6 more min, for a total analysis time of ca. 58 min. The Class-GC10 software (Shimadzu) was used to acquire and process the GC data.

Instrumentation and Procedures for Tocopherols and γ-Oryzanol

An HPLC system (Shimadzu) consisting of an automatic sampler (SIL-10AF), a solvent mixing module (LC-10 ALvp), an automatic degasser (FCV-10ALvp), a quaternary pump (DGU-14A), a column oven compartment (CTO-10ASvp), a UV–Vis spectrophotometric detector (SPD-10Avp), or alternatively a fluorescence detector (RF-10Axl), and a control system (SCL-10avp), was used. A Shim-Pak CLC-ODS column (3.9 cm × 150 mm, 4 μm particle size, Shimadzu) was also used.

The procedures for the determination of tocopherols and γ-oryzanol were adapted from Chen and Bergman [3]. Portions of ca. 250 and 500 mg oil for tocopherol and γ-oryzanol, respectively, were weighed and diluted with 5 mL of a 7:3 (v/v) isopropanol–acetonitrile mixture. To remove suspended solids, the mixtures were centrifuged at 9,000 rpm (7,245 g) for 6 min (NT-800 micro centrifuge, Nova Técnica, Piracicaba, Brazil), and the sample was transferred to a 1.5 mL vial. Aliquots of 20 to 40 μL were injected in the liquid chromatograph.

All HPLC separations were performed at 25 °C with a constant flow-rate of 1 mL min^{-1}. The UV–Vis spectrophotometric detector, set at 325 nm, was used for γ-oryzanol. Fluorimetric detection, with the excitation and emission wavelengths set at 290 and 330 nm, respectively, was used for the tocopherols. The initial and final mobile phases were a 50:40:10 (A) and a 30:65:5 (B) acetonitrile–methanol–isopropanol mixtures (v/v/v), respectively. Isocratic elution of phase A for 5 min, changing by a linear gradient for 10 min to phase B, followed by 5 min of isocratic elution with phase B, and then returning to phase A in 5 min, for both tocopherols and γ-oryzanol separation was used. Class-VP software (Shimadzu) was used to acquire and process the data. Standards of α-, γ- and δ-tocopherols, and γ-oryzanol were used to construct external calibration curves.

To identify the γ-oryzanol components, fractions of the HPLC eluate were manually collected in vials and injected in the electrospray ion source (ESI) of an HP 1100 series ion-trap mass spectrometer (ITMS) (Agilent, Waldbronn, Germany) through a 50 μm i.d. fused silica capillary (Supelco, Bellefonte, PA, USA). For this purpose, a syringe pump (kd Scientific, Holliston, MA, USA) at a constant flow-rate of 0.3 mL h^{-1} (5 μL min^{-1}) was used. The ITMS working conditions were: nebulizer gas pressure, 25 psi; drying gas flow, 5 L min^{-1} at 200 °C; capillary voltage, 3.5 kV; voltages of skimmers 1 and 2, −26.8 and −6.0 V, respectively. Nitrogen was used as the nebulizer and drying gas (Gaslab NG LCMS 20 generator, Equcien, Madrid, Spain). The mass spectrometer was scanned within the *m/z* 100–800 range in the negative-ion mode. The target mass was set at *m/z* 601 ($[M - H]^-$ peak of cycloartenylferulate). Maximum loading of the ion trap was 3×10^4 counts, and maximum collection time was 300 ms.

Statistical Analysis

Analysis of variance (ANOVA) and comparison of averages by the Tukey's test were carried out using the program

STATISTICA v. 6.0 [10]. A 5% significance level was used in all cases.

Results and Discussion

Changes of the Physicochemical Parameters

Data from the physicochemical parameters of crude, degummed, neutralized, bleached, dewaxed and deodorized RBO samples are shown in Table 1. According to the data, the moisture decreased during degumming, neutralization and bleaching, with a small residual moisture (<0.1%) remaining after the process.

