A Novel Process for Physically Refining Rice Bran Oil Through Simultaneous Degumming and Dewaxing

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ABSTRACT: A new process for the physical refining of rice bran oil through combined degumming and dewaxing was developed on a laboratory scale and then demonstrated on a commercial scale. The simultaneous degumming and dewaxing of the crude oil with a solution of water and $CaCl_2$, followed by crystallization at a low temperature (20°C), facilitated precipitation of the hydratable and nonhydratable phosphatides along with the wax, which enabled its separation and reduction to a greater extent. Bleaching and subsequent winterization (20°C) of this oil further reduced the phosphorus content to less than 5 ppm. Thus, these pretreatment steps enabled the physically refined rice bran oil to meet commercially acceptable levels for color, FFA content, and cloud point values (10–12 Lovibond units in a 1-in. cell, <0.25%, and 4–5°C, respectively) with very low neutral oil loss; this has not been observed hitherto. Rice bran oil is known for its high levels of bioactive phytochemicals, such as oryzanol, tocols, and sterols. The process reported here could retain more than 80% of these micronutrients in the end product.

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KEY WORDS: Calcium chloride, physical refining, rice bran oil, simultaneous degumming and dewaxing.

Rice bran oil (RBO) is one of the most nutritious and healthful edible oils owing to the presence of an abundance of natural bioactive phytoceuticals such as oryzanol, tocopherols, tocotrienols, and sterols with a balanced FA composition. Tocopherols and tocotrienols (tocols) belong to the vitamin E family, which occurs in plants and vegetable oils as ring-position isomers. These compounds, particularly tocotrienols, prevent lipid peroxidation and play a key role in delaying the pathogenesis of a variety of degenerative diseases such as cardiovascular disease, cancer, inflammatory diseases, neurological disorders, cataracts, age-related macular degeneration, and immunomodulation (1). Crude rice bran oil (CRBO) contains relatively high levels of tocols (up to 0.2%), of which about 70% are tocotrienols. This is rare among edible oils; however, the tocol content is often reduced through defective processing and storage (2).

Another bioactive molecule present in RBO is oryzanol, whose content ranges from 1.1 to 2.6% (3). Oryzanol is a mixture of ferulic acid esters of triterpene alcohols and phytosterols. The individual components were previously identified as cycloartanyl ferulate, 24-methylene cycloartanyl ferulate, campesteryl ferulate, β-sitosteryl ferulate, and cycloartenyl ferulate (4). Some of the beneficial biological effects reported for oryzanol are the reduction of blood cholesterol, improvement of the capillary action of blood vessels, anti-aging effects, and anti-itching and anti-dandruff properties (5). RBO is also rich in phytosterols, which are potent hypocholesterolemic agents, with contents that vary from 1.0 to 3.0% (6). Thus, RBO can be termed a nutritional oil, and it is of paramount importance to exploit this unique edible oil to its fullest potential for the health benefits.

India is the second-largest producer of rice in the world, with an estimated annual production of 130 million metric tons (MMT) and about 10 MMT of rice bran, a by-product of the rice-milling industry. It is estimated that <0.5 MMT of CRBO is produced in India, compared with a potential of about 1.5 MMT. Current problems related to the logistics of stabilization, collection, and timely processing of rice bran for RBO could be attributed to the underutilization of bran. Rice bran reportedly contains a very active lipase that destabilizes the oil, resulting in a high FFA content (5–25%) within a short time after milling (7). Hence, the timely processing of rice bran is an important step. Commercial CRBO, which is extracted using hexane, contains 2–3% wax, 1–2% phosphatides, and 5–25% FFA in addition to pigments that are often degraded, leading to color fixation. From a commercial perspective, the quality of bulk CRBO is therefore unsuitable for refining for direct edible use. The nonexploitation of rice bran for edible use can thus be attributed to processing and quality problems. Only 10–20% of CRBO is actually refined for edible use in India, and the major portion is used for industrial applications.

Currently, CRBO is chemically and physically refined through phosphoric acid degumming. Chemical refining results in excessive neutral oil loss and the removal of oryzanol and other bioactive compounds (8). The conventional phosphoric acid treatment is unable to remove the phosphatides, leading to a dark-colored refined oil (9). Thus, the conventional process has been unsuccessful in delivering high-quality refined RBO or in preserving its nutritional benefits through an economically viable process.

Alternative approaches have been tried by some workers to overcome the refining problems of RBO. Solvent refining was found to be inefficient and also resulted in a high neutral oil

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loss and darkening of the oil (8). The re-esterification of FFA through enzyme-mediated biorefining and by chemical methods was unsuccessful because of its poor efficiency and for other economic reasons (10,11). Pretreatment of CRBO to remove the gum and wax by membrane technology has been reported as a promising method (12). However, more work is required to make it economically viable. Considering the composition of the commercially available CRBO at present, only physical refining may be suitable for RBO from the perspectives of economics and nutrition. Oil containing low amounts of phosphatides must be physically refined to avoid darkening (13,14).

