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γ-Oryzanol and tocopherol contents in residues of rice bran oil refining

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ABSTRACT

Rice bran oil (RBO) contains significant amounts of the natural antioxidants γ-oryzanol and tocopherols, which are lost to a large degree during oil refining. This results in a number of industrial residues with high contents of these phytochemicals. With the aim of supporting the development of profitable industrial procedures for γ-oryzanol and tocopherol recovery, the contents of these phytochemicals in all the residues produced during RBO refining were evaluated. The samples included residues from the degumming, soap precipitation, bleaching earth filtering, dewaxing and deodorisation distillation steps. The highest phytochemical concentrations were found in the precipitated soap for γ-oryzanol (14.2 mg g^{-1}, representing 95.3% of total γ-oryzanol in crude RBO), and in the deodorisation distillate for tocopherols (576 mg 100 g^{-1}, representing 6.7% of total tocopherols in crude RBO). Therefore, among the residues of RBO processing, the deodorisation distillate was the best source of tocopherols. As the soap is further processed for the recovery of fatty acids, samples taken from every step of this secondary process, including hydrosoluble fraction, hydrolysed soap, distillation residue and purified fatty acid fraction, were also analyzed. The distillation residue left after fatty acid recovery from soap was found to be the best source of γ-oryzanol (43.1 mg g^{-1}, representing 11.5% of total γ-oryzanol in crude RBO).

1. Introduction

Rice is a very popular crop in Brazil, the annual production reaching ca. 11,661 million tons, the state of Rio Grande do Sul being responsible for 62.8% of this production (Conab – Companhia Nacional de Abastecimento, 2011). Rice bran, a by-product of rice processing, represents about 8–11% of the grain by weight, and contains 16–22% of lipids, thus being commonly used for rice bran oil (RBO) extraction (da Silva, Sanches, & Amante, 2006; Pestana, Zambiazi, Mendonça, Bruscatto, & Ramis-Ramos, 2009). Refining is decisive for improving stability, quality (flavour and colour) and functionality (fatty acid composition, vitamins and antioxidants) of RBO. Refining should be carried out so as to minimize costs, including reduced equipment and minimal energy, as well as minimal losses of neutral oil (Rodrigues, Pessôa Filho, & Meirelles, 2004).

The common chemical RBO refining process includes degumming, neutralisation, bleaching, dewaxing and deodorisation (Pestana et al., 2008) (Fig. 1). Degumming removes phospholipids and lipoproteins, through hydration, by adding water and either citric or phosphoric acid, followed by centrifugation (Baruffaldi & de Oliveira, 1998; Zambiazi, 1997). During neutralisation, free fatty acids are removed by precipitation with a sodium hydroxide solution (Araújo, 1999), and the sodium salts of the free fatty acids (soaps) are separated by centrifugation (Baruffaldi & de Oliveira, 1998). The pigments naturally present in the crude oil (including chlorophylls and carotenoids) are removed by adsorption on bleaching earth (Ferrari, 2001; Weiss, 1983). During dewaxing, the oil is maintained at low temperatures to provoke wax crystallisation; then solidified waxes are removed by filtration or centrifugation (Zambiazi, 1997). Finally, during deodorising, volatile substances that are responsible for undesirable odours are removed; for this purpose, the oil is heated to 200–250 °C at low pressures (3–5 mm Hg) (Kao & Luh, 1991; Baruffaldi & de Oliveira, 1998).

On the other hand, precipitated soap is further processed for fatty acid recovering. As illustrated in Fig. 2, acid hydrolysis is initially carried out; the resulting raw fatty acids are separated from a hydrosoluble fraction, mainly containing HCl and NaCl. Finally, the raw fatty acids are distilled at low pressure to recover a 99.9% pure fraction. Therefore, during fatty acid recovering, raw fatty acids (or hydrolysed soap as an intermediate product), purified fatty acids (final product), and two residues (hydrosoluble fraction from hydrolysis and distillation residue) are produced.

