Recovery of γ-Oryzanol from Rice Bran Oil with Silica-Based Continuous Chromatography[1]

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ABSTRACT: A simulated moving bed chromatography separator was tested for recovery of γ-oryzanol from degummed and dewaxed rice bran oil that contained 1.2 to 1.6% γ-oryzanol. A crude product with 12–15% of γ-oryzanol was obtained and a 90 to 95% pure product was recovered from the concentrate by crystallization from heptane. With the recycling of the crystallization liquor, an overall γ-oryzanol recovery of 85 to 90% is feasible and is potentially higher than the recovery in the conventional soapstock-based process.
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KEY WORDS: γ-Oryzanol, rice bran oil, simulated moving bed chromatography.

Industrial production of γ-oryzanol, a mixture of at least five ferulic acid esters with documented therapeutic properties, is done by extracting the soapstock residues of alkali-refined crude rice bran oil (RBO) (1–8). After acidification with carbon dioxide (1) or hydrochloric acid (2) the alkali cake with 1 to 3% γ-oryzanol is selectively extracted with diethyl-ether (2), trichloro ethylene, benzene, or *n*-hexane (1), and γ-oryzanol is recovered from the organic phase at 85–90% purity and recovery of about 1.5% on the original RBO. Alternatively, the non-γ-oryzanol components of the cake are selectively extracted with methanol, ethanol (3), or *n*-hexane (4) and the remaining precipitate is then acidified with sulfuric acid and washed with water to yield a crude γ-oryzanol product.

The concentration of γ-oryzanol in RBO depends on the extraction and processing conditions. Commercial oil produced in India was reported to contain 1.5 to 1.9% γ-oryzanol, hexane-extracted oil 1.1 to 1.4%, and oil extracted with chloroform/methanol 2.5 to 2.6% (2). About 1.5 to 2.9% oryzanol was reported for Japanese oils (9), and as little as 0.1% for commercial oils marketed in the United States (5,10). The commercial γ-oryzanol is sold at $50–120/kg levels depending on the grade and campesterol (campesteryl ferulate) content (5) and, at a recovery of 2% on RBO or about 0.03% on rice, represents a potential revenue of $15–36 per ton of rice.

If alkali refining is not done and crude RBO is either to be refined physically or not refined, the soapstocks are not available and an alternative process is needed. Several analytical chromatographic silicas with isooctane/ethyl acetate eluents were recently tested (11) for separation of tocopherols and γ-oryzanol from saponified RBO. Of the tested silicas, a 4 μm Nova-Pak provided optimal separation. Two fractions, γ-oryzanol I and γ-oryzanol II, were obtained.

Chromatographic separations of organic products on ion exchange resins, polymeric adsorbents, silica gels, alumina, or zeolites are used in laboratory and process-scale purifications. Many applications have been reported in a number of recent symposia on preparative chromatography (12–18) for purification of antibiotics, enzymes, vitamins, amino acids, sugars, etc. With conventional batch chromatography, the feed is applied to the top of a column packed with an appropriate adsorbent. It is then eluted with a solvent chosen such that most or all components elute from the column within a relatively short time (no strong adsorption) and are fully or partially separated into individual components. While the batch separation affords good flexibility, the solvent and adsorbent use are high and may prove cost-prohibitive for many applications. In order to improve productivity, a number of continuous chromatography designs have been proposed in the last 30 yr but the simulated moving bed (SMB) concept has become increasingly accepted as ideally suited for continuous industrial separations of binary and, with some limitations, even multi-component mixtures (19–22). The systems consist of either one column subdivided into sections, or more frequently, a number of columns arranged in series (Fig. 1) with ports (feed inlet, solvent inlet, and product outlets) between individual columns, and provision for moving (switching) periodically all the ports along the direction of the liquid flow. This port movement can be accomplished by opening or closing of standard valves that distribute the liquids into the various columns or by distribution of the liquid streams with a special multi-port sliding valve (23–27). In the CSEP® (Continuous Separator) design, patented by Advanced Separation Technologies (25–27), 10 to 20 columns or adsorbent chambers are mounted on a carousel (Fig. 2) that rotates continuously or intermittently with the adsorbent chambers moving through the various phases, i.e., stripping and enrichment (entrainment of the strongly adsorbed components), elution (desorption of the retained components), and reload (purification of the recycled solvent). The motion