Recently, novel approaches to physical refining have been developed in parallel, leading to a commercially viable physical refining process for RBO. Enzymatic degummming using phospholipase is reported to be successful in reducing the phosphorus content to the level of 5 ppm (15), but its economic viability has yet to be established. This paper deals with the novel approach of simultaneous degumming and dewaxing using a $CaCl_2$ treatment followed by controlled two-stage crystallization and separation of the gum and wax together to reach the phosphorus content of <5 ppm and wax content ≤50 ppm required for physical refining. The process was developed on a pilot scale and transferred to an industrial scale. The results presented in this paper include data from the laboratory-scale and commercial-scale operations.

MATERIALS AND METHODS

Raw materials and chemicals. Raw RBO for laboratory-scale refining was procured from M/s Mahabir Vegetable Oils Ltd. (Karnal, Haryana, India). Industrially processed oil samples were obtained from commercial factories (M/s Mahabir Vegetable Oils Ltd. and M/s Ammi Reddi Oils Ltd., Anaparthy, Andhra Pradesh, India). Commercially refined market samples of RBO were obtained from local markets. All chemicals and reagents were of analytical grade. Tocopherol, tocotrienol, β-sitosterol, campesterol, stigmasterol, and methyl heptadecanoate standards and lipid standards were obtained from Sigma (St. Louis, MO).

Refining method: *laboratory trials. (i) Simultaneous degumming and dewaxing.* A 500-g quantity of CRBO was added to a 1,000-mL beaker and heated to 75°C on a heating mantle with a magnetic stirrer, then filtered through ordinary filter paper using a Büchner funnel. Water (2% vol/wt) was added to the oil and stirred at 200 rpm for 30 min using the magnetic stirrer, with the heating mantle set at 75°C. To this water–oil mixture was added 1% (vol/wt) of $CaCl_2$ solution (6% wt/vol), with continued stirring at 200 rpm at a temperature of 75°C for 30 min. The mixture thus obtained was subjected to crystallization by cooling from 75 to 20°C at a rate of 0.4°C/min using chilled water. After cooling, gum and wax from the oil mixture were separated by centrifuging the mass in a cooling centrifuge (Remi C-30) at 7,500 × *g* for 20 min. The supernatant layer was then decanted.

(ii) Bleaching. The oil obtained after a single step of degumming and dewaxing was placed in a 1,000-mL round-bottomed flask, 0.25% (vol/wt) of aqueous citric acid solution (25% wt/vol) was added to the oil, and the mixture was dried under 3 mmHg pressure while heating the oil to 95°C. To this preheated oil, 4% (w/w) of fuller's earth and 0.4% (w/w) of activated charcoal were added and heated to 105°C under 3 mmHg pressure for 20 min. Bleaching was carried out by maintaining the temperature of the oil at 105°C while stirring with the magnetic stirrer. The bleached oil was then filtered under suction through a Büchner funnel.

(iii) Winterization. The bleached oil thus obtained was then cooled to 20°C in a time- and temperature-programmable crystallizing unit (REPROC 3085) and was maintained at that temperature for 24 h. The crystallized wax and gum were removed by vacuum filtration using a Büchner funnel.

(iv) Deodorization/deacidification. The winterized oil was placed in a 1,000-mL three-necked round-bottomed flask. A thermometer was inserted in the flask through one neck and the second neck of the flask was fitted with a bent adaptor, which was connected to a water condenser/distillate receiver (500-mL round-bottomed flask fitted with a bent vacuum adaptor) and vacuum pump in series. The steam produced by heating the water that had been placed in the round-bottomed flask (1000 mL) was passed through the other neck of the flask. The oil was heated on a sand bath to 180–200°C for 30 min under 3 mmHg pressure for deodorization. The temperature of the oil was then raised from 200 to 250°C for 60 min under 3 mmHg pressure for deacidification.

These operations were also conducted at a 10-kg level using a programmed crystallizer and reactor with process controls.

Refining method: commercial trials. Commercial trials were conducted at two separate edible oil refineries, one located in northern India (M/s Mahabir Vegetable Oils Ltd.) and the other in southern India (M/s Ammi Reddi Oils Ltd.). These refineries were designed for 100 metric ton/d of RBO processing. The crucial step of this process, the combined degumming and dewaxing, was conducted using a continuous mixing and holding system followed by crystallization in a crystallizer of 15 metric ton/batch capacity with a temperature program. After crystallization of the gum and wax (from 75 to 20°C at rate of 0.4°C/min), the oil with gum and wax was subjected to continuous desludging centrifugation. The desludged RBO was bleached under standard conditions (105°C for 20 min) using 2–4% (w/w) bleaching earth and 10% activated charcoal on bleaching earth weight. Before bleaching, the oil was dosed with 0.25% (vol/wt) citric acid solution (25%, wt/vol). The bleached oil thus obtained was separated using a standard filter press and was then subjected to winterization by holding the oil for 24 h at 20°C. The residual wax and phosphatides present in the winterized oil were filtered at 20°C by using a standard filter press. Subsequently, the RBO was subjected to deacidification and deodorization at 240–250°C at 1 Torr vacuum in a continuous deodorizer as practiced in the industry. Finally, the oil was passed through a polishing filter. Oil samples were collected for analysis at each step. Three trials were conducted at each refinery. A flow chart of the refining process is shown in Figure 1.

