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Enriching Oryzanol in Rice Bran Oil using Membranes

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Abstract Oryzanol present in rice bran is associated with various physiological functions. However, these beneficial ferulate esters are lost to the extent of 87% during conventional refining of crude rice bran oil. In the present investigation, oryzanol enrichment in rice bran oil was attempted using nonporous polymeric membranes under undiluted as well as hexane-diluted conditions with different (crude, refined, and model oil) systems varying widely in their oryzanol content. During membrane processing, oryzanol content in the refined rice bran oil increased from 2,420 to 7,340 mg/kg (approximately threefold enrichment). While processing crude oil and model oil systems, the oryzanol content in the oil improved from 17,600 to 27,300 mg/kg and 20,400 to 30,300 mg/kg, respectively. The enrichment of oryzanol was due to its moderate rejection by the nonporous hydrophobic membrane owing to the hydrophilic nature of the ferulic esters. Hexane dilution improved the oil flux by one order of magnitude but reduced the selectivity. Enriched rice bran oil may find wider applications in the pharmaceutical, therapeutic, and dietary preparations as well as in producing standard cooking oil with guaranteed oryzanol content.

Keywords Enrichment · Flux · Hexane · Nonporous membrane · Oleic acid · Oryzanol · Rejection · Rice bran oil

Introduction

Oryzanol is a group of compounds containing ferulate (4-hydroxy-3-methoxycinnamic acid) esters of triterpene alcohols and plant sterols (ferulic acid esterified to cycloartenol, 24-methylene cycloartanol, β-sitosterol, and campesterol). Rice bran is the major source of oryzanol, and it is extracted into oil fraction during the extraction process. Crude rice bran oil (CRBO) typically contains ~15,000 mg/kg of gamma-oryzanol. These ferulic acid esters also possess antioxidant properties similar to that of tocopherols. Various physiological functions

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have been associated with oryzanol intake including a decrease in plasma cholesterol and decreased platelet aggregation, hepatic cholesterol synthesis, and cholesterol absorption [1].

The method of refining crude oil affects the oryzanol content in refined oil. Alkali refining resulted typically in a decrease in total oryzanol content from 16,000 to 2,000 mg/kg, while physical refining retained ~66% of the oryzanol in the oil [1]. Although physical refining offers several other advantages over chemical refining, industries generally prefer to follow alkali refining method as it is a versatile process and produces acceptable quality oil from all types of crude oil. During chemical refining, most of the oryzanol are removed along with soapstock, a byproduct containing 1.3–3.1% oryzanol [2]. Therefore, soapstock of rice bran oil (RBO) obtained from chemical refining process is generally used as the primary source for recovering oryzanol. Over the decades, the methods of oryzanol isolation from soap stock have been improved, and selected methods have been recently summarized by Narayan et al. [3]. There were also attempts to isolate oryzanol directly from rice bran [4] by super critical fluid extraction (SCFE) and CRBO employing either column chromatography-based techniques [5] or SCFE (6).

Oryzanol is used in various applications in pharmaceutical, therapeutic, and dietary preparations. Besides, it has also been proposed to add back oryzanol to RBO [7]. However, it may be desirable to enrich oryzanol present in the oil rather than isolating it and then adding it back to the oil. The following are few reports on the enrichment of oryzanol in RBO.