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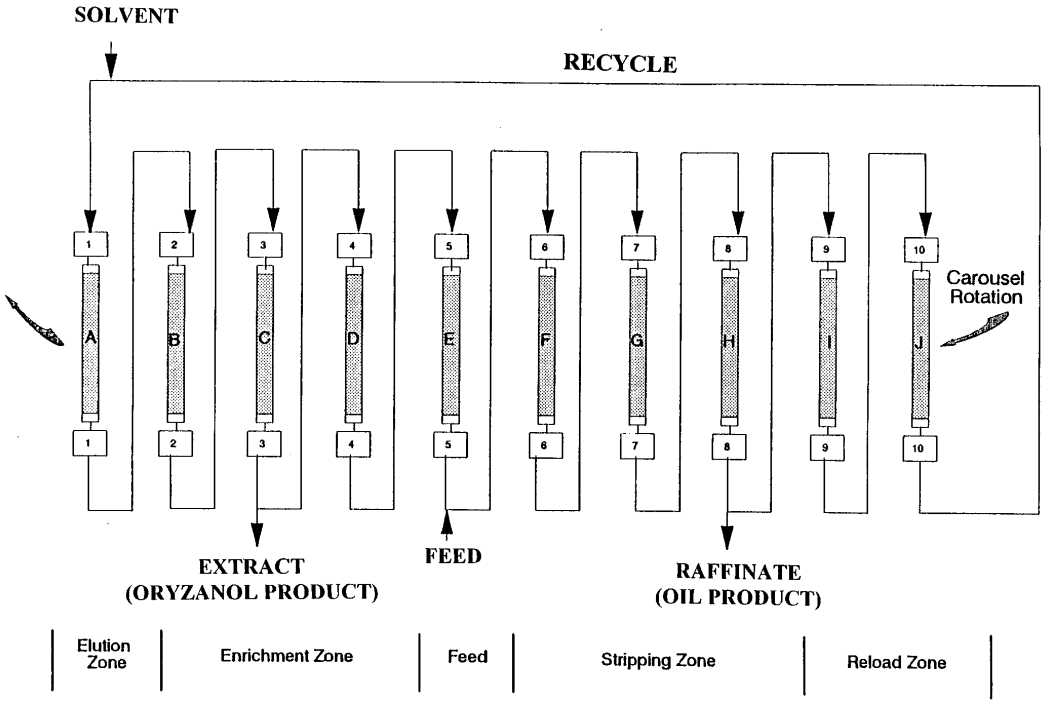

FIG. 1. Schematics of the operation of a 10-column simulated moving bed separator. The inlet and outlet ports 1–10 for addition of solvent (inlet port 1) and feed (inlet port 6) and withdrawal of extract (outlet port 3) and raffinate (outlet port 8) remain stationary, while the columns A–J mounted on a carousel rotate against the direction of liquid flow, achieving a simulated moving bed operation.

of the ports along the direction of the liquid flow effectively provides a countercurrent movement of the adsorbent bed, resulting in a continuous separation with reduced solvent and adsorbent use by a factor of 2 to 5 in comparison with the batch process. The efficiency of the stationary phase decreases with increasing particle size but, because of pressure limitations, the particle size of the stationary phase in industrial separations ranges between 100 and 300 μm. Because the SMB process is more efficient and cost-effective than discontinuous processes, larger-diameter particles can be used in the SMB process.

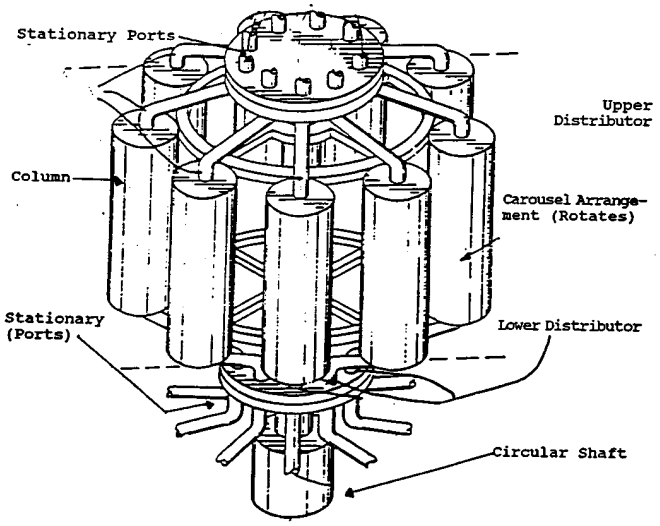

FIG. 2. Schematics of the Advanced Separation Technologies system (CSEP®) for continuous laboratory and industrial chromatography separations. The columns located on a carousel slowly rotate, resulting in a simulated moving bed countercurrent operation (23–26).

