REVIEW

Extraction and Purification of Oryzanol from Rice Bran Oil and Rice Bran Oil Soapstock

A.V. Narayan, R.S. Barhate, and K.S.M.S. Raghavarao*

Department of Food Engineering, Central Food Technological Research Institute (CFTRI), Mysore – 570 020, India

ABSTRACT: Oryzanol is an important value-added co-product of the rice and rice bran-refining processes. The beneficial effects of oryzanol on human health have generated global interest in developing facile methods for its separation from rice bran oil soapstock, a by-product of the chemical refining of rice bran oil. In this article we discuss the isolation of oryzanol and the effect that impurities have on its extraction and purification. Presented are the principles behind the extraction (solid-liquid or liquid-liquid extraction, and other methods) of these unit operations covered in selected patents. Methods other than extraction such as crystallization or precipitation-based or a combination of these unit operations also are reviewed. The problems encountered and the ways to solve them during oryzanol extraction, such as prior processing and compositional variation in soapstock, resistance to mass transfer, moisture content and the presence of surface active components, which cause emulsion formation, are examined. Engineering inputs required for solving problems such as saponification, increasing mass transfer area, and drying methods are emphasized. Based on an analysis of existing processes, those having potential to work in large-scale extraction processes are presented.

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KEY WORDS: By-product utilization, extraction, impurities, oryzanol, processing, purification, rice, rice bran, rice bran oil, rice bran oil soapstock.

Rice is the second-largest cultivated crop produced worldwide with current annual world production being approximately 583 million metric tons (1). Rice bran is one of the valuable by-products of the rice processing industry (2). In many instances, it is economically feasible to extract oil from rice bran and purify it by physical and chemical refining for either food or industrial use. Owing to several technical and nontechnical problems in oil refining, the actual annual production of rice bran oil (RBO) does not meet demand (3). The major difficulties in processing crude RBO for edible purposes are its high levels of FFA, waxes, gums, and pigments. Accordingly, most RBO is used in nonfood applications. Chemical refining of RBO produces soapstock by-product (RBOS). The typical soapstock from RBO contains ~65–70 wt% water, 20–22 wt% soap, 2–2.5 wt% glycerides (mainly TG), and 7–7.5 wt% unsaponified matter. The unsaponified fraction contains ~42% sterols, 24% higher fatty alcohols, 20% oryzanol (as ferulic acid esters), 10% hydrocarbons, and 2% unidentified compounds (4). Oryzanol represents ~15% (2% of 7.5 wt%) of the unsaponified matter. At present, the major use of the soapstock is the generation of soap for the toiletry and detergent industries. It could also be used for production of therapeutically active components such as oryzanol and tocopherols (5). In view of the amount of oryzanol present in RBOS, which is generated in large amounts, there is an opportunity for commercial production of oryzanol from this by-product.

Chemically, oryzanol is a mixture of ferulic acid esters of triterpene alcohols (phytosterols), i.e., ferulate (4-hydroxy-3-methoxy cinnamic acid) (6). The components of oryzanol were identified as Δ^7-stigmastenyl ferulate, stigmasteryl ferulate, cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, Δ^7-campestenyl ferulate, campesteryl ferulate, Δ^7-sitotenyl ferulate, sitosteryl ferulate, campestanyl ferulate, and sitostanyl ferulate (7). Important rice bran ferulates are cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, and campesteryl ferulate.

Kaimal (8) has summarized the beneficial health effects of oryzanol. One application of oryzanol in pharmaceuticals includes hypocholesterolemic activity (6). Oryzanol also is claimed to have a protective role in lipid peroxidation and thus finds applications in sunscreen agents, as an antioxidant and preservative in cosmetics and food preparations, in the treatment of atopic dermatitis, in senile xeroderma, and in the prevention of skin dryness (8). A safety assessment of oryzanol indicated no genotoxic or carcinogenic activity (9). Because of these beneficial effects of oryzanol on human health, a global interest in developing facile methods for separating oryzanol from RBOS has developed.

The isolation of oryzanol from RBOS has received much attention, particularly in rice-producing countries such as India, China, Japan, Thailand, and the United States. More than 40 patents have been filed worldwide on the isolation of oryzanol from RBOS. The isolation of oryzanol also has been the subject of more than 20 research articles. Because of its commercial importance, however, theories and principles behind the patented processes for separating oryzanol from soapstocks are proprietary. Also, problems encountered during large-scale separation of oryzanol from RBOS are rarely discussed. In view of these observations, we have attempted to consolidate information from selected patents and research articles pertaining to the extraction of oryzanol from RBOS. Emphasis is placed on gaining an understanding of the principles involved in the large-scale separation and processing of oryzanol and addressing selected problems encountered when using these technologies.