Cherukuri et al. [8] described an extraction procedure using lower aliphatic alcohols for obtaining enriched rice bran oil (ERBO) having enhanced anti-oxidant content (tocols and oryzanol) from CRBO. The yield of ERBO was in the range of 31–45% with an oryzanol enhancement of ~80–90%. Karan [9] reported that dimethylformamide extraction of CRBO after hexane dissolution was superior to alcohol extraction. The oryzanol content in RBO could be enriched by a factor of 7.1 with a yield of ~4% ERBO. Cherukuri et al. [8] also reported that short-path-vacuum-distillation (SPVD; 0.002 mm Hg and 235°C) of refined RBO could enhance the oryzanol content from 0.18% to 1.7% with a distillate percentage of 6.3%. Dunford and King [6] evaluated SCFE technique for reducing the free fatty acids (FFA) content in RBO and reported enrichment of phytosterol in the deacidified RBO fraction. When CRBO with 7% FFA and 1.3% oryzanol content was subjected to fractionation at 13.6 MPa, 45°C, and 1.2 l/min CO_2 flow rate, there was a decrease in FFA content (<1%) and increase in oryzanol content (1.78%) in the raffinate fraction. The methods proposed above are associated with some limitations such as employing either organic solvent for extraction or capital intensive equipment with lower throughput or severe conditions for separation in a capital intensive process.

Membrane technology has contributed significantly in the field of bioprocessing owing to its inherent advantages over the conventional processes. In the last three decades, a large number of attempts have been made worldwide towards degumming, dewaxing, deacidifying, and decolorizing edible oils using membrane technology with and without using solvents [10]. However, there is no membrane-based process approach reported for the separation/concentration of oryzanol. The differences in the molecular sizes among the oryzanol mixture (~600 Da) and that of triglycerides (>800 Da) are too small to use a porous membrane (nanofiltration) for separation based on size exclusion, whereas in nonporous membranes, the separation is due to solution-diffusion effect and such membranes may possess the required selectivity for the concentration of oryzanol. In the present study, performance of a nonporous denser membrane has been evaluated for the concentration of oryzanol in crude and refined RBO as well as in model oil systems.

Materials and Methods

Materials

Raw Materials

Two lots of CRBO were obtained from M/s M.K. Agrotech (Lot-1), Srirangapatna, India and M/s Habib Agro Industries (Lot-2), Mandya, India. Refined RBO was purchased from the local market.

Chemicals and Solvents

Oryzanol used as a reference in the analyses and in the preparation of model oil systems was of analytical grade (purity ~98%) and obtained from M/s Wako Pure Chemical Industries, Osaka, Japan. High-performance liquid chromatography (HPLC)-grade solvents, analytical-grade dichloromethane, and laboratory-grade hexane and oleic acid were procured from reputed manufacturers in the country.

Membrane

Two nonporous polymeric composite hydrophobic membranes, NTGS-2200 and NTGS-2100 with polydimethylsiloxane (PDMS) as active layer and polyimide as support layer, were used in the study (Nitto Denko, Kusatsu, Japan). The membranes were cut into circular discs (7.5 cm diameter with 32 cm^2 effective area) and fitted in the membrane cell in such a way that active surface comes into contact with the feed material.

Membrane Apparatus

Experimental runs were conducted in a self-stirred flat membrane test cell (model: C40-B, Nitto Denko) under nitrogen atmosphere [10]. The membrane cell was placed on a magnetic stirrer, and the magnetic spin bar fitted into the cell provided the agitation. Stirring was employed to minimize concentration polarization effect, and the speed was maintained at 800 rpm. Experiments were carried out at 2–4 and 2 MPa pressures with undiluted and hexane-diluted oil samples, respectively. All the experimental runs were conducted at room temperature (25–28°C). The unit was operated in batch mode by charging the cell with 50–80 g of oil or 150–200 g of hexane-diluted oil, and the run was terminated when a predetermined quantity of permeate had permeated.

Methods

Analysis of Diluted and Undiluted Oil Samples

Feed, permeate, and retentate samples of each experimental run were analyzed for oryzanol content. In the experiments conducted with hexane-oil systems, the samples were analyzed after evaporating the hexane under vacuum using a flash evaporator (40°C for 40 min) followed by flushing with nitrogen (5 min).

Spectrophotometric Analysis of Oryzanol

Oryzanol contents in the oil samples were determined by spectrophotometric method at a specific wave length of 315 nm [11] using dichloromethane as solvent.