Both the pH measurements and acidity values indicated that crude and degummed RBO were weakly acidic, due to the free fatty acids. Due to alkali addition, RBO became weakly basic after neutralization, then slightly acid after bleaching. Both the soap produced and the excess NaOH were removed during neutralization, but some free fatty acids probably remained, or appeared immediately after during further saponification and bleaching. This residual acidity is reduced during deodorization, due to volatilization of the free fatty acids. The final acidity was 0.11%. The amount found was close to the amounts reported by Rodrigues et al. [11], which found an acidity reduction from 9.34% in crude RBO to 0.07% in refined RBO. These values are also lower than the maximum values required by Brazilian legislation, i.e. 15, 0.8 and 0.3%, for extracted, degummed and neutralized RBO, respectively [12].

Crude RBO samples had the greatest peroxide value, which could be due to the presence of lipoxygenases in the bran. Peroxides in rice bran are produced by the joint action of enzymes and atmospheric oxygen during peeling and polishing of crude rice. According to the data in Table 1, peroxides are partially removed during degumming, but their concentrations remain constant during neutralization. It is interesting to observe that the peroxide content decreased during bleaching (using acid activated earth), but increased during dewaxing. Formation during dewaxing may be due to the presence of air. The peroxides are largely removed during deodorization. None of the peroxide values in Table 1 surpassed the limits established by the Brazilian legislation [12]. Finally, the content of unsaponifiable matter was not significantly modified by any of the steps of refining.

Changes in the Fatty Acid Profiles

The fatty acid profiles of crude, degummed, neutralized, bleached and dewaxed RBO are shown in Table 2. The profiles for crude RBO were not significantly different from those reported in the literature, with oleic, linoleic and palmitic acids showing the largest concentrations [5, 12]. The profiles were approximately constant during the whole refining process, not showing any significant variation.

Changes of the Concentrations of the Tocopherols

An HPLC chromatogram showing the profile of the tocopherols for a typical crude RBO sample is given in Fig. 2. The peaks of the δ-, ($\beta + \gamma$)- and α-tocopherols were identified by spiking a sample with the corresponding standards. According to literature, the largest peak in the middle location corresponds to the sum of the β- and γ-tocopherols, since these isomers are not resolved using RP-HPLC [13]. Calibration curves showed excellent linearity ($r^2 > 0.997$, $n = 6$ for all curves). Using α-tocopherol as a reference, the response factors (relative fluorescence sensitivities) were 4.6 and 3.5 for γ- and δ- tocopherol, respectively. The changes in the tocopherol concentrations during the various stages of oil refining is shown in Table 3. The concentrations found in crude RBO were lower than those reported by Krishna et al. [14], i.e. 12.6, 26.9 and 9.5 mg of α-, β- and γ-tocopherol per 100 g of refined RBO, respectively. However, they were within the ranges reported by Desai et al. [15], i.e. 17–33 mg α-tocopherol per 100 g of refined RBO.

As can be observed, the α-tocopherol concentration increased slightly along refining, particularly during degumming, neutralization and bleaching. It is also deduced that α-tocopherol was stable during the entire refining process, even during deodorizing, where temperatures of 230 °C were used, while the content of the other tocopherols decreased slightly along refining. However, the total

Table 1 Changes of the physicochemical parameters of RBO during the refining process

	Moisture (%)	pH	Acidity (% oleic acid)	Peroxide value (mequiv g kg^{-1})	Unsaponifiable matter (%)
Crude	1.01 a	5.79 a	8.14 a	8.54 a	2.28 a
Degummed	0.64 ab	5.56 a	6.55 b	5.71 b	1.88 a
Neutralized	0.34 b	8.12 b	0.08 c	5.79 b	1.92 a
Bleached	0.04 c	6.38 a	0.17 c	3.80 b	2.04 a
Dewaxed	0.04 c	6.29 a	0.21 c	5.91 b	1.98 a
Deodorized	0.08 c	6.09 a	0.11 c	0.28 c	2.01 a

