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ORIGINAL PAPER

Optimization of Physical Refining to Produce Rice Bran Oil with Light Color and High Oryzanol Content

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Abstract Crude rice bran oil (RBO) is rich in valuable minor components such as tocotrienols, phytosterols and γ-oryzanol. These compounds are well preserved during physical refining, but in current industrial practice, RBO is mostly refined chemically because this results in a lighter color. However this process removes most of the γ-oryzanol. The challenge is to develop a refining process which combines a high γ-oryzanol retention with the commercially desired light color. A modified physical refining process was developed, consisting of an acid degumming, prebleaching, dewaxing, physical removal of free fatty acids using packed column technology, a modified washing step, conventional bleaching and deodorization. A RBO with acceptable oryzanol retention of 39% had a Lovibond red color value (measured with a 5.25-inch cell) of 2.8, approaching very close the color of a chemically refined RBO (red = 2). At the process step where high (94%) retention of γ-oryzanol was achieved, a somewhat darker Lovibond red value of 5.2 was obtained.

Keywords Rice bran oil · Physical refining · Lovibond color · Oryzanol · Phytosterols · Tocopherol

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Introduction

Rice bran oil (RBO) is popular as a cooking oil in several countries such as India, Thailand, Japan, Korea, China and Taiwan [1]. This oil can be extracted from a byproduct (rice bran) of rice processing, and the production of this oil is gaining world-wide attention because of its interesting technological properties such as the excellent heat stability and high smoke point. Moreover, from a nutritional point of view, RBO is exceptionally rich in minor components, such as tocotrienols, phytosterols and γ-oryzanol. The latter component is unique in RBO and has a high value as a nutraceutical [2]. RBO and its main components have demonstrated an ability to improve the plasma lipid pattern of rodents, rabbits, non-human primates and humans, reducing total plasma cholesterol and triglyceride concentrations, and increasing the HDL cholesterol level. Other potential properties of RBO and γ-oryzanol, studied in both in-vitro and in animal models, include modulation of pituitary secretion, inhibition of gastric acid secretion, antioxidant action and inhibition of platelet aggregation [3]. Therefore, crude RBO is rich in health promoting minor components, and is used in food, cosmetic and pharmaceutic applications.

However, crude RBO needs careful refining due to the high content in free fatty acids, which is about 2–6% for high quality RBO [4–6] but usually in the range 5–10% [1, 6–9] or up to 30% [10, 11] and due to the high levels of other components such as partial glycerides (2–3% diacylglycerols; 1–2% monoacylglycerols), phospholipids (1–2%), glycolipids (0.8%), waxes (3–4%) and unsaponifiable matter (4%) [4]. Chemical refining seems the most appropriate and straightforward process to obtain a refined oil with bland taste, light color and good (cold) stability. It consists of degumming, dewaxing, alkali neutralization,

bleaching and deodorization [4, 12]. However, the operating costs of chemical refining are quite high due to high oil losses in the neutralization and environmental problems caused by the production of large soapstock waste streams. Moreover, high percentages of the valuable minor compounds are lost [7, 8, 12–14]. Consumer interest for minimally processed oils of high nutritional value necessitates continuous research for soft refining processes.

The problems of soapstock disposal and losses of oryzanol and neutral oil can be reduced, as described in US Patent 6,197,357, by using a weak acid salt (such as sodium bicarbonate) to achieve refined RBO with a high yield and with good oryzanol retention. Further, this oil can be treated with small amounts of concentrated caustic to yield a refined RBO and a nutraceutical-rich concentrate [15]. The color values of the final oil were not mentioned. Other alternative methods to chemical refining are miscella refining, mixed solvent refining and physical or steam refining [4]. The latter process is commonly used for high FFA feedstocks (palm oil, coconut oil) and has the advantage of producing no soapstock and yielding lower oil and micronutrient losses [14]. However, steam refining needs a rigorous pretreatment to remove interfering pigments, waxes and phospholipids [4, 9]. For this pretreatment, many different degumming and dewaxing methods are described, such as degumming with water or acid, super degumming, TOP degumming, enzymatic degumming, combined degumming/dewaxing, miscella dewaxing, and solvent dewaxing [4].