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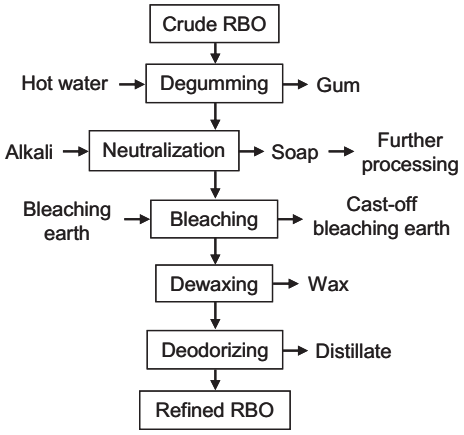

Fig. 1. Flow chart for RBO chemical refining.

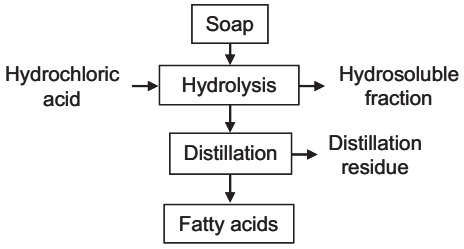

Fig. 2. Flow chart for the recovery of fatty acids from soap.

In a previous work we have investigated the variations of the concentrations of several phytochemicals, including γ-oryzanol and tocopherols, during the steps of industrial RBO refining (Pestana et al., 2008). These two compound classes are important antioxidants, being also of interest from a nutritional viewpoint (Ferrari, 2001; Pestana et al., 2008). During RBO refining, the concentration of γ-oryzanol is largely reduced. Therefore, the concentration of γ-oryzanol in refined RBO is merely 2% of its initial value in crude RBO (Pestana et al., 2008). On the other hand, the concentration of tocopherols in refined RBO is similar to or slightly lower than that in crude RBO; thus, taking into account that refined RBO represents less initial mass of crude RBO, it can be deduced that an important fraction of the tocopherols present in crude RBO is lost during refining. However, the concentration of γ-oryzanol and tocopherols in the by-products of the process were not evaluated. Therefore, there is still a lack of quantitative knowledge regarding the fate of these phytochemicals in the products and residues of RBO refining. Further, so far, the vast majority of the residues are ruled out in effluents. Thus, in this work, with the aim of supporting the development of industrial procedures for the recovery of γ-oryzanol and tocopherols, these phytochemicals were evaluated in the main products, in key intermediates, and in all the residues generated during RBO refining, and in the associated process of fatty acid recovery from soap. From the concentrations and amounts of products and residues produced, the mass distribution of the phytochemicals among them, throughout the refining process, was also estimated.

2. Materials and methods

2.1. Reagents and samples

Analytical grade isopropanol, acetonitrile and methanol (Vetec, Rio de Janeiro, Brazil), were used. To identify and quantify phytochemicals, standards of γ-oryzanol (analytical grade, TCI, Tokyo, Japan), α-tocopherol (99%, Merck, Darmstadt, Germany), γ-tocopherol (96%, Sigma) and δ-tocopherol (90%, Sigma), were used.

Samples of residues from RBO processing, provided by Irgovel Ltda (Industria Riograndense de Oleos Vegetais, Pelotas, Brazil), were collected directly from the processing line, immediately after each refining operation. According to the scheme of Fig. 1, these were the following: precipitated gum obtained by degumming with water at 72 °C; soap produced by neutralisation with NaOH solution at 80 °C; cast-off bleaching earth (recovered after oil filtration at 110 °C); wax from dewaxing at 12 °C; and deodorising distillate (residence time 3 h at 230 °C).

The residues taken from each step of soap processing (according to the scheme of Fig. 2), including the hydrosoluble fraction, the purified fatty acids (obtained at 230 °C and 1 mm Hg), and the distillation residue, were analyzed. The soap hydrolysate (containing raw fatty acids, an intermediate), obtained after soap hydrolysis with a 6:4 mixture of concentrated HCl and water (residence time 6 h at 220 °C) was also analyzed. In all cases, three different lots of samples were analyzed in triplicate. The samples were kept frozen at −18 °C in translucid plastic containers prior to analysis.