HPLC Analysis of Oryzanol

Oryzanol contents in oil samples were determined in the reversed-phase HPLC by the method followed by Xu and Godber [4] with slight modification. The mobile phase consisted of methanol, acetonitrile, dichloromethane, and acetic acid (50:44:3:3 *v*/*v*). HPLC conditions are: column C-18, 2.5×250 mm with 5 μm particles (Merck, Darmstadt, Germany); LC-10AT HPLC pump, SCL-10A system controller and SPD-10A UV-VIS detector (Shimadzu, Kyoto, Japan). Measurement conditions were: absorbance, 330 nm; column temperature, 30°C; flow, 1.4 ml/min; and analysis time, 40 min. Sample preparation: 0.5 g of oil sample was dissolved in 3 g of dichloromethane and then diluted with an equal amount of mobile phase before analysis.

Viscosity Measurement

Measurement of viscosity (apparent) of samples was carried out using disc spindle measuring system in a digital viscometer (Model DV-II+, version 2.0; Brookfield Engineering Laboratories, Stoughton, MA, USA) at room temperature (29.5°C).

Fatty Acid Composition

Analysis of fatty acid composition was done by gas chromatography (GC; Model: GC-15A, Shimadzu) as per the American Oil Chemists' Society method, Ce 1-62 [12]. Fatty acids present in oil were first converted to fatty acid methyl esters before injecting into GC column to obtain the fatty acid profile. Temperatures maintained in the analysis are: column oven—180°C, injection block—220°C, and detection block—230°C.

Performance Parameters

The performance of the membrane process was expressed in terms of percent observed rejection (R_o) and oryzanol enrichment (fold) in the processed oil.

The rejection is referred to as observed rejection to indicate the fact that in a batch process the feed concentration constantly changes during the process. R_o was determined, assuming that it was constant during each batch experimental run, by using Eq. 1 [13]:

$$R_o = \frac{100\left[\ln\left(C_{R,f}/C_{R,i}\right)\right]}{\ln\left(W_i/W_f\right)}(\%) \tag{1}$$

where $C_{R,i}$ and $C_{R,f}$ are the initial and final oryzanol contents in the retentate (mg/kg oil), and W_i and W_f are the initial (feed) and final weights of retentate (kilograms oil), respectively.

Enrichment is the ratio of the oryzanol contents in the retentate to the feed.

$$\text{Enrichment (fold)} = \frac{\text{Oryzanol content in the retentate (mg/kg)}}{\text{Oryzanol content in the feed (mg/kg)}} \tag{2}$$

Results and Discussion

Selectivity of Nonporous Membranes for Oryzanol in Real and Model Oil Systems

Studies were carried out using two nonporous membranes NTGS-2100 and NTGS-2200 to evaluate their selectivity for oryzanol in CRBO and various other model oil systems. The oryzanol content in the feed, observed rejection and permeate flux during membrane processing are presented in Table 1. The rejection of oryzanol in CRBO was 33.4% and 41.3% with NTGS-2100 and NTGS-2200 membranes, respectively. In the case of nonporous membranes, separation/permeation of components depends on their own solubility and diffusivity in the membrane material. In addition, it also depends on the coupling effect as well as solubility of individual components in other permeating components including the solvent being used [14]. All the above factors are also concentration-dependent besides solubility being greatly influenced by the polarity of individual components [15]. Oryzanol is composed of 25% to 50% of campesteryl, 10% to 40% of 2,4-methylene cycloartanyl, 15% to 30% of cycloartenyl, and 15% to 26% of β-sitosteryl ferulates. The molecular weight of this mixture of four compounds varying in composition is calculated as ~600 Da. The alcohol group present in the ferulate moiety of components of oryzanol gives rise to a relatively high polarity [4]. This may explain the moderate rejection of oryzanol in spite of its lower molecular weight (~600 Da) compared to triglycerides (>800 Da), since the active surface (PDMS) of the nonporous denser membranes used in the study is hydrophobic in nature. In our earlier study on the rejection of carotenoids [13], rejection of xanthophylls (oxygenated carotenoids) and permeation of β-carotene (hydrocarbon carotenoids) in a nonporous hydrophobic membrane revealed the influence of polarity of compounds on their transport. The above studies were extended to different model oil systems for assessing the membrane selectivity (Table 1). The rejections of oryzanol in triglyceride (refined RBO)–oryzanol system with NTGS-2100 and NTGS-2200 membranes were 29.7% and 30.9%,