Furthermore, studies on the use of different types of membranes show that they have the potential for use in all stages of the RBO refining [4, 16], and for increasing the retention of oryzanol in the RBO [17]. Despite these encouraging results, a major drawback of membrane processing, especially in an industrial environment, is the difficulty of preventing fouling and/or cleaning fouled membranes [4].

In industrial practice, rice bran is still mostly chemically refined. Earlier attempts to apply physical refining failed because it was not possible to produce a refined oil with a sufficiently light color.

The goal of this work was to develop a physical refining process resulting in a RBO with a light color similar to chemically refined RBO, and at the same time a maximal γ-oryzanol content. Manjula and Subramanian [16] noticed that Maillard reaction products are responsible for the color of RBO rather than β-carotene and chlorophyll. Furthermore, the removal of phosphorus-containing components is essential to avoid color fixation in the oil during physical refining [16]. Therefore, in physical refining, a proper pretreatment is crucial to remove phospholipids as well as waxes and metals [4]. A good to excellent retention of γ-oryzanol was obtained by different researchers using various pretreatment procedures. However, the final color of the physically refined oils was not mentioned [17, 18], or could not be decreased similar to the chemically refined RBO, as discussed in the following examples. In chemical refining, a Lovibond red color value of 2.2, measured with a 5.25-in. cell, is usually obtained [12]. Lovibond red values between 1 and 3 or between 10 and 12 were reported for physically refined RBO [6, 7], which were however all measured with a 1-in. cell and would correspond to much higher values if measured in the 5.25-in. cell. Making an estimation according to the general conversion table in the AOCS official method Cc13b-45, a red value of 1, measured with the 1-in. cell, corresponds approximately to a red value of 6, measured with the 5.25-in. cell, while oils with red color values above 2 in the 1-in. cell will be even out of range when measured with the 5.25-in. cell [19]. Similar observations apply for reports where red values of 0.7 were reported for physically refined RBO, but an even smaller 1-cm (=0.4 in.) cell was used [11]. It means that all those oils had a significantly darker color than a chemically refined RBO. Narayana et al. [9] mentioned the use of ethanolamines replacing the alkali refining, with diethanolamine resulting in a lighter color than triethanolamine. However they did not report the actual color values. Hwang et al. [10] reported a Lovibond red color value, after physical refining, measured with a 5.25-in. cell, as low as 3.6, but the effect on the oryzanol retention was not studied.

In this study, a modified physical refining process was established, consisting of an acid degumming, prebleaching, dewaxing, physical deacidification using packed column (PC) technology, an adapted washing step, conventional bleaching and finally deodorization. The influence of each step on the composition and color of the RBO is assessed. The choice of the optimal pretreatment is a trade-off between the desired color value and the maximal retention of valuable compounds. Particular attention is paid to the preservation of γ-oryzanol.

Materials and Methods

Physical Refining Processing Steps

Samples

The crude RBO was obtained from an industrial plant. Samples were taken after each stage of the modified physical refining process and refrigerated at 4 °C until analysis.

Acid Degumming

The crude oil was first heated to 85 °C and degummed using 30% H_3PO_4 solution thoroughly mixed to convert the

non-hydratable phospholipids to hydratable. Subsequently, a water-diluted base was also thoroughly mixed to neutralize the acid excess and to hydrate the phospholipids. Eventually the emulsion was centrifuged for 15 min at 2,000 g. The oil was then washed with water involving reheating of the oil to 85 °C, addition of 3 wt% water, thoroughly mixing, and finally centrifugation as described above.

Bleaching and Dewaxing

The degummed oil was next bleached as described in the AOCS official method Cc 8f-91 using 0.8% activated bleaching clay. Filter aid was added to the pre-bleached oil, and the oil was dewaxed by controlled cooling to 6 °C and desired holding time at this temperature, followed by membrane press filtration including cake squeezing to 6 bar.

Packed Column Stripping

The dewaxed oil was afterwards physically deacidified according to the procedure described by Vila Ayala et al. [20] under following constant conditions; 205 °C (highest temperature, achieved at the top of the stripping column), 2.5 mbar (highest pressure) together with a pressure drop of 1.25 mbar over the 2-m structured PC and 1.5% sparge steam injection.