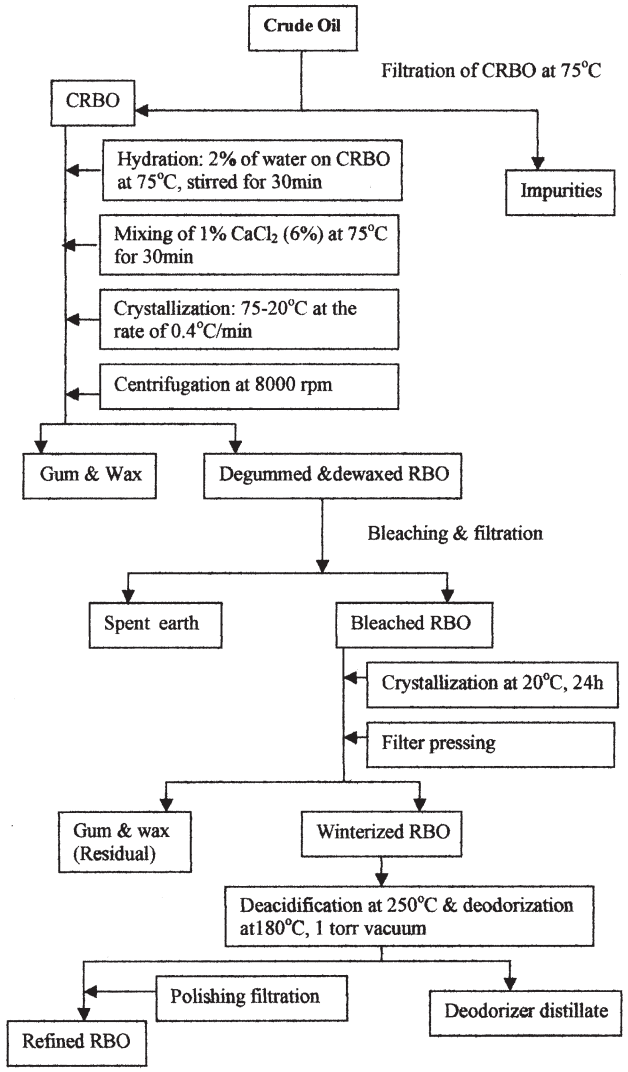

FIG. 1. Flow sheet for the physical refining of rice bran oil (RBO) through a simultaneous degumming and dewaxing process. CRBO, crude RBO.

Market samples. Local market samples of refined RBO were chemically refined, although no further details were available from the processors.

Physicochemical characteristics. Physicochemical characteristics such as FFA, phosphorus content, moisture and volatile matter, iodine value, saponification value, specific gravity, and refractive index were determined according to standard AOCS methods (16). The colors of the crude and processed oil samples were determined with a Lovibond tintometer (16).

The total gum and wax content was estimated as the percentage of acetone-insoluble matter in the oil (9).

Estimation of phytonutrients. (i) Tocopherols and tocotrienols (tocols). Tocols were determined by using an HPLC system equipped with an LC-10AD pump (Type 7125; Rheodyne, Cotati, CA), 20-µL sample loop, UV-vis detector (Shimadzu Corporation, Kyoto, Japan), and a Phenomenex NH_2 column (4 mm i.d. × 25 cm length; Phenomenex, Kyoto, Japan). The solvent system *n*-hexane/isopropanol (92:8 vol/vol), at a flow rate of 1 mL/min, was used for the separation of tocols. The detector was set at 297 nm and oil sample solutions prepared in *n*-hexane were injected for analysis (17).

(ii) Oryzanol. Oryzanol content was determined by using the same HPLC system. A Microbondapack C_{18} column (4 mm i.d. × 25 cm length; Waters, Milford, MA) filled with 5-µm particles was used in the reversed phase. The solvent systems acetonitrile/dichloromethane/acetic acid (22:1.5:1.5) and methanol/*n*-butanol/water (9:0.2:0.8) in a ratio of 75:25 (vol/vol), at a flow rate of 1 mL/min, were used for the separation of oryzanol isomers. Analysis was carried out at 325 nm. Twenty-milligram oil samples dissolved in 1:4.5:4.5 dichloromethane/acetonitrile/methanol were used for the analysis (18).

(iii) Sterols. Sterols and the sterol composition of RBO at different stages of refining were determined according to the HPLC method of Holen (19). The estimation was carried out with the same HPLC system used to estimate oryzanol and tocol contents. A Zorbax C_{18} reversed-phase column (4 mm i.d. × 25 cm length; Agilent Technologies, Palo Alto, CA) filled with 5-µm particles was used for the analysis. The solvent system MeOH/H_2O in a ratio of 96.5:3.5 (vol/vol) at a flow rate of 1.2 mL/min was used for the separation of sterol isomers. Peaks were detected at 206 nm. Calibrations of standard curves were done by using standard sterol solutions in the range of 1–20 µg/20 µL. For sample analysis, the oil was first saponified according to the standard AOCS method, and the unsaponifiable matter thus obtained was dissolved in 9:1 MeOH/$CHCl_3$ and injected into the high-performance liquid chromatograph for analysis (19).