Second author presently at Nanoscience and Nanotechnology Initiative, Faculty of Engineering, National University of Singapore, Singapore.

*To whom correspondence should be addressed. E-mail: fed@cftri.res.in

COMPLEXITIES IN EXTRACTION OF ORYZANOL

The problems encountered during extraction of oryzanol are mainly due to variations in the compositions of RBOS, which include surface-active impurities such as soaps, phospholipids, waxes, and glycolipids. The processing conditions used during oil refining and seasonal variations in oilseed composition dictate the type and quantity of the impurities extracted with the soapstocks. Thus, the amounts of individual impurities that are removed vary among soapstocks. Hence, an isolation procedure developed for one soapstock does not necessarily work well with another soapstock. The impurities that stabilize the soapstock dispersion (solid-liquid and liquid-liquid ratios) vary depending on the type and amount of impurities present. Impurities also affect processing conditions and hamper the equilibria governing the separation processing steps, such as extraction, isolation, and purification.

During alkali-refining (deacidification) of RBO, a significant amount of oil is retained within the soap. When the soap is centrifuged, the oil is also centrifuged with it (10). In this step, a significant amount of oryzanol is removed along with the oil into the soapstock as its sodium salts (ranging from 0.1 to 1.8%). An efficient degumming step prior to the alkai-refining step reduces the oil and gum content in the soapstock. This step is needed because of the micellar and flocculating properties of the gums that are responsible for entrapping oil in the soapstock.

IMPURITIES INTERFERING WITH EXTRACTION OF ORYZANOL

Knowledge of the types of impurities associated with oryzanol in RBOS is essential to the design of a large-scale process for isolation of oryzanol. During rice milling, impurities get into the rice bran, and subsequently the RBO and then the RBOS after alkali refining. The major impurities removed during isolation and recovery of oryzanol from soapstock or the acid oil derived therefrom include FFA, soap, glycerides, phospholipids, waxes, sterols, glycolipids, resinous matter, tocopherol derivatives, and pigments. The following paragraphs discuss these constituents and their role in oryzanol processing from RBOS.

Soap. The FA of RBO are about 77% unsaturated acids (mainly oleic and linoleic acids) and 23% saturated acids (palmitic acid) (11). The soapstock resulting from the chemical refining step of RBO contains approximately 65–70 wt% soap (sodium salts of FFA). RBO soaps are highly to moderately soluble in water and methanol, respectively, but insoluble in nonpolar solvents such as acetone and ethyl acetate. Oryzanol is soluble in acetone and ethyl acetate but insoluble in water and polar solvents such as methanol. Such contrasting solubility behaviors of oryzanol and soap in polar and nonpolar solvents form the basis for the separation of oryzanol by extraction [differential solubility during leaching and partitioning in liquid-liquid extraction (LLE)] (12).

Glycerides. The glycerides (glycerol esters of FA) present in soapstock are mostly in the form of TAG. The soapstock also contains DAG and MAG in minor amounts depending on the extent of hydrolysis of the oil. The TAG are soluble in most organic solvents such as hexane, isopropyl alcohol, chloroform, and ethyl acetate, but the DAG and MAG have lower solubilities in these solvents compared with TAG. The amount of TAG in RBOS varies depending on processing conditions used during the alkali-refining step. A suitable strategy for the removal of TAG from soapstock is to convert them to soaps by further saponification. The degree of saponification of all glycerides in soapstock varies with reaction conditions, but TAG saponify rapidly in aqueous alkali compared with the hydrolysis of oryzanol (10). Oryzanol is then separated from the soap by leaching (solid-liquid extraction) or LLE (12).

Phospholipids (gums). Both hydratable and nonhydratable gums are present in soapstock, and their relative proportions vary depending on the efficiency of the degumming step. The main component of hydratable gums is phosphatidylcholine (PC) whereas nonhydratable gums are calcium and magnesium salts of phosphatidic acid (PA) and phosphatidylethanolamine (PE) (13). The gums, especially the nonhydratable phospholipids, are the most interfering impurities affecting oryzanol separation from RBOS, probably owing to their high surface activity. During solvent extraction of RBOS, the gums stabilize the soapstock-solvent microemulsion and thereby decrease the rate of phase separation. To reduce their interference during the extraction step (solid-liquid and liquid-liquid) used for isolating oryzanol, efficient degumming of RBO is desirable prior to the alkali-refining step.