The objective of this research was the development of an industrial chromatography-based process to recover γ-oryzanol from partially or fully refined oil based on its adsorption on a silica stationary phase. Of particular interest was whether or not the degummed and dewaxed RBO may foul the silica packing in an extended operation. The replacement cost of the silica can be as high as $6,000/m^3 and its lifetime may be a decisive factor for the economical feasibility of a process.

MATERIALS AND METHODS

Crude RBO of Louisiana origin was degummed and dewaxed by addition of 2% citric acid, heated to 65°C for 30 min, and cold-filtered at 10°C. The oryzanol determination throughout this work was done with normal phase silica-based high-performance liquid chromatography (HPLC) (11) with the exception that 95:5 hexane/ethyl acetate was used as the mobile phase. A 60 Å Nova-Pak 3.9 × 150 mm column (Waters, Milford, MA) was used at an ambient temperature, in conjunction with a manual 10 μL Rheodyne (Cotati, CA) 7010 injection valve and a Spectra-Physics (San Jose, CA) 200 ultraviolet (UV) detector set at 295 nm. A high-purity commercial γ-oryzanol (Maypro Industries, Harrison, NY) specified by the supplier as 99.2% pure was used as a standard without any further purification. Both of the γ-oryzanol fractions, I and II (Fig. 3), which in turn consist of two or three components each, were assumed to have an identical extinction coefficient (10). The total oil concentration in the dilute products of the

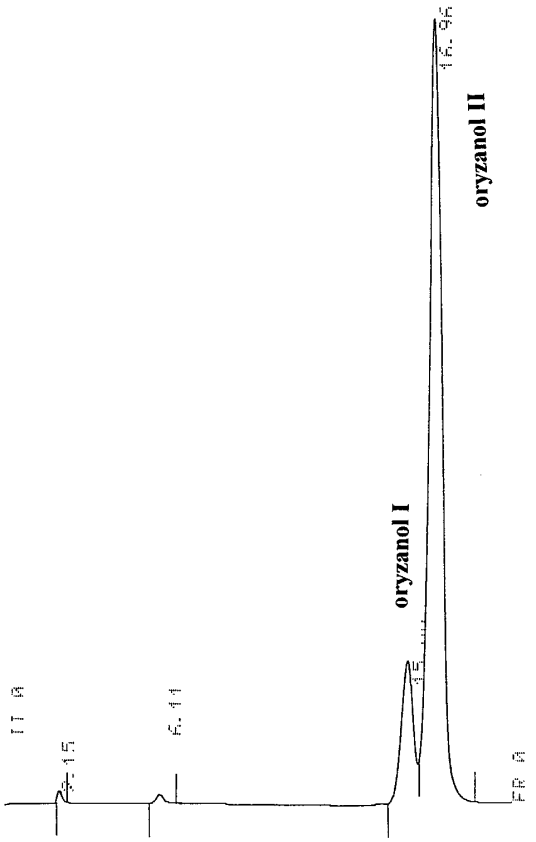

FIG. 3. A high-performance liquid (HPL) chromatogram, high-purity commercial γ-oryzanol product (Maypro Industries, Harrison, NY). A Nova-Pak 3.9 × 150 mm silica column, 295 nm ultraviolet (UV) signal. Other parameters as given in the text.

chromatography separation was determined from the weight loss after drying the sample in a stream of air at 90°C for 1 h.