Estimation of lipid classes and FA. Total lipids from each stage of refining were separated into lipid classes and estimated by GLC (20). The lipid classes TG, DG, MG, FFA, phospholipids (PL), and glycolipids (GL) were separated on a TLC plate (20 × 20 cm) using the solvent system *n*-hexane/diethyl ether/acetic acid (8:2:0.1 by vol). The separated bands were visualized by placing the plate in an iodine chamber. Lipid bands were identified with respect to the R_f values of lipid standards. Neutral lipids were eluted with chloroform. Polar lipids were first eluted with acetone to obtain GL, followed by methanol to extract PL. FAME of the separated lipid classes were prepared according to an IUPAC method (21) from samples at each stage of refining. Methylheptadecanoate was used as the internal standard. FAME were analyzed using a Hewlett-Packard 5890 series II model gas chromatograph equipped with FID. An HP-FFA capillary column (cross-linked FFAP; 30 m × 0.5 mm × 1 µm; Hewlett-Packard, Avondale, PA) was used for the separation of FAME. Injector and detector port temperatures were 250 and 300°C, respectively. The column temperature was maintained at 100°C for 1 min and increased to 180°C at the rate of 5°C/min, then maintained at that temperature for 15 min. Nitrogen at a flow rate of 20 mL/min was used as a carrier gas with air flow at the rate of 280 mL/min. FA were identified using standards. The amount of lipid sample was determined by relating the total area of the FAME peak to the peak

of the internal standard. Correction factors for each lipid class were calculated by dividing the M.W. of the heptadecanoic acid derivative of the respective lipid class by that of methylheptadecanoate (22).

Estimation of trace metals. The trace metals Cu, Fe, Zn, Mn, Ca, and Mg were determined by using an atomic absorption spectrometer (model 3100; PerkinElmer, Shelton, CT).

RESULTS AND DISCUSSION

Process development on a laboratory scale and a pilot scale. Details of the apparatus used for laboratory- and pilot-scale trials are presented in the Materials and Methods section. Hydratable phosphatides were precipitated using water at a 1, 2, or 3% (vol/wt) level on oil weight while stirring at a temperature range of 65–85°C for 30 min. Subsequently, aqueous $CaCl_2$ with concentrations ranging from 2 to 10% was added at a 1, 2, or 3% (vol/wt) level on oil. The addition was done gradually while stirring. The reaction mixture was held for 30 min after each experiment at the reaction temperature while stirring at 200 rpm. A white suspension was visible under these experimental conditions. However, the precipitate thus obtained was not in a state that could be separated. Each experiment described in the previous section was subsequently subjected to a controlled crystallization process. Crystallization was conducted at an initial temperature of 75°C at a cooling rate of 0.4°C/min. At the end of the crystallization period, the crystallized mass was separated using a cold centrifuge at $7{,}500 \times g$. The supernatant oil was decanted and taken for detailed analysis. Detailed analytical data are given in Tables 1–6. The maximum separation of phosphatides and wax, i.e., about 90–95% phophatides and 79–81% wax, was obtained at this stage under the optimal conditions of 2% (vol/wt) water on oil and 1% (vol/wt) $CaCl_2$ (6%, wt/vol) on oil at a cooling rate of 0.4°C/min from 75–20°C. We also observed that the separation of phosphatides after the initial hydration followed by crystallization in the absence of $CaCl_2$ resulted in less crystallization of the phosphatides and wax. $CaCl_2$ has been reported as one of the most effective degumming (23) and dewaxing agents (24). The addition of $CaCl_2$, followed by crystallization, facilitated the combined crystallization of gum and wax.

Physicochemical characteristics. Samples from each stage of refining were preserved for analysis. Samples from the commercial trials described in the Materials and Methods section were also analyzed; data on the samples from M/s Mahabir Vegetable Oils Ltd. are presented here. The physicochemical characteristics of crude, degummed/dewaxed, bleached, winterized, and refined (RRBO) RBO processed on a laboratory scale and an industrial scale, and that of market samples (RRBO) are given in Table 1. The results obtained show that single-step degumming and dewaxing of CRBO reduced the phosphorus content from 500–700 to 25–30 ppm and the total gum and wax content from 4–5 to 0.5–0.6%. That is, a 90–95% reduction of phosphorus content and a 79–81% reduction of wax content were observed by this simultaneous degumming/dewaxing method using a $CaCl_2$ solution followed by crystallization, which ensured the effective separation of gum and wax at the preprocessing stage.