2.2. Determination procedures for γ-oryzanol and tocopherols

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

The procedures for the determination of γ-oryzanol and tocopherols were taken from literature (Chen & Bergman, 2005; Pestana et al., 2008). Sample portions of ca. 250 mg were weighed and diluted with 5 ml of isopropanol. After centrifugation at 9000 rpm (7.245*g*) for 6 min (NT-800 micro centrifuge, Nova Tecnica, Piracicaba, Brazil), the upper layer was transferred to a 1.5 ml vial. Aliquots of 10–20 μl were injected. Separations were performed at 25 °C with a 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 tocopherols. The mobile phases were 50:40:10 (A) and 30:65:5 (B) acetonitrile–methanol–isopropanol mixtures (v/v/v). For the separation of both γ-oryzanol and tocopherols, isocratic elution with phase A for 5 min, followed by a 10 min linear gradient from phase A to 100% phase B, with a final 5 min isocratic elution with phase B, was used (adapted from Chen & Bergman, 2005). Class-VP software (Shimadzu) was used to acquire and process the data. To construct the calibration curves, standard solutions of γ-oryzanol, and α-, γ- and δ-tocopherols, were used.

2.3. Statistical analysis

Analysis of variance (ANOVA) and comparison of averages by Tukey's test were carried out using the programme Statistica v. 6.0 (Statsoft Tulsa, OK, USA). A 5% significance was used in all cases. All means and standard deviations of data in Tables 1 and 2 were obtained with $n = 9$.

3. Results and discussion

3.1. General

Typical chromatograms obtained for γ-oryzanol and tocopherols in two different residues of the RBO refining process are shown

Table 1
Contents of γ-oryzanol and tocopherols in residues from RBO refining,[a] and recovery values (in percentages with respect to the contents of the phytochemical in 100 mass units of crude RBO).

Products	Ref. mass [b]	γ-Oryzanol, mg g^{-1}, and recovery (%)	Tocopherols, mg 100 g^{-1}, and recovery (%)			
			δ-	(β + γ)-	α-	Total
Crude RBO[c]	100	12.4; 100	0.49; 100	9.73; 100	16.1; 100	26.4; 100
Gum	3.0 + 6.5	2.92 ± 0.69a; 2.2	0.23 ± 0.05a; 4.5	1.40 ± 0.37a; 1.4	2.87 ± 0.16a; 1.7	4.50 ± 0.22a; 1.6
Soap, S	26.2 + 57	14.2 ± 1.24b; 95.3	0.37 ± 0.40a; 62.8	1.13 ± 0.03a; 9.7	2.49 ± 0.16a; 12.9	3.99 ± 0.52a; 12.6
Clarified RBO, C[c]	69.7	0.49; 2.7	0.34; 48.4	8.43; 60.4	18.3; 79.2	27.1; 71.8
Maximal recov. from S + C (%)	–	100.2	111.2	71.5	93.8	86.0
Cast-off bleaching earth	1.5	0.12c ± 0.12; 0.01	0.07 ± 0.11a; 0.2	0.29 ± 0.30a; 0.04	0.04 ± 0.08a; 0.004	0.40 ± 0.20a; 0.02
Wax	11.8	0.28c ± 0.16; 0.27	0.44 ± 0.27a; 10.6	3.95 ± 1.37a; 4.81	5.25 ± 1.54a; 3.87	9.64 ± 1.63a; 4.33
Deod. distillate	0.3	0.24c ± 0.01; 0.01	10.2 ± 0.44b; 6.2	172 ± 0.13b; 5.30	393 ± 2.61b; 7.32	576 ± 9.59b; 6.57
Refined RBO[c]	57.6	0.29; 1.35	0.38; 44.7	7.74; 45.8	21.5; 76.92	29.7; 65.05
Maximal recov. from C (%)[d]	–	1.6 from 2.7	61.7 from 48.4	56.0 from 60.4	88.1 from 79.2	75.97 from 71.8
Total loss (%)	–	−1.1	–	−4.4	–	–

[a] Means followed by an identical letter in the same column did not differ beyond a 5% significance (test of Tukey, $p < 0.05$).
[b] Mass obtained by processing 100 mass units of crude RBO, which in the cases of degumming and soap precipitation was increased by the addition of water and HCl or NaOH solutions, respectively; the values of this column were used to calculate mass recoveries of each phytochemical from the found concentrations.
[c] Data from Pestana et al. (2008); clarified RBO is an intermediate product.
[d] Maximal recovery from clarified RBO: sum of the percentages found in the bleached earth, wax, deodorisation distillate and refined RBO.