Several commercial silicas were tested for their efficiency in separating γ-oryzanol from triglycerides and free fatty acids, and results with two of them, an 80 µm particle size IMPAQ® RG 10 (BTR Separations, Wilmington, DE) and a 200–500 µm UETICON C490-D (CU Chemie Ueticon AG, Ueticon, Switzerland) are described in this publication. Both are unmodified irregular (nonspherical) silicas, with comparable physical properties with the exception of particle size (Table 1). Low-pressure glass 10 × 100 mm and 10 × 300 mm Omnifit columns (Supelco, Bellefonte, PA) were slurry-packed with the silica and equlibrated by pumping through the heptane/ethyl acetate or hexane/ethyl acetate mobile phase. Various concentrations of ethyl acetate were tested, but the 5 (vol/vol) to 15% (vol/vol) range was found most suitable. After equilibration, 0.1 to 5 mL of the sample, prepared by dissolving the degummed/dewaxed RBO in hexane, heptane, or the mobile phase, typically at a 10 to 40% wt/vol concentration, was injected into the preparative silica column and eluted with the mobile phase at a 2 to 5 mL/min flow rate. The refractive index and 295 nm UV signals were recorded with a chart recorder. In some tests the effluent from the column was sampled and the total oil and γ-oryzanol concentrations determined.

TABLE 1
Characteristics of the Preparative Silica Used in Simulated Moving Bed Recovery of Oryzanol from Degummed/Dewaxed Rice Bran Oil (suppliers' data)

	IMPAQ*RG10[a]	UETICON C490-D[b]
Particle size, µm	40 or 80	200–500
Particle shape	Irregular	Irregular
Specific surface (BET), m^2/g	360–440	400
Pore volume, mL/g	1.0–1.3	0.9
Median pore diameter, Å	85–120	90

[a]BTR Separations, Wilmington, DE.
[b]CU Chemie Ueticon AG, Ueticon, Switzerland.

A C912 lab scale SMB separator (Advanced Separation Technologies, Lakeland, FL, and licensee Knauer GmbH, Berlin, Germany) with either six 10 × 300 mm or twelve 25 × 300 mm glass columns and two different silicas (40 and 80 µm particle size IMPAQ® RG10 and 200–500 µm UETICON C490-D) was tested to establish the basic operational parameters required for design of an industrial process. The experiments involved varying, preferably one at a time, several parameters, *viz.* solvent composition, feed composition (concentration of RBO in the solvent), flow rates (feed, solvent, products), and the step time, i.e., time between advancing the positions of the input and output ports, such as to optimize the γ-oryzanol purity and its recovery in the γ-oryzanol-rich product.

Tho two products, termed, in order to conform with majority of chromatography literature, extract (γ-oryzanol-rich product) and raffinate (γ-oryzanol-poor product), respectively, were desolventized under vacuum, and oryzanol crystallization was studied from the former. Although other solvents may be suitable for purification of crude γ-oryzanol (6), crystallization from heptane solutions, studied in this work as heptane (with ethyl acetate as a modifier), appears the preferred mobile phase for the chromatographic separation. The solubility of pure 99% γ-oryzanol in heptane is low, approximately 0.3% at room temperature, but it increases with increasing contents of oil, and is higher the lower the purity of the crude γ-oryzanol. The crude γ-oryzanol (desolventized extract) was redissolved in heptane at 60–85°C and cooled at varying rates. The final cooling temperature was varied from ambient to 0°C.

RESULTS AND DISCUSSION

The clear filtered RBO used for the separation tests contained 1.2–1.6% γ-oryzanol, similar to contents reported for Indian (2) and Japanese (9) oils, but substantially higher than some oils marketed in the United States (5,10). From the bulk of the oil, *viz.* triglycerides and free fatty acids, γ-oryzanol is well resolved on even very low efficiency silica supports (Figs. 4 and 5) and its relative retention can be readily manipulated by adjusting the polarity of the eluent. The higher the polarity of the mobile phase (ethyl acetate concentration), the weaker the adsorption of γ-oryzanol on the silica, and the shorter the elution time. The retention in Figures 4 and 5 of a