De and Bhattacharyya (9) suggested that single-step degumming/dewaxing at a low temperature is a better approach than the conventional two-stage process of degumming and dewaxing (9). They were successful in reducing the total gum and wax contents of low-FFA oil (4%) from 3.8 to 0.48% after a combined water degumming/dewaxing (10°C), bleaching, and physical refining process. Bhattacharya and Bhattacharya (23) tried various degumming agents such as an organic acid, an inorganic acid, and an inorganic salt to degum CRBO, and these degumming agents reduced the phosphorus content of CRBO to 52–72 ppm (23). The degumming and dewaxing carried out with an inorganic acid required further neutralization and water

TABLE 1
Physicochemical Characteristics of Rice Bran Oil (RBO) at Different Stages of Processing on a Laboratory Scale and an Industrial Scale and of Market Refined RBO (RRBO) Samples

Samples		Phosphorus[a] (ppm)	Gum and wax[a] (% w/w)	PL[a] (% w/w)	Wax[a] (% w/w)	FFA[a] (% w/w)	Color (1-in. cell)	Moisture[a] (% w/w)	Cloud point (°C)
Laboratory scale	CRBO	675.1 ± 19.9	4.41 ± 0.15	1.69 ± 0.05	2.72 ± 0.10	7.85 ± 0.04	83.2	0.34 ± 0.08	
	DRBO	25.5 ± 1.6	0.63 ± 0.03	0.06 ± 0.01	0.57 ± 0.03	7.74 ± 0.08	56.2	0.53 ± 0.05	
	BRBO	5.7 ± 0.7	0.44 ± 0.05	0.01 ± 0.00	0.43 ± 0.05	7.88 ± 0.05	30.2	0.13 ± 0.01	
	WRBO	5.4 ± 0.6	0.02 ± 0.00	0.01 ± 0.00	<0.005	7.28 ± 0.04	13.9	0.15 ± 0.02	
	RRBO	3.3 ± 0.3	0.01 ± 0.00	<0.01	<0.005	0.23 ± 0.02	10.0	0.01 ± 0	4
Industrial scale	CRBO	509.7 ± 10.0	4.66 ± 0.09	1.27 ± 0.03	3.39 ± 0.12	4.56 ± 0.04	85.9	0.25 ± 0.04	
	DRBO	30.2 ± 2.2	0.72 ± 0.11	0.08 ± 0.01	0.64 ± 0.10	4.63 ± 0.02	72.2	0.39 ± 0.01	
	BRBO	7.8 ± 0.5	0.48 ± 0.05	0.02 ± 0.00	0.46 ± 0.05	4.62 ± 0.02	45.7	0.09 ± 0.01	
	WRBO	3.6 ± 0.2	0.02 ± 0.00	<0.01	<0.005	4.61 ± 0.05	14.8	0.04 ± <0.01	
	RRBO	3.5 ± 0.06	0.01 ± 0.00	<0.01	<0.005	0.24 ± 0.01	12.0	0.02 ± <0.01	5
Market samples of RRBO	I	1.9 ± 0.50	<0.01	<0.005	<0.005	0.22 ± 0.01	12.1	—	5
	II	0.8 ± 0.2	0.24 ± 0.03	<0.005	0.24 ± 0.03	0.23 ± 0.02	12.8	—	7
	III	4.7 ± 0.3	0.27 ± 0.05	<0.01	0.26 ± 0.05	0.24 ± 0.02	12.4	—	8

[a]Mean values of four replications ± SD. PL, phospholipids; CRBO, crude RBO; DRBO, degummed and dewaxed RBO; BRBO, bleached RBO; WRBO, winterized RBO. A dash (—) indicates "trace".

washing (9,14), which resulted in a loss of neutral oil and the formation of soap. The degumming agent $CaCl_2$ reduced the phosphorus content of CRBO to 56 ppm after degumming (23). Divalent calcium ions in the presence of water are known to cause the thermotropic phase transfer of lecithins, and water molecules form liposomes with hydratable PL, which facilitates crystallization and their subsequent separation from oil on cooling (14). Degumming and dewaxing the RBO without passing it through a cooling cycle was not effective.

Bleaching of the combined degummed/dewaxed oil obtained by the present method with activated charcoal and fuller's earth further reduced the phosphorus content from 25–30 to <10 ppm. Removal of PL at the bleaching step could be attributed to physical adsorption of these components on the bleaching earth (13). Winterization of the bleached oil reduced the phosphorus content of the oil to <5 ppm and the color value to <15 Lovibond units (1-in. cell). The FFA content of RBO showed no variation up to the winterization step. Deacidification of the winterized oil as practiced in edible oil refining (250°C) was effective in reducing the FFA to the acceptable level. After physical refining, the color of CRBO (83–85 Lovibond units in a 1-in. cell) decreased to 10–12 Lovibond units (1-in. cell). The results obtained showed that the laboratory results were comparable with those of the industrial trials. The results further established that the neutral oil loss by this method was about 1.7 to 2.0 times the gum and wax content, including loss at the bleaching and deacidification steps, which is far less than that of conventional chemical and physical refining methods. The cloud point for oil refined by the present method on a laboratory and industrial scale was low (4–5°C) when compared with that of the chemically refined market samples (5–8°C). The other parameters, such as FFA content and color value, were comparable with those of market samples. The physicochemical characteristics were identical for both laboratory- and industrial-scale operations and established the commercial feasibility of this novel approach to RBO refining.