Values followed by an identical letter in the same column did not differ beyond a 5% significance (test of Tukey, $p < 0.05$)

Table 2 Changes in the fatty acid profile during the refining stages of RBO

FAME	Crude	Degummed	Neutralized	Bleached	Dewaxed	Deodorized
C6:0	tr	0.09	0.12	tr	tr	tr
C12:0	tr	0.13	0.13	tr	tr	tr
C14:0	0.25	0.33	0.11	tr	0.07	0.24
C14:1	0.04	0.20	0.34	0.23	0.02	0.01
C16:0	17.5	20.0	18.5	18.3	18.7	19.2
C16:1	0.15	0.15	0.14	0.13	0.13	0.14
C17:0	2.07	1.12	4.30	1.43	0.89	0.86
C17:1	tr	tr	tr	0.33	tr	tr
C18:0	1.47	1.52	1.45	1.53	1.66	1.60
C18:1	33.3	34.4	36.1	36.0	38.2	37.9
C18:2	30.2	31.9	31.4	30.9	33.2	33.1
C18:3	4.26	3.11	3.20	4.47	2.91	2.66
C20:0	0.73	0.48	0.53	0.52	0.64	0.59
C20:1	1.21	0.77	0.75	0.47	0.65	0.61
C20:2	2.02	1.32	1.26	2.52	1.16	1.19
C20:4	tr	0.32	tr	tr	tr	tr
C22:0	3.17	1.17	0.86	1.82	0.88	0.97
C22:2	0.23	tr	tr	tr	tr	tr
C22:3	0.50	0.12	tr	tr	tr	tr
C22:4	0.75	0.70	tr	tr	tr	tr
C24:0	1.23	1.40	0.52	1.11	0.60	0.66
C24:1	0.95	0.81	0.32	0.30	0.32	0.25
Saturated (total)	26.5	28.6	29.0	26.7	25.5	26.0
Unsaturated (total)	73.5	71.4	71.0	73.3	74.5	74.0

From the GC peaks of the FAMEs, calculated as relative percentages; *tr* below the quantification limit (traces)

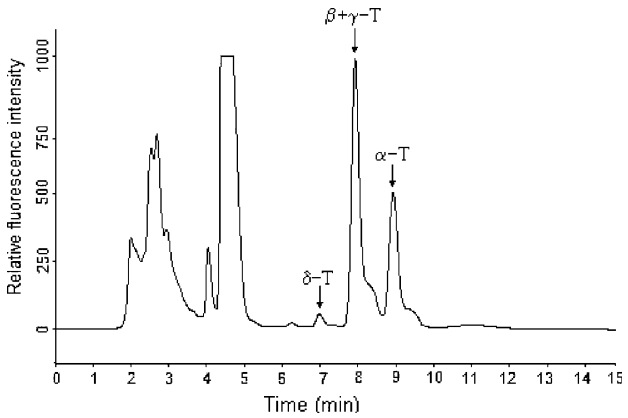

Fig. 2 HPLC chromatogram with fluorimetric detection showing the profile of the tocopherols for a typical crude RBO sample (*T* tocopherol)

tocopherol contents slightly increased during refining, due to the increase of the α-tocopherol concentration.

The same tendency for the tocopherol changes was observed by Hoed et al. [16]. These authors found a slight increase in the concentration of α-tocopherol and a slight decrease of γ-tocopherol during refining of rice bran oil. They observed almost no changes of the tocopherol concentration during deodorization at 238 °C for 90 min.