Table 1 Rejection of oryzanol in RBO systems using nonporous membranes[a].

Type of system	Feed			NTGS-2100		NTGS-2200	
	Oryzanol[b] (%)	Oleic acid[b] (%)	Oryzanol[c] (mg/kg)	R_o (%)	Flux [kg/(m^2.h)]	R_o (%)	Flux [kg/(m^2.h)]
Real system							
CRBO	–	–	15,200	33.4	0.03	41.3	0.03
Model systems							
Triglyceride–oryzanol	1	–	13,600	29.7	0.08	30.9	0.05
Triglyceride–oryzanol	2	–	23,600	26.7	0.07	–	–
Triglyceride–oleic acid–oryzanol	1	49.5	12,400	22.2	0.14	32.3	0.09
Triglyceride–oleic acid–oryzanol	2	49.0	21,500	–	–	30.7	0.10
Oleic acid–oryzanol	1	99.0	10,300	27.7	0.51	45.8	0.37
CRBO–triglyceride (1:1)	–	–	9,600	39.0	0.03	44.1	0.03

[a] CRBO (Lot-1); 20% permeation of feed; operating pressure 3 MPa; R_o, observed rejection

[b] Addition

[c] Estimated value

respectively. The lower rejections observed with the above model system as compared to CRBO could be attributed to the basic differences between the real and model systems in terms of their composition. Inclusion of oleic acid in the model oil system increased the permeation rate, and the highest permeation rate was observed in oleic acid–oryzanol system (Table 1). Oleic acid exhibited higher differential permeability over triglycerides in a non-porous denser membrane owing to the cumulative effect of its higher solubility as well as diffusivity in the membrane material [13]. However, it appears that the permeability of the solute (oryzanol) was not influenced much by its solubility in oleic acid resulting in its higher rejection in oleic acid–oryzanol system. In this system, cumulative effect of higher molecular weight and relatively higher polarity of oryzanol seemed to be responsible for their rejection. Our earlier studies on degumming showed that these two membranes possess excellent selectivity in near-complete elimination of phospholipids (PL) in crude vegetable and model oils [13]. Although the rejection of oryzanol was not as high as that of PL, the moderate rejection of oryzanol by these membranes evoked interest. NTGS-2200 membrane exhibited higher selectivity for oryzanol compared to NTGS-2100 in crude as well as model oil systems. Although these two membranes were prepared from the same membrane materials (active and support layers), the difference in their observed rejection values were significant which could be probably attributed to the differences in the thickness of their active (PDMS) layers. Relatively lower flux values obtained with NTGS-2200 compared to NTGS-2100 membrane (Table 1) also suggested differences in their active layer thickness. Considering the higher selectivity for oryzanol, NTGS-2200 membrane was used in further experiments.

Processing Undiluted Crude and Model Oil Systems

The performance of NTGS-2200 membrane was assessed at different levels of permeation during batch processing of CRBO and triglyceride–oryzanol model system. Oryzanol content in the process streams and enrichment fold at various stages along with the overall observed rejection and permeate flux for CRBO and triglyceride–oryzanol system are presented in Tables 2 and 3, respectively. Palmitic, oleic, and linoleic are the major fatty acids in RBO, and their contents in CRBO and model oils used in the present study were 23.6%, 44.2%, and 28.7%; and 20.6%, 41.1%, and 33.0%, respectively. The oryzanol content in the final retentate (ERBO) increased to a level of 27,300 mg/kg from an initial value of 17,600 mg/kg in CRBO. The oryzanol content increased during the batch process, and its enrichment steadily increased at various levels of permeation, in spite of the increased solute diffusion

Table 2 Enrichment of oryzanol in CRBO at various levels of permeation[a].