The hydratable gums in RBO can be removed by either water or acid degumming, or by the use of surface-active agents such as lauryl sulfate, sodium oleate, alkylated phenol ethylene oxides, or alkyl aryl sulfonates (14). These methods, however, are not capable of reducing the phosphorus content in RBO below 10 ppm. Methods for the removal of the nonhydratable phospholipids from RBO include superdegumming and enzymatic degumming. A superdegumming method developed and patented by Unilever is based on the principle that the nonhydratable phospholipids present in the RBO can be converted to a hydratable form by heating the oil to 70–80°C and treating with citric acid for about 20 min at this temperature (15). The hydratable phospholipids formed are allowed to crystallize as calcium and magnesium salts, waxes, and glycerol. Final neutralization gives oil with a phosphorus content of ≤5 ppm.

Enzymatic degumming has been proposed for treating the nonhydratable phospholipids of vegetable oils (16). The enzyme used, a phospholipase, hydrolyzes the ester bond of the phospholipids at the oil-water interface thereby converting the nonhydratable phospholipids into fully hydratable phospholipids and FFA. The hydratable phospholipids are then removed by conventional water degumming. This enzyme degumming method was extended to RBO, and after bleaching the oil had a phosphorus content of ≤5 ppm (17). Gums are partially soluble in hot acetone but insoluble in cold acetone. The solubility of phospholipids in hydrocarbon solvents decreases in the order pentane, hexane, and *n*-heptane.

Waxes. RBO waxes are esters of saturated FA (C_{16} to C_{26}) and saturated fatty alcohols (C_{24} to C_{30}) (3). The amount of wax present in RBO depends on extraction conditions, and levels can range as high as 8%. The common range in RBO is 2–4% with a level of <0.5% being desirable (3). Rice bran waxes can be classified arbitrarily into two classes: soft waxes (m.p. <75°C) and hard waxes (m.p. >80°C). The majority of waxes (about two-thirds) exist in polymeric form with the remainder being monomeric. Waxes tend to form stable emulsions during oil refining and thus reduce oil yields during processing (3). Common dewaxing methods include: gravity settling, combined degumming and dewaxing, and post-neutralization dewaxing (18). A combined degumming and dewaxing method was found to be best for reducing phosphorus content in RBO to ≤5 ppm. In the superdegumming process a part of the wax also crystallizes with the gums, which increases wax removal (18). Waxes are insoluble in acetone, ethyl acetate, and isopropyl alcohol but soluble in hot hexane (3).

Sterols. Sterols constitute a major portion of the unsaponified matter of RBOS (19). Sterols in RBO are mainly present as neutral sterols (free sterols and steryl esters), with some polar sterols (steryl glycosides and acylated steryl glycosides). The major sterols in RBO are β-sitosterol, campesterol, and stigmasterol. During alkali refining, considerable amounts of steryl esters and steryl glycosides are extracted into the soapstock from the crude oil (20).

The content of individual types of sterols in soapstock varies depending on processing conditions during alkali refining. Harsh processing conditions, such as high temperature and high pH during isolation of oryzanol, promote several chemical reactions of sterols such as hydrolysis, oxidation, dehydrogenation, and isomerization. Temperature, pH, and time of processing also contribute to the rate of sterol degradation. Alkaline hydrolysis cleaves the ester bond of steryl esters and releases its FA into the reaction mixture along with the sterol (21). Acid hydrolysis cleaves the acetal (glycosidic) bond of steryl glycosides and releases the carbohydrate from the sterol or steryl ester (21).

Neutral sterols (free sterols and steryl esters) are soluble in organic solvents such as acetone, chloroform, and ethyl acetate. Steryl glycosides contain carbohydrate moieties (sugar units) attached to sterol or steryl esters and therefore require relatively more polar solvents to solubilize them. This difference in solubility of esters and steryl glycosides forms the basis for the separation of sterol glycosides from steryl esters during the isolation process.

Resinous materials. Resinous materials in soapstock are thought to be formed by polymerization of wax components. The resinous materials can be saponified to triacontanol and soaps. The waxy-like components are significant interfering impurities during the purification of oryzanol, particularly in the chromatographic and crystallization processes. These impurities interfere with oryzanol separation during chromatography because they strongly adsorb onto the support along with oryzanol. These impurities also interfere in crystallization of oryzanol by disrupting crystal growth. During crystallization of oryzanol from the unsaponified fraction of soapstock, the waxy-like (mucilaginous) impurities precipitate out first when the temperature of solvent miscella is reduced from about 60–70°C (that is, the reflux temperature) to 25–30°C. At this temperature (25–30°C), oryzanol also crystallizes, thus making the separation of wax and oryzanol difficult (22). Hence, the separation of supernatant miscella from mucilaginous impurities should be carried out judiciously.