Table 3 Changes in the tocopherol concentrations during the refining stages of RBO (in mg per 100 g oil)

RBO	α-tocopherol	(β + γ)-tocopherol	δ-tocopherol	Total
Crude	16.1 a	9.73 a	0.49 a	26.3
Degummed	16.9 a	9.63 a	0.48 a	27.0
Neutralized	18.3 ab	8.43 a	0.34 b	27.1
Bleached	20.0 c	8.62 a	0.38 ab	29.0
Dewaxed	19.6 bc	8.65 a	0.38 ab	28.6
Deodorized	21.5 c	7.74 a	0.38 ab	29.7

Values followed by an identical letter in the same column did not differ beyond a 5% significance (test of Tukey, $p < 0.05$)

Some losses of tocopherols in the refining of sunflower oil have been reported in the literature, where the concentrations of total tocopherols were reduced to a ca. 26% of the values found in unrefined sunflower oil; however, the β- and γ-tocopherol losses were greater than the α-tocopherol losses [17]. Reductions of the concentrations of the tocopherols during deodorization of soybean oil have been also reported. Ferrari [18] found losses of 10.2, 8.1 and 3.9% for α-, γ- and δ-tocopherol, respectively, during deodorization at 210 °C for 1 h.

Changes of the Concentrations of γ-Oryzanol

A typical chromatogram of γ-oryzanol in a crude RBO sample is shown in Fig. 3. Resolution of peaks 5A and 5B was rather poor in the crude and deodorized oil samples; therefore, the sum of the areas of these two peaks was used for quantification purposes.

In order to identify the γ-oryzanol components, fractions of the eluate were collected and analyzed by mass spectrometry. On Fig. 3, the arrows indicate the retention times at which the collection of a new elution fraction began. As shown in the figure, single fractions were collected for the peak pairs 5A, 5B and 6, 7. The fractions were directly infused in the ESI source of the mass spectrometer.

The fractions showed the predominant $[M - H]^-$ peak of a single component of γ-oryzanol. Retention times, *m/z* values and identification of the components are shown in Table 4. Significant peaks corresponding to known γ-oryzanol components were not identified in fractions 1 and 2. Since the two components, Δ^7-campestenyl ferulate and campesteryl ferulate, have the same *m/z* value, a single predominant peak was observed in the mass spectrum of the fraction corresponding to peaks 5A and 5B. For the same reason, a single predominant peak was also observed in the mass spectrum of the elution fraction corresponding to peaks 7 and 8. Thus, in addition to the *m/z* value, literature data [19] were used to assign the individual components of peaks 5A, 5B, 7 and 8. Both the elution order and the relative proportions of the components coincided with the data reported by Xu and Godber [19].

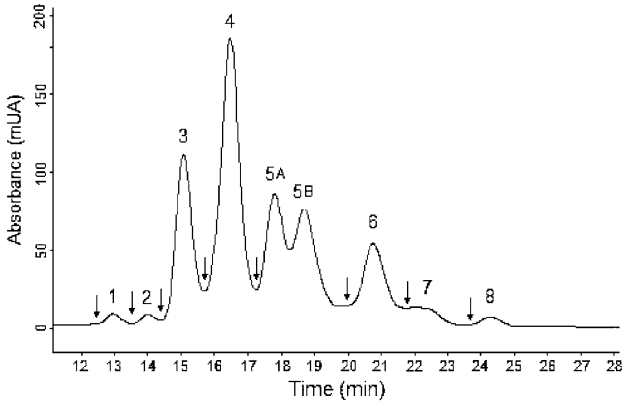

Fig. 3 HPLC–UV–Vis chromatogram of a typical crude RBO showing the peaks of the γ-oryzanol components; the arrows indicated the beginning of the fractions collected for mass spectrometry. The numbers on the peaks correspond to the peak assignments of Table 4

Table 4 Identification of γ-oryzanol components

Peak no.[a]	Retention time (min)	*m/z* of $[M - H]^-$ peak	Compound
3	15.1	601	Cycloartenyl ferulate
4	16.5	615	24-Methylene cycloartenyl ferulate
5A	17.8	575	Δ^7-Campestenyl ferulate
5B	18.7	575	Campesteryl ferulate
6	20.8	589	Δ^7-Sitostenyl ferulate
7	22.3	589	β-Sitosteryl ferulate
8	24.3	591	Sitostenyl ferulate

[a] According to the peak numbering in Fig. 3

The total γ-oryzanol concentration in crude RBO was similar to other reported values, which in mg per 100 g oil are: 980 [19], 1,220 [6], 1,720 [11] and 1,630–2,720 [14].