Caustic Washing and Second Bleaching

The deacidified oil was then caustic washed at 90 °C by thorough mixing of a water-diluted base, different amounts of pure NaOH were used ranging from 0 to 0.65% for this step. The emulsion was centrifuged for 15 min at 2,000 g. The oil was subsequently washed with water as described above. The caustic-washed oil was next bleached as described above but using 3% of a 90/10 mixture of activated bleaching clay/active carbon.

Deodorization

Finally the bleached oil was deodorized as described by Petrauskaitè et al. [21] at 255 °C, 3 mbar, 1% sparge steam injection and a 60-min residence time.

Materials

Standards of cholesterol (99+%) and squalene were purchased from Sigma-Aldrich (Bornem, Belgium). All solvents and reagents were of analytical grade and purchased from Acros Organics (Geel, Belgium), VWR (Leuven, Belgium) and Sigma-Aldrich (Bornem, Belgium).

Analytical Methods

The FFA content using as indicator phenolphthalein and bromothymol blue, the fatty acid composition, the concentration of iron, phosphorus, calcium and magnesium, the determination of the color, the amount of diglycerides, the level of γ-oryzanol, the concentrations of the different tocopherol and tocotrienol isomers, the total sterols content and the squalene content were all determined as described by Van Hoed et al. [12]. The soap content was determined according to AOCS Official Method Cc 17-95, the chlorophyll content was analyzed according to AOCS Official Method Cc 13d-55 and the cold test at 0 °C was determined according to AOCS Official Method Cc 11-53. The β-carotene content was determined spectroscopically at 446 nm [22]. Reported results are means of triplicate analyses.

Results and Discussion

The physical refining process established in this study consisted of acid degumming, pre-bleaching, dewaxing, physical neutralization (FFA stripping on a PC), a caustic washing step and finally conventional bleaching and deodorization. After the PC treatment, six different routes were followed: five different conditions of caustic washing and one reference sample where no caustic was used (Fig. 1). A caustic washing was introduced into the process since it is known from industrial practice that this is an effective way to reduce the color. Only this step was different, while all other processing steps were performed using the same conditions for all samples (degumming, pre-bleaching, dewaxing, FFA stripping in PC, final bleaching and deodorization). The influence of each refining step on a number of quality parameters was studied (Tables 1, 2, 3, 4). The optimal concentration of NaOH in the caustic washing step will mainly depend on the desired color values and oryzanol retention.

FFA Content

As described earlier [12], because of its oryzanol content, the measured FFA value of RBO depends on the indicator used for its determination. Using phenolphthalein, the acidity of oryzanol is titrated as well, leading to an overestimation of the real FFA. This does not occur when bromothymol blue is used as the indicator. Both values are reported in Table 1. It is clear that the PC stripping reduced the FFA to about 10% of the original value (bromothymol blue). Caustic washing, irrespective to NaOH concentration (0.244–0.65%), reduced further the FFA content to 0.03–0.04%, and to 0.09% for the CW1 (0.122% NaOH). The final FFA content, after bleaching and deodorization, was

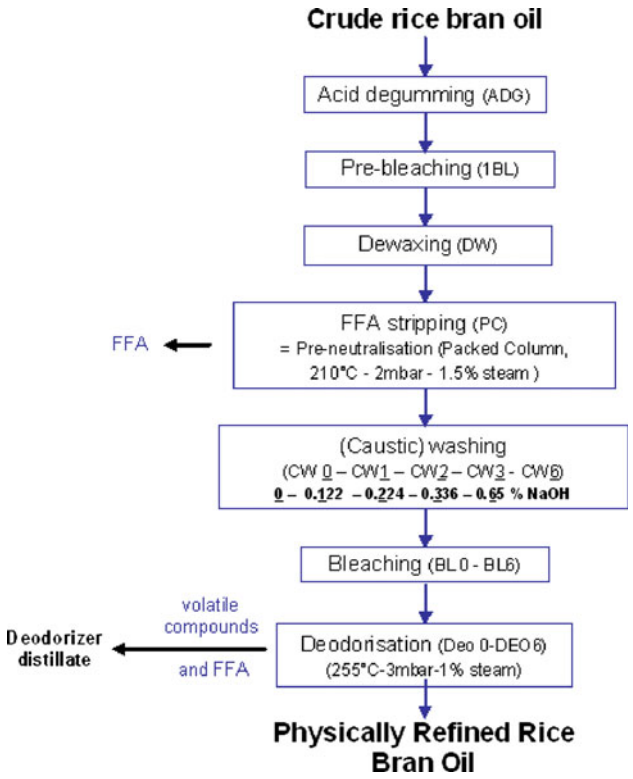

Fig. 1 Process flow chart: physical refining of RBO for light color and oryzanol retention

similar to chemically refined RBO, ranging from 0.02% for DEO6 to 0.1% for DEO1.