Tocopherol derivatives. Other impurities in RBO that are carried into the soapstock include tocopherol derivatives (i.e., tocopherols and tocotrienols or vitamin E). They are a family of compounds possessing a hydroxychromane ring and a terpenoid side chain (23). These components have been separated by normal-phase chromatography (23) and reversed-phase HPLC (24). In normal-phase chromatography, the elution order is tocopherols, tocotrienols, then γ-oryzanol by using 2.5% of ethyl acetate in isooctane as mobile phase at 1.6 mL/min (23). However, in reversed-phase HPLC, the tocotrienols elute first, followed by tocopherols, and γ-oryzanol. The mobile phase used was acetonotrile/methanol/*tert*-butyl methyl ether (65:30:5 by vol) using isocratic conditions at 1 mL/min. Subsequent analysis by CI MS identified the major oryzanol components as cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, campesteryl ferulate, β-sitosteryl ferulate, and cycloartanyl ferulate (24).

Pigments. Crude RBO contains pigments such as chlorophyll, carotenoids (lutein, xanthophylls), and protein degradation products (19). Among these pigments, concentrations of chlorophyll compounds are very high in crude RBO. The content of peptides in RBO varies depending on the degree of heating during stabilization of the rice bran (3). The common method of color removal from crude RBO is by bleaching using adsorbents such as activated clay and phosphoric acid (25). If these impurities are not removed during bleaching, they are carried into the soapstock during alkali refining.

Glycolipids. Glycolipids (phosphoglycolipids) have been identified in RBO (26). These compounds are critical interfering impurities affecting the purification of oryzanol. They interfere with the degumming step of RBO refining as a result of their very high surface activity, which leads to high oil losses during the deacidification step. It is recommended that glycolipids be kept to as low a content as possible in crude RBO. One approach is enzymatic pretreatment with Lipase G, which has a hydrolysis function similar to that of a phospholipase (26). This enzyme hydrolyzes glycerides, phospholipids, and glycolipids and reduces the phosphorus level to about 5 ppm (26).

APPROACHES FOR PURIFICATION OF ORYZANOL

When developing large-scale processes, it is mandatory to consider productivity (time, energy, and human resources requirements), environment- and health-related problems, and unit operations/processes investment (equipment and recovery of solvents) apart from separation efficiency (purity and yield of product). Many patented processes report equilibrium-governed unit operations such as extraction, precipitation, and crystallization for the

TABLE 1
Summary of Extraction Methods, Feedstocks Used, Purity, Yield of Oryzanol, and Potential for Commercial Application of the Processes

Method description	Feedstock	Purity (%)	Yield (%)	Potential	Reference
Extraction-based:					
Solid-liquid	Wet soapstock	85	—	Yes	27
	Wet soapstock	—	0.1	No	28
	Dry soapstock	40–45	80	Yes	29
Liquid-liquid	RBO[a]	60	2.5	No	30
Supercritical fluid	RBO	—	1.8	No	32
		—	1.11 mg oryzanol/g rice bran	No	33
Crystallization-based or precipitation-based:					
	Oryzanol	—	(Fractionated cycloartenol ferulic acid ester)	—	34
	Second soapstock	98	1.9% of second soapstock	—	35
	Soapstock	65	70	Yes	22
Other:	Acid oil	98.3	32.6	Yes	36
	Acid oil	—	—	No	37
	Soapstock	90	—	—	38
	Soapstock	90	56–70	No	39
	Soapstock	51.4	75.7	No	6

[a]RBO, rice bran oil.

isolation of oryzanol. A summary of the different methods used for extracting oryzanol from RBOS is listed in Table 1 and discussed below.

Extraction-based processes: Solid-liquid extraction (leaching). Leaching is probably the simplest operation for the separation and purification of oryzanol from RBOS. Leaching is the extraction of soluble components from an insoluble permeable solid phase using a suitable solvent. Leaching operations typically used in patented processes are for two purposes: (i) leaching of oryzanol from the dried soapstock and (ii) leaching of impurities from the oryzanol-enriched fraction. The choice of solvent intended for leaching differs depending upon the intent of the process. The basis for selection of a solvent for leaching is the differential solubility of oryzanol and the impurities in the leaching solvent. Critical process parameters that need to be considered for a given leaching process are solid-to-solvent ratio, temperature, and time.