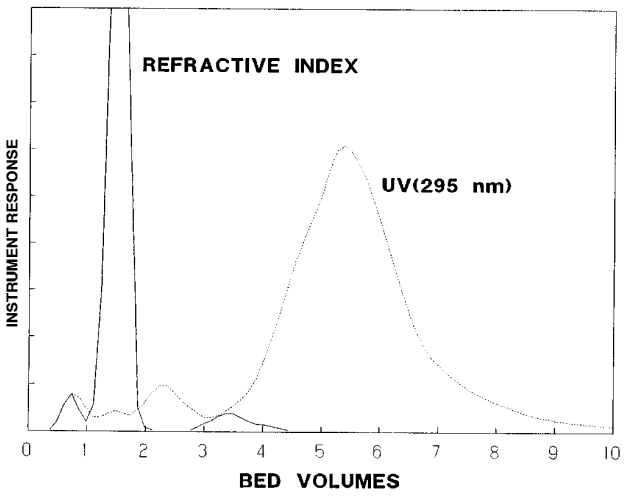

FIG. 4. Separation of degummed/dewaxed rice bran oil (RBO) on a 10 × 100 mm column of 80 µm IMPAQ® RG 10 silica (BTR Separations, Wilmington, DE), 92.5:7.5 hexane/ethyl acetate mobile phase, 4 mL/min, injection of 0.1 mL of 40% wt/vol of oil solution in hexane. Refractive index and UV 295 nm signals. Triglycerides elute 1–2 bed volumes (BV), γ-oryzanol at BV 4–8. For abbreviation see Figure 3.

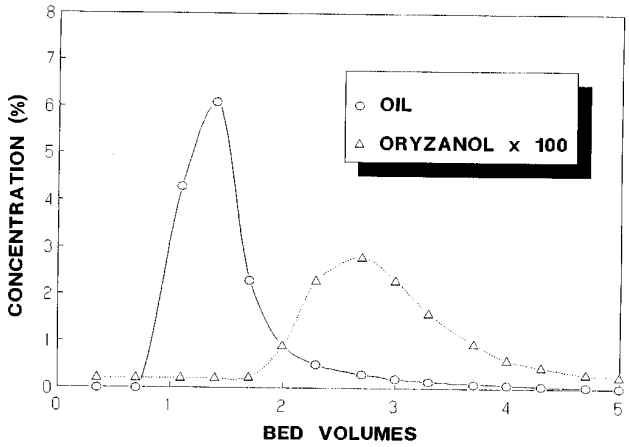

FIG. 5. Separation of degummed/dewaxed RBO on a 10 × 300 mm column, 200–500 µm UETICON C490-D silica (CU Chemie Ueticon AG, Uetican, Switzerland), 85:15 heptane/ethyl acetate mobile phase, 3.1 mL/min, injection of 4 mL of 40% wt/vol of RBO in the mobile phase. For abbreviations see Figure 4.

component is expressed as volume of the eluent in bed volumes, i.e., multiples of the volume of the chromatography column, 8 mL for the 10 × 100 mm column (Fig. 4) and 24 mL for the 10 × 300 mm column (Fig. 5). This makes the retention time independent of the flow rate and column size and provides for a ready comparison between systems with different sizes and flow rates.

The bulk of the oil, *viz.* the weakly retained triglycerides with negligible UV absorption, elute between bed volumes (BV) 1 and 2, while the more strongly retained and strongly absorbing γ-oryzanol elutes from the column later, between BV 4 and 8 (Fig. 4). Because of its low concentration, around 1.5% on RBO, γ-oryzanol is invisible with the refractive index detector but its concentration is proportional to the 295 nm signal. With a higher-polarity solvent (Fig. 5) γ-oryzanol elutes earlier, at 2–4 BV, and yet is still relatively well-resolved from the bulk of the oil despite the larger particle size of the silica. The tailing of the oil peak that is caused by the large silica particle diameter employed limits the purity of the crude γ-oryzanol that can be recovered from single-stage chromatography.

Simulated moving bed separation of γ-oryzanol. The performance of a CSEP® separator (Advanced Separation Technologies) with six (Table 2) and twelve (Table 3) columns was tested over a period of about 8 wk. These were not nonstop

TABLE 2
Recovery of γ-Oryzanol from Degummed/Dewaxed RBO with a Six-Column CSEP® Continuous Chromatography Separator[a]