β-Carotene and Chlorophyll Content

As shown in Table 1, the crude oil contained 143.6 mg/kg of β-carotene and 51.9 mg/kg of chlorophyll, which are high values for crude RBO [23]. During acid degumming, 88% of the β-carotene was lost, while the chlorophyll content was unchanged. After the pre-bleaching, only 2.8 and 6.5 mg/kg respectively of both pigments was left. The slight increase of β-carotene during the dewaxing and PC treatment might be explained by the removal of waxes and FFA respectively, resulting in a net increase of other compounds in the oil. However, values are close to detection limit and therefore subject to relatively high variability. During the different caustic washing treatments a slight decrease was noticed for both pigments, more or less relative to the NaOH concentration, and after the second bleaching step they were practically completely removed.

Lovibond Color Values

The crude oil had Lovibond yellow and red values (Y/R) of 70/3, measured with the 1-in. cell. The first bleaching step, which removed an important part of the β-carotene and chlorophyll, did not have an important reducing effect on the Lovibond color values (Table 2). Manjula and Subramanian [16] pointed out that Maillard products are mainly responsible for the RBO color, rather than β-carotene and chlorophyll. The dewaxing reduced both color values by about 50%, suggesting that the main coloring compounds in the RBO were entrained during the dewaxing step. After the caustic washing step itself, no clear relationship between the increasing NaOH concentrations and the decreasing effect on the oil color values was observed. However, the difference was more obvious in the next steps: after the 2nd bleaching, the color values decreased with increasing NaOH concentration used in the previous caustic washing step. For the lowest NaOH concentration (0.122%, BL1) the color values (Y/R: 14/2) were identical to the sample which was bleached immediately after the FFA stripping (BL0). Increasing the NaOH concentration (BL2-3-5) gradually decreased the color values. In the fully refined RBO samples the effect of the caustic concentration was even more obvious: the Lovibond red value (for DEO samples measured with the 5.25-in. cell) decreased from 5.2 (DEO0) to 2.5 (DEO6). By using the highest NaOH concentration (0.650%), the red color value (2.5, DEO6) approached very close to the value (2.2) which can be obtained by chemical refining [12].

Trans Fatty Acids Formation

It is known that the formation of trans-fatty acids (*t*-FA) is specific to the deodorization step in vegetable oils refining [24], which was reported as well in the case of RBO refining [12]. The *t*-FA observed in the fully refined oils was limited to 0.7% (Table 1), which was similar to the value reported for chemically refined RBO (0.8%) [12].

Cold Test

All fully refined oils passed the cold test at 0 °C during the prescribed testing period of 5.5 h, making them suitable as salad oils.

Elemental Analysis

A prerequisite for the process of physical refining is a very low content of phosphatides prior to the physical deacidification. P-content has to be less than 15 mg/kg, preferably less than 10 mg/kg, and ideally less than 5 mg/kg [9]. As indicated in Table 2, the degumming step decreases the P-content to 6 mg/kg, which is reduced in the further steps to below 1 mg/kg, an excellent value for oils to be steam refined. From the P-content point of view, the degummed oil is already within the limits for steam refining. However further steps are needed to remove pigments, metals and

Table 1 Content of FFA, pigments, and trans fatty acids at different stages of the physical refining