Table 2
Contents of γ-oryzanol and tocopherols, and recovery values (in percentages with respect to the phytochemical contents in 100 mass units of soap), in the residues of soap processing for fatty acid recovery.[a]

Products	Ref. mass [b]	γ-Oryzanol, mg g^{-1}, and recovery (%)	Tocopherols, mg 100 g^{-1}, and recovery (%)			
			δ-	(β + γ)-	α-	Total
Soap, S	26.2 + 57	14.2 ± 1.02a; 95.3	0.37 ± 0.52a; 62.8	1.13 ± 0.33a; 9.7	2.49 ± 0.35a; 12.9	3.99 ± 0.39a; 12.6
Hydrosol. fraction, H	60.7	0.20 ± 0.02b; 1.0	NDa ± 0.00; 0	NDa ± 0.00; 0	NDa ± 0.00; 0	NDa ± 0.00; 0
Hydrolysed soap [c]	22.5	27.3c ± 1.36; 49.5	1.08 ± 0.05a; 49.6	2.89 ± 0.37a; 6.7	3.50 ± 0.45a; 4.9	7.47 ± 1.13a; 6.4
Purified fatty acids, P	19.2	0.14 ± 0.05b; 0.2	0.60 ± 0.66a; 23.5	2.16 ± 0.28a; 4.3	1.42 ± 0.64a; 1.7	4.18 ± 0.62a; 3.0
Distillation residue, D	3.3	43.1d ± 1.70; 11.5	1.95 ± 0.00a; 13.2	26.3 ± 0.34b; 9.0	69.3 ± 1.43b; 14.3	97.5 b ± 1.77; 12.3
Max. rec. from H + P + D (%)	–	12.7	36.7	13.3	16.0	15.3
Max. rec. from all res. (%)[d]	–	14.3	98.4	69.3	104.1	91.2

[a] Means followed by an identical letter in the same column did not differ beyond a 5% significance (test of Tukey, $p < 0.05$); ND = not detected.
[b] Mass units of the residue obtained by processing 100 mass units of soap; the values of this column were used to calculate the mass recoveries of each phytochemical from soap.
[c] Intermediate product, further processed to obtain the purified fatty acids and the distillation residue.
[d] Maximal recovery which can be achieved using all the residues of the RBO and soap processing; for instance for γ-oryzanol: 0.91 + 1.6 = 2.51%.

in Fig. 3. The chromatograms of γ-oryzanol showed nine peaks (Fig. 3A); however, due to difficulty in accurately measuring peaks 5A and 5B in some samples, the sum of the areas of these two peaks was measured. The nine peaks of γ-oryzanol, obtained using similar chromatographic conditions and mass spectrometry detection, were identified by Xu and Godber (1999). These nine peaks were also identified by Pestana et al. (2008), using the same chromatographic conditions as those adopted in this work and mass spectrometry detection. Therefore, according to these literature sources, the γ-oryzanol peaks were identified as indicated in the caption of Fig. 3.

The tocopherols were detected within the 5.6–7.1 min range (Fig. 3B), in the expected retention time order: δ < γ < α. According to literature (Pestana et al., 2008, and other authors), β-tocopherol, present in minor concentrations in RBO, was measured jointly with γ-tocopherol, since this pair of isomers is not usually resolved using RP-HPLC.