Date	γ-Oryzanol in extract (wt% oil)	γ-Oryzanol in raffinate (wt% oil)	Recovery (%)	Comments
4/27	4.5	0.75	54	
4/28	15.1	0.43	69	
5/1	20.7	0.38	49	40 µm silica
5/2	10.9	0.30	65	Solvent 12 mL/min
				Solvent 14 mL/min
5/3	22.2	0.42	72	
5/4	5.5	0.45	74	
5/5	6.0	0.34	75	Washed columns with solvent
5/8	5.6	0.52	69	
5/9	5.8	0.45	72	Step time 780 s
5/10	5.6	0.3	81	Step time 740 s
5/12	6.6	0.24	84	
5/15	7.9	0.2	85	Step time 640 s
5/16	8.3	0.27	81	Step time 580 s
5/17	10.5	—[b]	—	New 40 µm silica
5/19	11.6	—	80	
5/23	12.6	—	80	New 80 µm silica
5/24	9.9	—	80	
5/25	10.0	—	79	
5/26	10.4	—	82	
5/30	10.0	—	80	
5/31	9.1	—	72	Feed conc. 70% wt/vol
6/1	9.6	—	69	
6/2	8.9	—	—	Feed 1.5 mL/min
6/9	8.9	0.51	79	
6/12	10.0	0.32	87	
6/13	9.6	0.31	87	
6/14	15.5	0.29	90	

[a]Other parameters: Solvent 92.5:7.5 vol/vol heptane/ethyl acetate, feed 40% wt/vol rice bran oil (RBO)/solvent, flow rates: feed 1 mL/min, solvent 14.0 mL/min, extract 10.5 mL/min, recycle 3.6 mL/min, step time 900 s. Columns 11 × 300 mm, IMPAQ® RG10 Silica. For manufacturer see Table 1.
[b]Missing data are indicated with a em-dash.

TABLE 3
Recovery of γ-Oryzanol from Degummed/Dewaxed RBO with a 12-Column CSEP® Continuous Chromatography Separator[a]

Date	γ-Oryzanol in extract (wt% oil)	γ-Oryzanol in raffinate (wt% oil)	γ-Oryzanol recovery (%)	Comments
10/6	12.0	0.10	—[b]	
10/31	10.9	0.62	87	Step time 10.5 min
	15.8	0.05	103	Step time 8.0 min
11/1	16.0	0.13	81	Feed 60% wt/vol
11/2	16.8	0.07	86	

[a]Other parameters: solvent 85:15 vol/vol heptane/ethyl acetate, feed 40% wt/vol RBO/solvent, flow rates: feed 10.5 mL/min, solvent 80 mL/min, extract 63 mL/min, recycle 4.0 mL/min, step time 480 s. Columns 25 × 300 mm, 200–500 µm UETICON C490-D silica. For manufacturers and abbreviation see Tables 1 and 2.
[b]Missing data are indicated with an em-dash.

continuous tests, but typically 6–12 h tests during the day, stopped for the night, and re-started the next morning. Daily averages are given in Tables 2 and 3. The lower-polarity components of the feed oil with the shorter retention times in the HPLC chromatograms (Fig. 6), *viz.* triglycerides, free fatty acids, tocopherols, etc. are concentrated in the raffinate product, while the extract contains 70 to 90% of the γ-oryzanol in the feed. The parameters, *viz.* the feed flow rate and concentration, extract and solvent flow rates, step time, etc. were varied occasionally to optimize the separation. Particular attention was paid to concerns about potential fouling of the silica, which could evidence itself as either a steady loss of separation or an increase of the pressure drop across the columns. Following the drop-off in performance on May 4, the silica was washed extensively without significant improvement in the performance. This indicated that the loss of separation was due to the nonoptimal parameters (flow rates and step time) rather than deterioration of the silica. All silica was replaced on May 17, and again on May 23 with new 80-µm IMPAQ® RG10. Surprisingly, the performance was not impaired by switching to a larger particle diameter and the pressure was cut in half to below 3 bars (~40 psi). The performance with the 80-µm silica was satisfactory but the pressure, although manageable, was considered quite high for a large-scale separation. Therefore, a still larger particle diameter commercial silica was chosen (200–500 µm) and tested in single-column and SMB experiments. Since it was expected that the larger particle size would result in a reduced separation, six additional columns were added to the separator. The flow rates were recalculated to take into account the fact that larger columns, 25 × 300 mm, were used, to give the same flow velocity (cm/min) within the columns as with the smaller diameter 10 × 300 mm columns. The performance was further improved (Table 3) and the final parameters on November 1–2 are the ones used for the tentative process design in Figure 7. Despite some discoloration of the silica at the top of the columns, fouling that would result in a noticeable drop in the separation was not observed in these tests. As a precaution, prefiltration through a bed of lower-grade silica would be expected in an industrial process as a means of protecting the expensive chromatographic packing.