Sample	Soaps (mg/kg)	FFA (as % oleic acid)		β-carotene (mg/kg)	Chlorophyll (mg/kg)	*t*-FA (%)
		Phen	Bromo			
Crude (CR)	nd	9.98 ± 0.01	8.6 ± 0.03	143.6 ± 2.6	51.9 ± 0.2	–
Acid degummed (ADG)	nd	9.63 ± 0.04	–	17.6 ± 0.3	52.6 ± 0.2	–
1st Bleached (1BL)	nd	10.55 ± 0.03	–	2.8 ± 0.1	6.5 ± 0.0	–
Dewaxed (DW)	nd	9.38 ± 0.05	7.76 ± 0.03	5.3 ± 0.2	7.3 ± 0.0	–
Packed column FFA stripped (PC)	210 ± 16	1.36 ± 0.12	0.72 ± 0.03	10.2 ± 0.3	4.6 ± 0.0	–
Caustic washed (CW) (% NaOH)						
CW1 (0.122)	350 ± 17	0.97 ± 0.12	0.09 ± 0.03	4.9 ± 0.2	3.3 ± 0.0	–
CW2 (0.244)	300 ± 10	0.61 ± 0.13	0.04 ± 0.04	5.5 ± 0.1	2.6 ± 0.0	–
CW3 (0.366)	575 ± 14	0.48 ± 0.16	0.04 ± 0.04	3 ± 0.2	0.2 ± 0.0	–
CW6 (0.650)	500 ± 17	0.14 ± 0.17	0.04 ± 0.04	–	–	–
Bleached (BL)						
BL0	nd	1.65 ± 0.11	0.66 ± 0.03	1.6 ± 0.2	0.06 ± 0.0	–
BL1	nd	0.95 ± 0.12	0.15 ± 0.01	6 ± 0.3	nd	–
BL2	nd	0.56 ± 0.14	0.11 ± 0.03	1 ± 0.3	nd	–
BL3	nd	0.58 ± 0.10	0.14 ± 0.04	1.1 ± 0.2	nd	–
BL6	nd	0.21 ± 0.10	0.11 ± 0.02	–	–	–
Deodorized (DEO)						
DEO0	–	0.21 ± 0.08	0.07 ± 0.01	3.4 ± 0.2	nd	0.6 ± 0.0
DEO1	–	0.8 ± 0.11	0.10 ± 0.03	2.2 ± 0.2	nd	0.5 ± 0.0
DEO2	–	0.43 ± 0.10	0.04 ± 0.02	1.2 ± 0.3	nd	0.6 ± 0.0
DEO3	–	0.33 ± 0.09	0.04 ± 0.01	1.1 ± 0.2	nd	0.6 ± 0.0
DEO6	–	0.12 ± 0.06	0.02 ± 0.05	–	–	–

Phen: FFA content measured with Phenolphthalein as indicator

Bromo: FFA content measured with Bromothymol Blue as indicator

– not measured, *nd* not detected

waxes in order to decrease the final color of the oil. The degumming removes most of the other measured elements Fe, Ca, Mg, Na, K as well, which are further reduced in the next steps to values below 0.05 mg/kg for the Fe and below 0.5 mg/kg for the other elements before the steam refining step. This is important as low concentrations of metals would substantially increase the chance of darkening of the oil upon heating during the deodorization [4].

Oil Losses

During caustic washing, the oil losses, relative to the stripped oil weight, increased with the NaOH concentration. Higher losses at higher NaOH dosage are due to the neutralization of more FFA, higher oryzanol losses and also to possible partial saponification of triacylglycerols at higher NaOH dosage.

Oryzanol, Phytosterol and Squalene Content (Table 3)

The total oryzanol content was measured spectrophotometrically. The processing steps before the caustic washing did not have much influence on the oryzanol content. However, depending on the NaOH concentration, the γ-oryzanol content dropped slightly (for CW1: to 2.01 mg/100 g) to drastically (CW6: 0.25 mg/100 g). After caustic washing with 0.655% NaOH (CW6), the residual γ-oryzanol was even lower than the reported residual content after neutralization in chemical refining (0.4 g/100 g) [12].