3.2. Quantification of phytochemicals along the RBO refining main process

The contents of phytochemicals in all the residues of RBO refining and soap hydrolysate, fatty acid recovery from soap, calculated from the peak areas, are shown in Tables 1 and 2, respectively. In the same Tables, the distribution of each phytochemical among the residues (recovery values), using its total amount in a batch of crude RBO as reference (100% of initial compound present in 100 arbitrary mass units of crude RBO), is also indicated. In this way, the fate of the phytochemical during the process was established. According to the data given in Table 1, the samples of cast-off bleaching earth, wax and deodorisation distillate showed small γ-oryzanol contents, without any significant difference between them. In comparison with these residues, gum presented a significantly higher γ-oryzanol content; however, the highest content was found, by far, in the soap samples, largely differing from that of the other residues (14.2 mg g^{-1}, representing 95.3% of the total γ-oryzanol distribution). This value agreed with reported γ-oryzanol contents in crude RBO (12.4 mg g^{-1}, Pestana et al., 2008), thus confirming that almost all the γ-oryzanol was precipitated during neutralisation. As indicated in Table 2, the sum of the amounts of γ-oryzanol found in all the residues, as well as in the final products of RBO refining (refined RBO and purified fatty acids), represented ca. 12.7% of the initial amount of this phytochemical in crude RBO.

The data given in Table 1 for γ-oryzanol agree with other reported values. Thus, according to Krishna, Khatoon, and Shiela (2001), the reduction of the γ-oryzanol content in RBO during neutralisation can be as large as 93–95.8%, whereas only small percentages are lost during degumming and dewaxing (1.1–2.3% and 2.0–5.9%, respectively). According to Orthoefer (1996) and Mishra, Gopalakrishna, and Prabhakar (1988), during neutralisation, high losses of neutral oil (18–22%), maximized by the synergistic effect of the precipitated soap and γ-oryzanol, occur. Both neutral oil and γ-oryzanol dragging, during soap precipitation upon

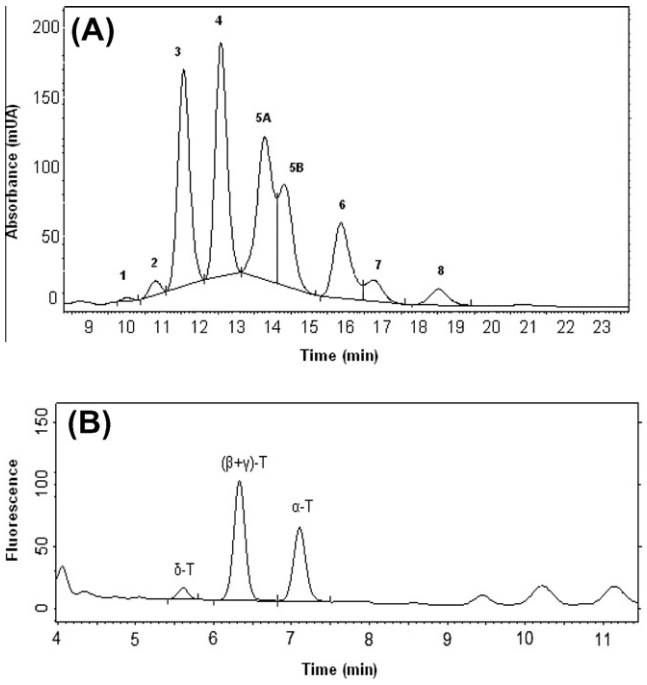

Fig. 3. Typical chromatograms for γ-oryzanol (A) and tocopherols (B) in the samples. The numbers on the peaks (A) correspond to the Δ7-stigmastenyl ferulate (1), stigmasteryl ferulate (2), cycloartenyl ferulate (3), 24-methylene cycloartenyl ferulate (4), Δ7-campestenyl ferulate (5A), campesteryl ferulate (5B), Δ7-sitostenyl ferulate (6), β-sitosteryl ferulate (7), and sitostanyl ferulate (8).

neutralisation, can be due to the surfactant-like nature of soap, and probably the formation of emulsions in the precipitate. The γ-oryzanol content of 14.2 mg g^{-1} in the precipitated soap, given in Table 1, also agrees with the reported 12.2 mg g^{-1} (Scavariello, 1997). This author also extracted γ-oryzanol from soap using acetone at 10 °C for 60 min, obtaining an extract with 62.5 mg g^{-1} of γ-oryzanol. Finally, Krishna et al. (2001) indicated that bleaching and deodorising do not affect the γ-oryzanol content.