Tocols, oryzanol, and phytosterols. The micronutrient content (tocopherols/tocotrienols and oryzanol) of the oil samples from different stages of processing on a laboratory and industrial scale and that of market samples are shown in Table 2. The results showed the presence of major vitamin E isomers such as α-tocopherol, α-tocotrienol, γ-tocopherol, γ-tocotrienol, and δ-tocotrienol. The most abundant of these was γ-tocotrienol, followed by α-tocotrienol and α-tocopherol, with other isomers in smaller proportions. The tocol content decreased during laboratory- and industrial-scale operations. The most significant reduction (12 to 13%) occurred at the bleaching step, which may be due to the preferential adsorption of these components on bleaching material. Further reduction (6–9%) of the tocol content in the physical refining step resulted from the distillation of tocols at a high temperature (250°C) under vacuum (25). Thus, about 80% of the tocols were retained in the final product, and no preferential loss was observed for any of the isomers (25). The tocol content of market samples was far lower than those in the refined oils processed on a laboratory and industrial scale.

Results for oryzanol content, as influenced by processing, are also presented in Table 2. Cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, campesteryl ferulate, β-sitosteryl, and cycloartanyl ferulate were the major ferulate esters identified in RBO using the HPLC technique and the oryzanol standard. These values showed that 24-methylene cycloartanyl ferulate and campesteryl ferulate were the major ferulate esters among the five components of oryzanol; they constituted more than 70% of the total oryzanols. The concentration distribution of the components of oryzanol in the CRBO and in the degummed/dewaxed oil followed the order 24-methylene cycloartanyl ferulate > campesteryl ferulate > cycloartenyl ferulate, β-sitosteryl, and cycloartanyl ferulate. This order changed to campesteryl ferulate > 24-methylene cycloartanyl ferulate > cycloartenyl ferulate, β-sitosteryl, and cycloartanyl ferulate in the bleaching step of all samples (processed on both a laboratory

TABLE 2
Tocopherols, Tocotrienols, and Oryzanol Contents of RBO at Different Stages of Processing on a Laboratory Scale and an Industrial Scale and Market Samples

		Tocopherols/tocotrienols[a] (ppm)							Oryzanol[a] (%)				
Samples		αT	αT_3	βT	γT	γT_3	δT_3	Total	CF	MCF	CMF	BSCF	Total
Laboratory scale	CRBO	129	170	5	73	628	47	1052	0.18	0.60	0.54	0.17	1.49
	DRBO	120	166	4	69	621	42	1022	0.22	0.59	0.50	0.19	1.50
	BRBO	94	154	4	55	581	36	924	0.21	0.31	0.69	0.20	1.41
	WRBO	101	157	4	66	588	42	958	0.21	0.33	0.69	0.19	1.42
	RRBO	88	126	—	60	546	23	843	0.20	0.31	0.66	0.19	1.36
Industrial scale	CRBO	126	174	4	74	610	50	1038	0.21	0.62	0.51	0.25	1.59
	DRBO	119	160	3	70	601	47	1000	0.22	0.61	0.52	0.26	1.61
	BRBO	98	148	—	58	561	36	901	0.20	0.39	0.71	0.21	1.51
	WRBO	102	153	—	61	566	40	922	0.21	0.39	0.72	0.21	1.53
	RRBO	86	126	—	58	547	24	841	0.20	0.37	0.70	0.20	1.47
Market	I	25	94	—	75	273	38	505	0.08	0.09	0.13	0.04	0.34
samples	II	48	33	—	99	495	21	696	0.08	0.03	0.01	0.01	0.13
of RRBO	III	42	31	—	102	442	26	643	0.15	0.23	0.35	0.10	0.83

[a]Means of four replications. T, tocopherol; T_3, tocotrienol, CF, cycloartenyl ferulate; MCF, 24-methylene cycloartanyl ferulate; CMF, campesteryl ferulate; BSCF, β-sitosteryl and cycloartanyl ferulate; for other abbreviations see Table 1. A dash (—) indicates "trace".

TABLE 3
Phytosterols[a] (%) of RBO at Different Stages of Processing on a Laboratory Scale

Sample	β-Sitosterol	Campesterol	Stigmasterol	Total
CRBO	0.76	0.55	0.47	1.78
DRBO	0.77	0.56	0.48	1.81
BRBO	0.75	0.54	0.45	1.74
WRBO	0.74	0.54	0.45	1.73
RRBO	0.73	0.52	0.44	1.69

[a]Values are averages of four determinations. For abbreviations see Table 1.

and an industrial scale). Since the antioxidant property of oryzanol is mainly due to the ferulate moiety, this change may not affect the nutritional value of the oil. Even though the relative composition of these individual components changed, about 90% of the oryzanol present in the CRBO was retained in the refined oil processed by the present method on a laboratory and industrial scale. A significant reduction of oryzanol in the bleaching step (5–6%) and a slight reduction in the deacidification step (2–3%) were observed. The relative composition of individual components of oryzanol in market samples also showed some variation. In RRBO samples I and III, the concentration distribution order was campesteryl ferulate > 24-methylene cycloartanyl ferulate > cycloartenyl ferulate > β-sitosteryl and cycloartanyl ferulate, and in RRBO II the order was cycloartanyl ferulate > 24-methylene cycloartanyl ferulate > campesteryl ferulate > β-sitosteryl and cycloartanyl ferulate. Oryzanols were the most affected by chemical refining, and the very low levels of oryzanols in the market samples indicated that they were alkali refined.