Fraction no.	Permeation	Oryzanol content (mg/kg)		R_o (%)	Enrichment (fold)	Permeate flux [kg/(m^2.h)]
		Permeate	Retentate			
1	First 25%	16,100	19,800	–	1.13	–
2	25–44%	13,430	21,900	–	1.11 (1.24)[b]	–
3	44–58%	13,900	24,500	–	1.12 (1.39)[b]	–
4	58–68%	13,930	27,300	–	1.11 (1.55)[b]	–
5	0–68%	–	27,300	36.9	1.55	0.028

[a] Crude (Lot-2); oryzanol content in feed, 17,600 mg/kg; feed viscosity, 62 mPa.s; operating pressure, 4 MPa; for abbreviations, see Table 1.

[b] Cumulative enrichment

Table 3 Enrichment of oryzanol in a model oil system at various levels of permeation[a].

Fraction no.	Permeation	Oryzanol content (mg/kg)		R_o (%)	Enrichment (fold)	Permeate flux [kg/(m^2.h)]
		Permeate	Retentate			
1	First 17%	12,500	22,000	–	1.08	–
2	17–38%	16,900	23,600	–	1.07 (1.16)[b]	–
3	38–57%	19,300	25,600	–	1.08 (1.25)[b]	–
4	57–85%	22,300	30,300	–	1.18 (1.49)[b]	–
5	0–85%	–	30,300	19.9	1.49	0.046

[a] Oryzanol content in feed, 20,400 mg/kg; feed viscosity, 61 mPa.s; operating pressure, 2 MPa; for abbreviations, see Table 1.

[b] Cumulative enrichment

owing to its increased concentration in the feed. The overall rejection of oryzanol by the membrane after 68% permeation was 36.9% with an enrichment fold of 1.55.

As in the case of CRBO, the oryzanol enrichment steadily increased, and its concentration reached a level of 30,300 mg/kg from an initial value of 20,400 mg/kg in the model system as well. The improvement in oryzanol content in the model oil was 1.49-fold after 85% permeation. The overall rejection of oryzanol was 19.9%, which was lower than the rejection observed with crude oil (36.9%). This could be due to the basic differences between the real and model systems as discussed earlier. The average PL content of CRBO is in the range of 4–5% [1]. From our earlier study on characterization of PL reverse micelles [13], it can be construed that the PL content in CRBO is above critical micelle concentration. The hydrophilic polar heads of PL are inward in the reverse micelles formed in the system, and these inner polar regions would have affinity for other polar components such as oryzanol present in the system resulting in their increased rejection. The model oil (triglyceride–oryzanol) did not contain PL which could be the probable reason for the lower rejection of oryzanol. The HPLC analysis of the process streams of model oil system showed all the ten components of oryzanol in their chromatograms. The four major peaks were identified as ferulate esters of cycloartenol, 24-methylenecycloartanol, campesterol, and β-sitosterol (retention time, 19.5, 21.0, 23.9, and 26.2 min, respectively). These major components were rejected by the membrane to the extent of 19.4%, 23.7%, 20.5%, and 25.4%, respectively, without deviating much from the rejection of total oryzanol estimated based on HPLC (20.0%) and spectrophotometric (19.9%) analyses. The permeate flux obtained with crude oil system was lower than that of model oil system which could be due to the higher viscosity as well as the greater effect of concentration polarization owing to the presence of various other impurities in the crude oil.