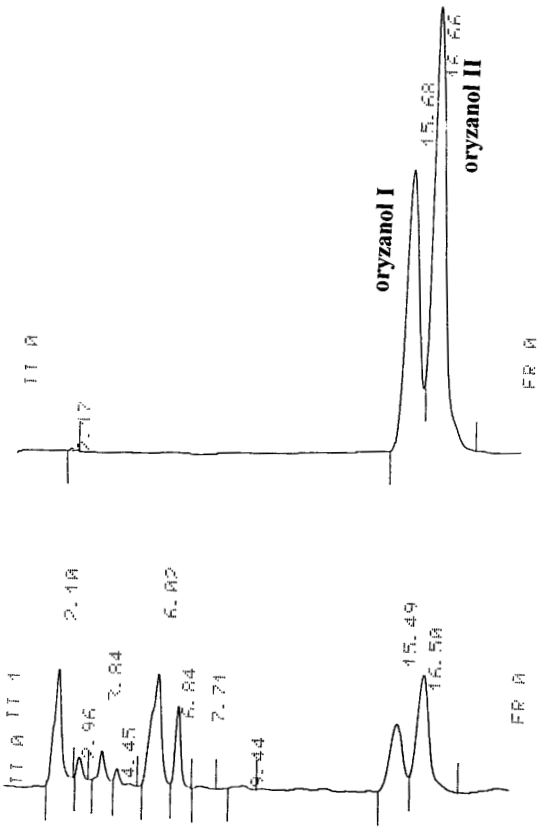

FIG. 6. HPL chromatograms. Top: A γ-oryzanol-rich chromatography product (extract). Bottom: a γ-oryzanol-poor chromatography product (raffinate). A Nova-Pak 3.9 × 150 mm silica column, 295 nm UV signal. Other parameters as given in the text. For abbreviations see Figure 3.

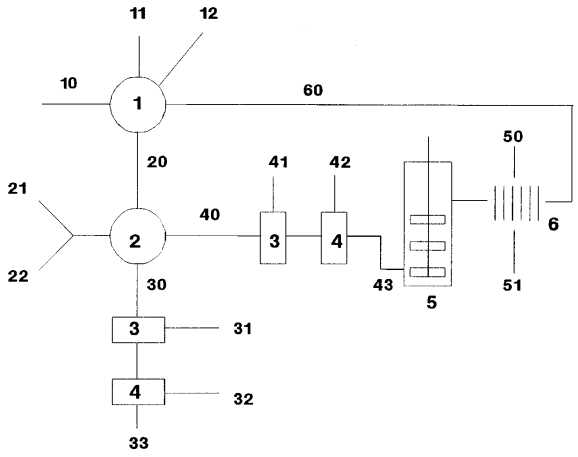

FIG. 7. Schematic diagram of a process for production of γ-oryzanol from degummed/dewaxed RBO with CSEP® continuous separation. Throughput ~9,200 kg/d (~2,600 gal/d) RBO. The crude γ-oryzanol with 12–15% purity is purified (99% purity) by crystallization from heptane. Codes to unit operations: 1, mixing of the feed RBO; 2, CSEP® separator; 3, ethyl acetate recovery; 4, heptane recovery; 5, γ-oryzanol crystallization; 6, filtration. Description of the streams and mass flow rates in kg/d of the principal components are given in Table 4.

The γ-oryzanol-depleted RBO (raffinate) contains 20 to 23 wt% oil (80% heptane/ethyl acetate) and 0.1–0.2 wt% γ-oryzanol (on weight of oil). After stripping the solvent, the oil was evaluated for marketing as a low γ-oryzanol RBO. With the exception of the γ-oryzanol content, it was found indistinguishable from the original degummed/dewaxed RBO. The γ-oryzanol-containing product (extract) is more dilute, with about 1.5 wt% oil (98.5% heptane/ethyl acetate) and, after the solvent has been recovered, it forms a yellow waxy solid with 12 to 15 wt% γ-oryzanol. The crude product can be further purified with a second-stage chromatography system or, preferably, by crystallization.