The phytosterols present in RBO, as analyzed and identified by HPLC and as affected by the refining process, are presented in Table 3. Three major phytosterols were identified; their relative proportions were 42.7, 30.9, and 26.4% for β-sitosterol, campesterol, and stigmasterol, respectively. Retention of phytosterols in the final RRBO product was about 95% by this process. A slight reduction of phytosterols occurred only in the bleaching and deacidification steps. The phytosterol content of RBO, as affected by refining, has not been reported previously. However, refining studies on soybean and corn oils reported by other workers have indicated that up to 95% of phytosterols can be retained (25,26).

The effect of refining on total oryzanol and tocols also was studied by a few workers (9,27). De and Bhattacharyya (9) obtained a retention of >90% oryzanol and 34% tocols by a physical-refining method. Yoon and Kim (27) reported far lower values for oryzanol (49%) and tocols (66%) in RBO refined by a chemical method. The physical refining process reported here, however, was found to retain about 80% tocols, 90% oryzanol, and 95% sterols. Further, for the first time we report a comprehensive refining study that includes a detailed characterization of all the micronutrients present in RBO as influenced by refining on a laboratory and commercial scale.

Lipid classes and FA composition. High levels of FA and PL of the commercial CRBO and consequent alteration in the lipid classes and the distribution of FFA at various stages of processing were studied here. Changes in the glyceride content during refining are important in terms of the final lipid profile of the refined oil. The lipid class composition at each stage of RBO processing is shown in Table 4. The water and $CaCl_2$ treatment of oil in this simultaneous degumming/dewaxing process removed 92% of the PL, 50% of the GL, and 56% of the MG, which resulted in increases in TG (1%) and DG (3%). Further reductions of 83% PL, 58% DG, 20% GL, and 30% MG were observed at the bleaching step. This may be due to the adsorption of these components on the bleaching earth. As explained in the preceding discussion, no significant reduction in the FFA content was observed up to the winterization step. Deacidification of oil at a high temperature and low pressure removed low-M.W. components such as FFA and MG by distillation. Consequently, the TG content increased to 93.7% in the RRBO. As a result, with respect to crude oil, total reductions of 96.8% of the FFA, 38.5% of the DG, 87.4% of the MG, 99.3% of the PL, and 79.3% of the GL were observed in the refined oil obtained by this process.

The FA composition of RBO from each refining step and of the separated lipid classes is presented in Table 5. No significant variation was observed in the FA composition between CRBO and RRBO. The FA 18:1, 18:0, and 16:0 were the major acids present in RBO used here; 80% of the acids were unsaturated and 20% were saturated. This 80:20 unsaturated/saturated ratio was retained in the oil samples from all processing steps. The highly unsaturated FA present in RBO, particularly 18:2, could make the oil prone to oxidation. However, the excellent oxidative stability observed for RBO could be attributed to the high levels of tocols, oryzanol, and sterols (27). The FA profile of lipid classes also was found to be similar to that of total lipids, with deviations in the relative percentage compositions (Table 5). The relative abundance of FA for each lipid class could be summarized as follows: For TL, 18:1 > 18:2 > 16:0; for TG, 18:1 > 18:2 > 16:0; for FFA, 18:1 > 18:2 > 16:0; for DG, 18:1 > 18:2 > 16:0; for MG, 16:0 > 18:1 > 18:2; for GL, 18:1 > 18:2 > 16:0; for PL, 16:0 > 18:1 > 18:2. During process-

TABLE 4
Effect on the Lipid Classes[a] (wt%) of RBO of Processing on a Laboratory Scale

Sample	TG	FFA	DG	MG	PL	GL
CRBO	76.07 ± 1.50	8.01 ± 0.50	5.98 ± 0.60	2.37 ± 0.10	1.36 ± 0.08	2.17 ± 0.20
DRBO	77.08 ± 1.30	7.21 ± 0.20	9.06 ± 0.70	1.05 ± 0.10	0.12 ± 0.02	1.08 ± 0.10
BRBO	83.43 ± 2.00	7.11 ± 0.40	3.78 ± 0.20	0.74 ± 0.09	0.02 ± 0.01	0.86 ± 0.20
RRBO	93.76 ± 1.00	0.26 ± 0.10	3.68 ± 0.09	0.30 ± 0.04	0.01 ± 0.005	0.45 ± 0.10

[a]Mean of four replications ± SD. GL, glycolipids; for other abbreviations see Table 1.