A hydrolysis of the γ-oryzanol into a free sterol and ferulic acid during caustic washing would lead to increased levels of free phytosterols in the oil. However, the results indicate that the γ-oryzanol was removed with the caustic washing water without being hydrolyzed. Pestana et al. [1] suggested that the gamma-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. This is reflected in the losses of total phytosterols, which are quantified after saponification, as the sum of free sterols, sterols esterified with fatty acids and sterols esterified with ferulic acid (γ-oryzanol). As reported elsewhere for chemical refining as well, the bleaching and deodorization step had only a small decreasing effect on the oryzanol and sterol content

Table 2 Content of elements and Lovibond color values at different stages of the physical refining

Sample	Elements (mg/kg)						Lovibond color 1 in.; DEO: 5.25 in.[a]	
	P	Fe	Ca	Mg	Na	K	Y	R
Crude (CR)	210.0 ± 1.3	4.4 ± 0.2	5.7 ± 0.1	33 ± 0.5	10.6 ± 1.1	110 ± 1.9	70.0 ± 2.1	6.0 ± 0.2
Acid degummed (ADG)	6 ± 0.1	0.3 ± 0.0	1 ± 0.1	1 ± 0.2	4 ± 0.4	3 ± 0.5	73.3 ± 2.0	6.0 ± 0.2
1st Bleached (1BL)	3.2 ± 0.1	0.4 ± 0.0	<0.5	<0.5	1 ± 0.2	2 ± 0.5	69.0 ± 2.0	6.9 ± 0.2
Dewaxed (DW)	1.7 ± 0.1	<0.5	1.5	<0.5	1.5 ± 0.2	0.5	35.0 ± 0.5	3.5 ± 0.2
Packed column FFA stripped (PC)	1.7 ± 0.1	0.2 ± 0.0	1.6 ± 0.1	1 ± 0.2	8 ± 0.8	1 ± 0.5	36.7 ± 0.5	4.1 ± 0.3
Caustic washed (CW) (% NaOH)								
CW1 (0.122)	<1	0.13 ± 0.0	6 ± 0.1	<0.5	15 ± 0.2	1 ± 0.5	[b]	[b]
CW2 (0.244)	<1	0.16 ± 0.0	7 ± 0.1	<0.5	11 ± 0.1	1 ± 0.5	[b]	[b]
CW3 (0.366)	<1	0.12 ± 0.0	2 ± 0.0	<0.5	36 ± 0.4	<0.5	[b]	[b]
CW6 (0.650)	<1	0.08 ± 0.0	<0.5	<0.5	33 ± 0.4	<0.5	[b]	[b]
Bleached (BL)								
BL0	<1	<0.05	<0.5	<0.5	<0.5	<0.5	14 ± 0.5	2.0 ± 0.1
BL1	<1	<0.05	<0.5	<0.5	<0.5	<0.5	14 ± 0.5	2.0 ± 0.1
BL2	<1	<0.05	<0.5	<0.5	<0.5	<0.5	11 ± 0.5	1.7 ± 0.1
BL3	<1	<0.05	<0.5	<0.5	<0.5	<0.5	11 ± 0.5	1.6 ± 0.1
BL6	<1	<0.05	<0.5	<0.5	<0.5	<0.5	9.6 ± 0.5	1.5 ± 0.1
Deodorized (DEO)								
DEO0	–	–	–	–	–	–	70 ± 2.0	5.2 ± 0.1
DEO1	–	–	–	–	–	–	70 ± 2.0	4.7 ± 0.1
DEO2	–	–	–	–	–	–	46 ± 2.0	3.0 ± 0.1
DEO3	–	–	–	–	–	–	46 ± 2.0	2.8 ± 0.1
DEO6	–	–	–	–	–	–	34 ± 1.7	2.5 ± 0.1

[a] Deodorized samples: Lovibond color value measured with a 5.25-in. cell, all other samples with a 1-in. cell

[b] No measurement was done as water interferes with the analysis

– not measured, *nd* not detected

[12]. Considering the relative levels of the individual phytosterols, important changes were observed only for 24-methylene cycloartanol, both due to isomerization of this sterol (as had been observed in chemical refining as well [12]) and due to losses, as it is the main sterolic component of γ-oryzanol, which was selectively lost in the alkaline washing water (Table 3).

As expected from its volatile nature, the squalene content mainly decreased during the FFA stripping in the PC treatment and the final deodorization. However after the caustic washing the squalene content was higher than after the preceding PC step. It seems improbable that the squalene would be lost or not detectable after the PC treatment, but return after the caustic washing step. Therefore, all of the squalene found in the caustic washed sample must have been present right after the PC treatment. Since storage conditions until analysis were identical for all samples (a few weeks, 4 °C, dark), the explanation must be found in the composition of the oil after PC treatment. Probably the presence of pro-oxidants, which are removed during caustic washing, were responsible for the lower stability of the oil after PC treatment. Further research is needed to confirm this hypothesis.