Also according to literature reports, RBO refining produces large amounts of soap as a consequence of enzymatic activity of lipases, which largely increases the free fatty acid concentrations of crude RBO (De & Bhattacharyya, 1998). As illustrated in Fig. 2, the abundant soap residue is further treated to recover purified free fatty acids, which are then used by the cosmetics and cleaning industries. Thus, in order to support the development of procedures for γ-oryzanol recovery, its contents in the residues of soap processing should also be established. This point is further discussed in the next section.

As also shown in Table 1, the δ-, (β + γ)- and α-tocopherol contents were separately quantified. With the exception of cast-off bleaching earth, all the other residues of RBO refining showed the following relative contents of the individual tocopherols: δ < (β + γ) < α. This order agreed with the reported values for crude RBO (Pestana et al., 2008). The exceptionally large retention of (β + γ)-tocopherol by bleaching earth can be explained by the particular selectivity of solid surfaces with respect to positional isomers. However, since the amount of bleaching earth was very small (1.5 mass units per 100 mass units of crude RBO), the percentage of this phytochemical retained in this residue was also very small.

Among the main products and residues, the largest tocopherol amount, ca. 65%, was found in refined RBO. In addition, the largest tocopherol concentration, by far, was that found in the deodorisation distillate (576 mg 100 g^{-1}). As deduced from Table 1, in comparison to crude RBO (data from Pestana et al. (2008)), tocopherols are concentrated by a factor of ca. 22 times that in the deodorisation distillate. For this reason, and in spite of the small amount of this residue (only 0.3 mass units per 100 mass units of crude RBO), tocopherols in the deodorisation distillate represented ca. 7% of the tocopherol distribution. Thus, deodorisation distillate could be of interest for tocopherol recovery. Concentration of tocopherols in the deodorisation distillate has also been observed by other authors, and should be attributed to volatilization of these phytochemicals at high temperatures (Hoed et al., 2006). Soon-Nam, Sun-Mi, and In-Hwan (2008) found 1490 mg 100 g^{-1} of tocopherols in the deodorisation distillate of RBO. These authors also recovered tocopherols with acetonitrile at −20 °C, obtaining an extract with 2140 mg 100 g^{-1}. Hoed et al. (2006) found 1100 mg 100 g^{-1} of tocopherols in the deodorisation distillate of RBO. The large differences of total tocopherol contents in the deodorisation distillate found in this work (576 mg 100 g^{-1}), and in other literature reports, may be related to both natural variations of the phytochemical contents in crude RBO, and the different industrial conditions used during deodorisation.

It is interesting to observe that soap, which retained most of the γ-oryzanol (95.3% of the total amount found in crude RBO), dragged only moderate percentages of tocopherols (ca. 13%). Thus, tocopherols are less soluble in the soap than is γ-oryzanol, and probably also less prone to form mixed micelles or emulsions with the neutral oil and the fatty acid sodium salts than is γ-oryzanol. Most tocopherols were thus retained in the clarified RBO (ca. 86%). From this intermediate, ca. 7% was concentrated in the deodorisation distillate, but most of it reached the refined RBO (ca. 65%).