Processing Hexane-Diluted Crude Oil System

Our recent studies [16, 17] showed that significant improvement in oil flux could be achieved in nonporous denser membranes by hexane dilution of crude vegetable oils without practically affecting the rejection of PL. The same approach was examined to assess the membrane selectivity for oryzanol under hexane-diluted conditions and to improve the oil flux, keeping in view the process economics. The permeate flux increased by nearly tenfold at 1:1 dilution and 18-fold at 1:3 dilution while processing hexane-diluted CRBO (Table 4). However, the rejection of oryzanol decreased to 17.9% in hexane-diluted system (1:1 dilution) compared to undiluted system (39.6%), and the rejection decreased further with increase in

Table 4 Enrichment of oryzanol in hexane-diluted CRBO[a].

Oil–solvent ratio	Feed viscosity (mPa.s)	Oryzanol content[b] (mg/kg)		R_o (%)	Enrichment (fold)	Yield (%)	Oil flux [kg/(m^2.h)]
		Permeate	Retentate				
1:1	3.2	15,400	24,100	17.9	1.37	17	0.32
1:3	1.9	16,600	21,900	12.5	1.24	17	0.51

[a] Crude (Lot-2); oryzanol content in feed, 17,600 mg/kg; 90% permeation; operating pressure, 2 MPa; for abbreviations, see Table 1.

[b] Hexane-free basis

hexane dilution. Similar behavior was observed in the rejection of color compounds in hexane-diluted crude vegetable oils [16–18]. The rejection in a multi-component system is controlled by several factors. Besides membrane parameters, the interaction between the components in the feed also plays a significant role. Further, it appears that hexane seemed to have positive coupling with other oil constituents that are soluble in hexane during its transport across the nonporous denser membrane increasing their permeation or in other words lowering their rejection. Although hexane dilution affected the rejection of oryzanol, this could be the practical approach considering the oil flux improvement.

Processing Undiluted and Hexane-diluted Refined Oil System

The ability of nonporous membranes was examined in producing refined RBO with guaranteed oryzanol content that would conform to the efficacy levels of oryzanol in dietary and therapeutic applications. The oryzanol content in chemically refined RBO was 2,420 mg/kg, which improved to 7,340 mg/kg after membrane processing under undiluted conditions. The overall rejection of oryzanol by the membrane after 90% permeation was 44.2% with an enrichment fold of 3.03 (Table 5). Similar to the observations made with crude oil, hexane dilution of refined oil improved the oil flux (15- to 18-fold) but affected the selectivity of the membrane as evidenced from the lower rejection values (Table 5). The results indicated that it may be possible to use membrane processing in conjunction with conventional refining process to guarantee a minimum oryzanol content in the finished product.

Table 5 Enrichment of oryzanol in undiluted and hexane-diluted refined RBO[a].

Oil–solvent ratio	Feed viscosity (mPa.s)	Oryzanol content[b] (mg/kg)		R_o (%)	Enrichment (fold)	Yield (%)	Oil flux [kg/(m^2.h)]
		Permeate	Retentate				
1:0	58.0	1,880	7,340	44.2	3.03	10	0.068
1:1	1.53	2,150	4,480	26.0	1.85	9.4	1.03
1:2	1.25	2,220	4,940	26.1	2.04	6.5	1.24

[a] Oryzanol content in feed 2,420 mg/kg; 90% permeation; Operating pressure 2 MPa; For abbreviation see Table 1

[b] Hexane-free basis

Conclusions

The present study revealed that the nonporous hydrophobic membrane possesses a reasonable selectivity towards enriching oryzanol in RBO due to the hydrophilic nature of the ferulic esters leading to their moderate rejection. Owing to the preferential permeation of FFA, the enriched oryzanol fraction (retentate) obtained in the process would have reduced FFA content. Membrane processing could be used for concentration/enrichment of oryzanol present in RBO by physical means under mild process conditions thus offering advantages over the methods proposed earlier (solvent extraction, SCFE, and SPVD). Although hexane dilution affected the selectivity, this could be the practical approach considering the oil flux improvement until suitable membranes with high selectivity and flux are developed.

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