Tocopherols and Tocotrienols (Table 4)

As in many vegetable oils, the main tocopherol is γ-tocopherol, but RBO is relatively rich in tocotrienols as well, mainly γ-tocotrienol. The original tocol (tocopherol + tocotrienol) content of about 0.06 g/100 g was only slightly affected by the acid degumming, pre-bleaching, dewaxing and free fatty acids stripping step. The lower tocopherol content in the oil after the PC treatment might be caused by the same reason as its lower squalene content. The effect of the NaOH concentration is clearer: the bleached sample that was not caustic washed has the highest tocopherol content, followed by the 0.122, 0.244, 0.366 and 0.65% treated samples. Only in the latter, the decrease was highly significantly, reaching more than 50%. The deodorization step as such, had a minor influence on the tocols content in the case of the samples treated with no or lower NaOH concentration, while in the more strongly caustic washed samples (DEO3,

Table 3 Content of oryzanol, squalene and sterols at different stages of the physical refining of rice bran oil. Values are means of triplicate analyses

Sample	Oryzanol (g/100 g)	24-Methylene cycloartanol (g/100 g)	Total sterols (g/100 g)	Squalene (g/100 g)
Crude	2.11 ± 0.14	0.86 ± 0.01	3.54 ± 0.04	0.015 ± 0.001
Acid degummed	–	0.88 ± 0.01	3.63 ± 0.02	0.016 ± 0.001
1st Bleached	1.98 ± 0.12	0.83 ± 0.01	3.49 ± 0.03	0.018 ± 0.001
Dewaxed	2.00 ± 0.12	0.87 ± 0.02	3.59 ± 0.03	0.015 ± 0.000
Packed column FFA stripped	2.12 ± 0.13	0.90 ± 0.02	3.73 ± 0.05	0.008 ± 0.000
Caustic washed				
CW1 (0.112% NaOH)	2.01 ± 0.14	0.87 ± 0.03	3.62 ± 0.09	0.015 ± 0.000
CW2 (0.224% NaOH)	1.16 ± 0.08	–	–	–
CW3 (0.336% NaOH)	0.70 ± 0.04	–	–	–
CW6 (0.65% NaOH)	0.25 ± 0.03	0.45 ± 0.01	2.47 ± 0.05	0.015 ± 0.001
Bleached				
BL0	2.11 ± 0.15	0.64 ± 0.01	3.51 ± 0.01	0.014 ± 0.000
BL1	2.00 ± 0.12	0.54 ± 0.00	3.23 ± 0.01	0.009 ± 0.002
BL2	1.18 ± 0.11	0.40 ± 0.05	3.04 ± 0.07	0.014 ± 0.000
BL3	0.94 ± 0.11	0.39 ± 0.00	2.87 ± 0.02	0.015 ± 0.000
BL6	0.28 ± 0.04	0.38 ± 0.01	2.46 ± 0.05	0.016 ± 0.001
Deodorized				
DEO0	1.99 ± 0.09	0.66 ± 0.00	3.53 ± 0.03	0.007 ± 0.000
DEO1	1.91 ± 0.12	0.58 ± 0.00	3.38 ± 0.05	0.007 ± 0.000
DEO2	1.10 ± 0.09	0.40 ± 0.03	2.62 ± 0.08	0.003 ± 0.000
DEO3	0.83 ± 0.06	0.39 ± 0.01	2.52 ± 0.05	0.004 ± 0.000
DEO6	0.23 ± 0.04	0.36 ± 0.02	2.23 ± 0.05	0.004 ± 0.000

– not measured

Table 4 Changes in tocopherol and tocotrienol content and composition during the physical refining of rice bran oil