3.3. Quantification of phytochemicals along fatty acid recovery from soap

The contents of phytochemicals in the soap hydrolysate (intermediate product), and in the residues obtained during fatty acid recovery from soap, are shown in Table 2. Owing to the reduction of the total mass by removing water and hydrosoluble materials (as glycerol), soap hydrolysis allowed the γ-oryzanol concentration to increase from 14.2 to 27.3 mg g^{-1}. However, ca. 60% of the γ-oryzanol precipitated with soap was lost during soap hydrolysis with HCl at 220 °C for 6 h. Although this should be confirmed, it is reasonable to assume that γ-oryzanol is largely hydrolysed into ferulic acid and free phytosterols during this treatment. Hydrolysis of this γ-oryzanol (almost ca. 60% of the total γ-oryzanol in crude RBO), would probably enrich soap hydrolysate with free phytosterols. On the other hand, the purified fatty acids showed minimal amounts of γ-oryzanol; therefore, of γ-oryzanol still present in the soap hydrolysate was possibly hydrolysed during distillation of the fatty acids, which is carried out at 230 °C at low pressures. In fact, distillation produced a residue with a dark oily appearance, possibly containing lipid polymers and products of Maillard reactions (Chichester, 1986). Thus, the possible presence of free phytosterols in the soap hydrolysate and hydrosoluble fraction, as well as their subsequent concentration increase or destruction during distillation of the fatty acids, should be further investigated. In addition to the likely presence of large phytosterol concentrations, the distillation residue still had a large γ-oryzanol concentration (43.1 mg g^{-1}, representing only ca. 11.5% of total γ-oryzanol in crude RBO). In comparison to reported γ-oryzanol contents for rice bran (1.68 mg g^{-1}, Pestana et al., 2009), and crude and refined RBO (see Table 1), the concentration factors of γ-oryzanol in the distillation residue were 26, 3.5 and 149, respectively. As the distillation residue has currently no industrial application, a large amount of γ-oryzanol is wasted. Thus, the development of processes capable of profitably recovering this potent antioxidant,

from the hydrolysed oil (before distillation) or from the distillation residue, is of interest.

On the other hand, soap contains ca. 12.6% of total tocopherols in crude RBO. Soap hydrolysis produced an increase in the tocopherol concentrations, but the total amount of tocopherols in the soap hydrolysate was significantly reduced. Tocopherols were not detected in the hydrosoluble fraction, in agreement with the marked hydrophobic character of these phytochemicals. Therefore, most of the tocopherols present in the soap were also destroyed during soap hydrolysis. Further processing of the hydrolysed soap led to concentration of the remaining tocopherols in the distillation residue, which showed a total tocopherol content of 97.5 mg 100 g^{-1}. This was 3.7 times higher than the total concentration of tocopherols in crude RBO (Pestana et al., 2008). For this reason, it could be of interest to also recover tocopherols from the distillation residue; however, the amount found in this residue corresponded to a maximal recovery of ca. 7% of the total tocopherols in crude RBO. Therefore, recovery of tocopherols is potentially more productive by processing RBO at any stage of the main refining process, between degumming and final refined RBO, rather than by processing soap or any other product or residue produced upon soap processing. Finally, with the exception of the purified fatty acids, α-tocopherol showed higher concentrations than did the other tocopherols in all the samples.

4. Conclusion

Important criteria, to decide if a product or by-product can be of interest to recover a phytochemical, are preconcentration factor, absolute concentration, and total amount of product or by-product per batch. These latter two determine the maximal percentage of recovery of the phytochemical which can be achieved by further processing of the product or by-product. Preconcentration of the phytochemical in the by-product makes recovery and purification easier, and the total amount to be processed by batch determines the scale of the industrial operation to be designed. Among the by-products obtained during the industrial chemical refining of RBO, the highest γ-oryzanol concentration was found in the distillation residue from fatty acid recovery (43.1 mg g^{-1}, which represented ca. 11.5% of total γ-oryzanol in crude RBO). Then, the hydrolysed soap, either before or after distillation of the fatty acids, can be advantageously used for γ-oryzanol, recovery.

On the other hand, most tocopherols are retained by the refined RBO (ca. 65%), but the highest concentration of total tocopherols was found in the deodorisation distillate (576 mg 100 g^{-1}), representing ca. 7% of total tocopherols in crude RBO. Thus, advantageous recovery of tocopherols can be achieved from the deodorisation distillate. Thus, the deodorisation distillate, which is commonly discarded, could be used for a better exploitation of RBO as a natural resource. In our research group, further studies, in order to recover γ-oryzanol, free phytosterols and tocopherols from intermediates and wastes, to be used for pharmaceutical and nutritional purposes, are in progress.

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