The changes of the contents of the γ-oryzanol components during refining is detailed in Table 5, and the total γ-oryzanol concentration is also given in the last row of this table. As deduced from this table, all the γ-oryzanol components decreased in similar proportions during the various stages of refining. The concentrations of the main γ-oryzanol components did not decrease significantly during degumming. This agreed with the values reported in the literature [6]. However, neutralization produced substantial losses of all the γ-oryzanol components. Neutralized RBO contained only 4% of the total γ-oryzanol concentration in crude RBO. Krishna et al. [14] also reported losses of the same order during neutralization (5.4–7.0% of the initial values). The γ-oryzanol components are probably more soluble at the alkaline medium or may react and precipitate during neutralization, the polar free ferulic acid being dragged along with the sediments.

Significant reductions of the concentrations of all the γ-oryzanol components, which amounted to a ca. 25% reduction of the total concentration, were produced during bleaching. A significant further reduction of the total γ-oryzanol concentration was produced during deodorizing (a ca. 19% decrease), but not during dewaxing (only a 2% decrease). These data differed from that reported by Krishna et al. [14], who found no losses during bleaching and deodorizing, and a decrease of only a 5.9% during dewaxing. These differences can be attributed to the processing conditions employed by the industry. Thus, the filtering medium used for clarifying, and temperatures, pressures and processing times used for dewaxing and deodorizing, may differ greatly among industries.

As a consequence of the whole refining process, the final level of γ-oryzanol was reduced to a 2.3% of its original value in crude RBO. The γ-oryzanol concentration found in refined RBO, 29.0 mg per 100 g, was within the range reported by Rogers [20], i.e. 10–80 mg per 100 g oil. Higher values have been reported by other authors, i.e. 190–200 mg [14] and 120 mg [5]. Again, the differences could be due to the dissimilar processing conditions.

Table 5 Changes in the concentrations of the components of γ-oryzanol during the refining stages of RBO (mg per 100 g oil)

Component[a]	Crude	Degummed	Neutralized	Bleached	Dewaxed	Deodorized
1	11.1	12.3	0.81	0.48	0.72	0.57
2	14.7	14.5	0.32	0.16	0.30	0.20
3	292	267	9.67	6.18	7.19	5.77
4	493	486	20.4	4.72	8.39	7.44
5	220	215	8.63	17.9	12.6	9.63
6	159	157	6.57	5.03	4.80	3.59
7	31.0	38.4	1.81	1.10	0.96	0.79
8	21.4	22.6	1.07	0.96	0.85	0.99
Total	1,240 a	1,210 a	49.3 b	36.6 b	35.8 b	29.0 b

Values followed by an identical letter in the same column did not differ beyond a 5% significance (test of Tukey, $p < 0.05$)

[a] According to peak numbering in Fig. 3 and Table 4

Conclusions

Moisture, and the acidity and peroxide value of RBO, were largely reduced during refining, whereas the concentration of unsaponifiable matter was not altered significantly. Both the absolute and relative concentrations of the fatty acids and the tocopherols, were not reduced significantly either. On the contrary, the concentrations of all the γ-oryzanol components decreased greatly and in similar proportions. The main losses of γ-oryzanol occurred during neutralization. At this stage, the total γ-oryzanol concentration was reduced to 4% of its initial value in crude RBO. Further decreases during bleaching and deodorization reduced the total γ-oryzanol concentration to 2.3% of its initial value.

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