γ-Oryzanol crystallization. During cooling of the crude γ-oryzanol heptane solutions, γ-oryzanol has a tendency to precipitate with oil in the form of a gel that is hard to filter and that, after drying, does not yield a pure product. The solution concentration (crude γ-oryzanol/heptane ratio), seeding technique, rate of cooling of the heptane miscella, and final temperature affect the quality of the γ-oryzanol precipitate.

The following conditions consistently gave a good crystalline (nongelatinous) γ-oryzanol precipitate that, although fine (<5 μm particle size), was easy to filter and wash: 40% solution in heptane, final temperature ~10°C, and a residence time of 3–5 h. Inducing crystallization by seeding the solution is important and was accomplished best by emulsifying with about 2% of water, at the point when the final cooling temperature was reached. This is similar to previous report (6) for crystallization of γ-oryzanol from aqueous furfural. It is likely due to the insolubility of γ-oryzanol in water that pure γ-oryzanol crystal seeds form at the surface of the microscopic water droplets in the emulsion. During crystallization, water coalesces at the bottom of the crystallizer and can be easily separated from the heptane miscella.

The purity of the crystalline γ-oryzanol varied from 90 to 95% with respect to the commercial high-grade product (Maypro Industries) that was used as the standard for the analysis and was reported by the supplier as 99.2% pure. It is interesting to note that the γ-oryzanol II/γ-oryzanol I ratio was consistently higher in the crystal product than in the feed liquor (data not shown). This implies that the solubility in heptane/oil miscellas of the higher polarity γ- oryzanol II is lower than that of γ-oryzanol II. Obviously, if the crystallization liquor is recycled as indicated in the diagram in Figure 7, the γ-oryzanol II/γ-oryzanol I ratio in the product would be equal or close to that in the feed RBO. This was noted to be about 2 in this work and substantially less than that in the high-purity commercial product (Fig. 3).

A schematic of the complete process and an approximate material balance for processing of 9,200 kg/d of dewaxed/degummed RBO is given in Figure 7 and Table 4. The oil is diluted with the 85:15 vol/vol heptane/ethyl acetate solvent in the tank, **1**, to about 60% oil concentration and processed in the 12-column CSEP® separator, **2**; 90% of the incoming oil is recovered in the raffinate stream, **30**, which is further desolventized and either refined or marketed directly as a low γ-oryzanol (~0.1%) RBO. The extract with about 10% of the incoming oil and 90% of γ-oryzanol is first stripped completely of ethyl acetate in evaporator **3** and then, partially, of heptane in evaporator **4** to an oil concentration of about 40%, chilled, and crystallized in the crystallizer, **5**. The pure crystalline product is recovered with filter **6**, and the noncrystallized liquor is recycled back to the separator, **2**.

TABLE 4
Mass Balance (kg/d) of a 9,200 kg/d (2,600 gal/d) RBO Process for Recovery of γ-Oryzanol with Continuous Chromatographic Separation

Stream no.[a]	Description	Oil	γ-Oryzanol	Heptane	Ethyl acetate
10	Feed RBO	9,100	137	0	0
11	Ethyl acetate	0	0	0	855
12	Heptane	0	0	3,646	0
20	CSEP® feed	10,110	198	5,841	855
21	Ethyl acetate	0	0	0	15,641
22	Heptane	0	0	66,679	0
30	Raffinate	9,100	10	24,041	5,615
31	Ethyl acetate	0	0	0	5,615
32	Heptane	0	0	24,041	0
33	RBO	9,100	10	0	0
40	Extract	1,010	187	48,479	11,372
41	Ethyl acetate	0	0	0	11,372
42	Heptane	0	0	46,683	0
43	Crystallizer feed	1,010	187	1,796	0
50	Wash	0	0	400	0
51	γ-Oryzanol	0	126	0	0
60	Crystallizer liquor	1,010	61	2,196	0

[a]For definitions of the stream numbers, see Figure 7. For abbreviation and for manufacturer see Figure 2.

In contrast with the recovery from the cake that extracts 40–60% of the total RBO γ-oryzanol that is in the soapstock, the present process targets γ-oryzanol in the oil and has therefore a higher recovery potential than the soapstock-based extraction.

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