TABLE 5
FA Composition[a] (wt%) of Total Lipids (TL) and Lipid Classes of RBO Processed on a Laboratory Scale

Sample	Lipid class	12:0	14:0	16:0	18:0	18: 1	18: 2	18: 3	20:0
CRBO	TL	—	Trace	19.35	1.39	45.19	33.57	0.49	Trace
	TG	0.50	1.00	15.17	1.84	47.36	32.93	0.46	Trace
	FFA	0.32	0.93	23.69	2.49	40.77	30.17	0.54	Trace
	DG	Trace	1.02	26.24	2.05	42.62	27.51	0.51	Trace
	MG	1.72	1.87	38.94	2.80	31.81	20.87	0.66	1.26
	GL	2.11	2.16	26.58	3.35	35.12	29.82	0.92	Trace
	PL	2.60	2.32	39.56	2.09	26.88	25.35	Trace	Trace
DRBO	TL	—	Trace	18.90	1.49	46.05	33.17	0.42	Trace
	TG	0.22	1.01	13.32	2.01	48.58	33.52	0.51	Trace
	FFA	0.82	1.06	25.65	3.55	41.58	26.11	0.45	Trace
	DG	Trace	1.33	27.41	2.61	46.40	30.93	0.29	Trace
	MG	1.41	2.27	38.25	5.45	33.13	15.56	Trace	3.94
	GL	2.28	1.00	24.49	4.88	36.45	30.22	0.56	Trace
	PL	Trace	Trace	38.58	3.93	31.46	24.13	Trace	1.89
BRBO	TL	—	Trace	18.32	1.42	45.32	33.69	0.87	Trace
	TG	0.22	0.91	12.90	2.00	52.74	32.58	0.67	Trace
	FFA	1.51	1.59	23.83	3.24	40.45	28.69	0.70	Trace
	DG	Trace	0.84	26.06	2.20	41.54	28.01	0.65	0.74
	MG	1.06	5.10	38.53	7.28	27.85	16.47	Trace	3.71
	GL	2.43	2.21	21.07	6.54	38.20	29.61	Trace	Trace
	PL	2.03	2.78	38.79	5.55	28.44	20.50	Trace	2.00
RRBO	TL	—	Trace	18.66	1.54	47.57	32.00	0.19	Trace
	TG	—	0.63	13.15	3.00	54.80	31.25	0.47	Trace
	FFA	—	—	12.01	5.55	39.53	39.95	0.67	0.84
	DG	—	Trace	26.34	2.75	42.01	28.90	Trace	Trace
	MG	—	—	36.08	6.61	33.51	20.61	Trace	3.19
	GL	Trace	2.52	22.77	5.42	35.57	28.76	Trace	3.31
	PL	—	—	39.53	5.30	28.77	23.59	Trace	2.82

[a]All values are averages of four determinations. A dash (—) indicates "not detected." For abbreviations see Tables 1 and 4.

ing, no significant change in this order was observed for the oil samples from different processing steps.

Trace metal content. The amounts of trace metals in each stage of refining are shown in Table 6. These values indicate that the amounts of metals decreased significantly at the bleaching, winterization, and deacidification steps. The metals Ca and Mg were present along with PL such as PA and PE (nonhydratable phosphatides). Reduction in the amounts of these metals indicated the removal of nonhydratable PL. Metals such as Cu and Fe are known to catalyze the oxidation of oil, leading to rancidity. Removal of these pro-oxidants also is a purpose of refining. The steps optimized in the present process were capable of reducing the pro-oxidant metals such as Cu and Fe to levels that could not affect the oxidative stability of RRBO.

Quality parameters. The oils obtained after refining (RRBO) were subjected to a quality assessment to establish whether they met the legal quality specifications for refined edible oils. The quality parameters of saponification value, iodine value, refractive index, and specific gravity were determined for samples from the laboratory- and commercial-scale processes. The saponification value, iodine value, specific gravity, and refractive index were 186.5, 94.0, 0.916, and 1.4610, respectively, for laboratory-scale samples and 187.4, 96.1, 0.915, and 1.4623, respectively, for commercial-scale samples. These values were within the prescribed legal standards specified in India.

Based on these data, we concluded that a $CaCl_2$ and water treatment of RBO, followed by crystallization at a low temperature (20°C), effectively removed the PL and wax contents from the crude oil in laboratory- and commercial-scale trials. After bleaching and winterization of this oil, the phosphorus content was reduced to ≤5 ppm and the wax content to ≤50 ppm, making the oil suitable for physical refining. The deacidification of this oil at optimized conditions removed 95–97% of the FFA, reduced the color value to 10–12 Lovibond units (1-in. cell), and retained 80% of the tocols, 90% of the oryzanol, and 95% of the sterols. Thus, the CRBO, which is considered the most difficult oil to refine, could be processed on a commercial scale to edible-grade RBO with high levels of bioactive phytochemicals, as demonstrated here.

TABLE 6
Trace Metal Content[a] of RBO at Different Stages of Processing on a Laboratory Scale

Trace metals (μg/100 g)	CRBO	DRBO	BRBO	WRBO	RRBO
Cu	11.8	11.0	7.5	3.7	0.7
Fe	1149.5	1132.5	400.9	245.2	58.8
Zn	444.3	257.5	200.4	33.7	28.0
Mn	279.1	48.6	32.8	15.2	11.5
Ca	3627.5	2141.3	1456.7	284.7	235.8
Mg	2896.3	767.1	766.7	93.0	62.7

[a]Means of four replications. For abbreviations see Table 1.

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