Sample	Total tocols (g/100 g)	α-tocopherol (%)	α-tocotrienol (%)	γ-tocopherol (%)	γ-tocotrienol (%)	δ-tocotrienol (%)
Crude	0.055 ± 0.006	8.22 ± 0.27	1.70 ± 0.19	23.95 ± 0.14	62.19 ± 1.23	3.95 ± 1.01
ADG	0.064 ± 0.000	8.57 ± 0.09	1.98 ± 0.07	23.12 ± 0.12	62.67 ± 0.39	3.65 ± 0.28
1st BL	0.065 ± 0.001	7.10 ± 0.14	1.91 ± 0.08	21.22 ± 0.22	65.57 ± 0.10	4.21 ± 0.10
Dewax	0.064 ± 0.001	6.59 ± 0.05	1.85 ± 0.17	21.51 ± 0.15	66.01 ± 0.27	4.05 ± 0.21
PC	0.042 ± 0.000	8.70 ± 0.09	5.59 ± 0.38	25.64 ± 0.70	58.82 ± 1.71	1.25 ± 0.23
CW1	0.062 ± 0.001	13.26 ± 0.11	4.95 ± 0.08	19.50 ± 0.10	62.30 ± 0.11	nd
CW6	0.053 ± 0.001	13.69 ± 0.26	4.22 ± 0.24	19.56 ± 0.18	62.06 ± 0.61	0.47 ± 0.12
BL0	0.070 ± 0.001	18.49 ± 0.13	4.45 ± 0.14	17.88 ± 0.05	59.19 ± 0.11	nd
BL1	0.057 ± 0.000	15.13 ± 0.08	4.42 ± 0.12	19.28 ± 0.40	61.17 ± 0.53	nd
BL2	0.059 ± 0.000	16.69 ± 0.06	4.61 ± 0.34	18.30 ± 0.05	60.39 ± 0.25	nd
BL3	0.048 ± 0.001	15.70 ± 0.16	5.19 ± 0.18	19.04 ± 0.15	60.07 ± 0.22	nd
BL6	0.024 ± 0.001	18.63 ± 0.07	6.23 ± 0.89	20.52 ± 2.25	53.72 ± 0.80	0.89 ± 0.10
DEO0	0.070 ± 0.001	19.42 ± 1.21	12.96 ± 0.17	18.51 ± 0.38	49.11 ± 0.24	nd
DEO1	0.059 ± 0.001	18.02 ± 0.31	16.87 ± 0.05	17.81 ± 0.12	47.30 ± 1.41	nd
DEO2	0.047 ± 0.001	17.86 ± 0.35	12.29 ± 0.18	17.45 ± 0.27	49.19 ± 0.24	3.20 ± 0.18
DEO3	0.039 ± 0.002	18.08 ± 1.86	4.51 ± 0.51	17.46 ± 1.08	57.95 ± 4.10	2.00 ± 0.27
DEO6	0.018 ± 0.001	25.29 ± 1.90	5.25 ± 0.36	20.39 ± 0.80	45.25 ± 2.33	3.81 ± 0.22

Values are means of triplicate analyses

CW caustic washed, *BL* bleached, *DEO* deodorized, *nd* not detected

DEO6), the decrease was more pronounced. This may be linked to the disappearance of a protective antioxidant effect of γ-oryzanol, which was lost as well, more severely in sample 3 and, the most in sample 5.

The tocols relative composition however clearly changed during the PC treatment, and subsequent steps. The main losses were noted for the γ-tocotrienol and γ-tocopherol, which are known as the main antioxidant isomers, thus protecting the oil against oxidation during storage. This is reflected in a relative increase in the α-tocopherol and α-tocotrienol content. From a nutritional point of view this is rather positive, as a higher vitamin E activity is attributed to the α-isomer.

Discussion

In the literature, no other refining process of RBO has been described, combining Lovibond red color values between 5.2 and 2.5 (5.25-in. cell) with an excellent γ-oryzanol retention. The strongest NaOH concentration (0.65%) for the caustic washing, did not fulfill the requirements, as it removed—during the refining process—about 90% of the original γ-oryzanol content from the crude oil. This is a similar loss as that during chemical refining, which includes the neutralization with a very high NaOH concentration. However, in the physical refining method developed in the current study, using 0.122% of NaOH in the washing step, a final red color value of 4.7 was reached, with 90% retention of oryzanol. With 0.244% NaOH, a red value of 3 and oryzanol retention of 52% can be obtained. In conclusion, by carefully adjusting the NaOH concentration and process conditions in the washing step of the proposed physical refining process, an oil of excellent color with 80–90% oryzanol retention can be produced.

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