Masao and Yoshizane (27) reported a patented process for obtaining 85% pure oryzanol from RBOS using a two-step leaching process. In the first step, the major impurities, mainly soap, were separated from the soapstock by solvents such as methanol or ethanol while simultaneously sparging carbon dioxide through the mixture. Sparging with carbon dioxide facilitated preferential leaching of the soap, leaving oryzanol in the purified medium. Thus, carbon dioxide sparging improves selectivity of extraction and reduces oryzanol losses in the leaching medium. In the second leaching step, weak alkali salts such as sodium bicarbonate or sodium carbonate (which were associated with the residue during the first leaching step) were removed by methanol or ethanol washing. This process has the advantage of using fewer unit operations and sufficient quantity of solvent for efficient removal of impurities.

Takeshi (28) reported a patented process involving eight steps for obtaining oryzanol from soapstock. RBOS was initially converted to acid oil with sulfuric acid; the acid oil was esterified to methyl esters with a mixture of sulfuric acid and methyl alcohol; and the FAME were removed by distillation. The residue remaining after distillation of the FAME was subjected to successive leaching with *n*-hexane, methanol, and methanolic alkali. In each of the leaching steps, the residue obtained from the previous step was taken as the raw material for the next step. Here the distillation process was improved by esterifying FFA, since the esters distill off at a lower temperature than FFA, which results in an energy saving process. The major limitation of this process is the number of unit operations involved.

Recently, Indira *et al*. (29) reported a process for obtaining oryzanol of 40–45% (w/w) purity and 80% (w/w) recovery. The raw material used in this study was RBOS that had been saponified for a second time and dehydrated. The latter material was subjected to a leaching step using solvents such as ethyl acetate, acetone, or their mixtures to extract the oryzanol. This process offers several advantages such as reduced scale of operation (as considerable reduction of moisture in soapstock is achieved by dehydration), increased interfacial area for efficient leaching by micromixing, and hence, ease of scaleup. It was noted that milder operating conditions, with regard to temperature and pH, could be used to minimize degradation of oryzanol during processing. The limitation of this process is that it results in oryzanol of varying purity (33–43% w/w) and recovery (57–80% w/w).

LLE. The performance of LLE-based processes mainly depends on the differential partitioning of oryzanol and the other soapstock components into the two immiscible liquid phases. The criteria for selection of the two phases and conditions for

LLE are differential partitioning of the product and impurities into each phase and rapid phase separation after mixing. Important process parameters that need to be considered during LLE are solid content of the two-phase system, phase ratio, temperature, and tie-line length, which is a line joining the two points of a binodial in a phase diagram describing the two solvent phases. The tie-line length gives the composition of solvents required for LLE. Selected patented processes based on LLE are discussed in this section.

Tsuchiya *et al*. (30) obtained oryzanol of 60% (w/w) purity and 2.5% (w/w) yield from RBO (100 kg). The process uses two saponification steps, hydrolysis of the residue obtained after a second saponification with HCl, and finally a two-step LLE with ether and alkali. The first saponification step allows for obtaining soapstock from RBO whereas the second saponification step converts oryzanol containing neutral oil into its salt form. The residue remaining after the second saponification step is acidified, thus converting the salts of oryzanol into their free acid forms. The product obtained after hydrolysis is subjected to a two-step LLE using ether and aqueous alkali. In the first extraction, the impurities are extracted into the ether phase while the aqueous phase the oryzanol acids are converted to sodium salts. In the second extraction the target product (oryzanol) is extracted into the ether phase. The drawback of this process is the number of steps and the low yield of oryzanol.

Supercritical fluid extraction (SFE). SFE of RBO with carbon dioxide (CO_2) has certain advantages, namely, the low price of CO_2, its noncombustibility, and its safety in both foods and the environment (31). The work on CO_2 SFE of RBO has been done mainly with respect to extractability, and to some extent the scale-up operations, of various unsaponifiable components in RBO such as FFA, TAG, and sterols.

Recent studies have shown that the yield of oryzanol by SFE is higher than that achieved by solvent extraction using different solvents and processing conditions (32,33). These authors carried out experiments on a small scale and attempts are in progress for scaling up the process. However, to our knowledge, no reports are available using unsaponified matter from RBO as the raw material for SFE. The limitation of SFE is that the fluctuations in flow rate and pressure cause variations in results, and equipment and installation are expensive (33).

Crystallization or precipitation-based methods. During crystallization the parameters considered are temperature of the solvent or mixture of solvents along with their proportions, rate of nucleation, and rate of crystal growth. In most of the patent literature related to oryzanol crystallization, the authors do not fully explain purity and yield of oryzanol, which makes it difficult to interpret the results and draw conclusions. In this section a few patents pertaining to crystallization of oryzanol are discussed.

Koji and Tokuo (34) described a two-step procedure for obtaining a purified and concentrated form of oryzanol, namely, cycloartenol ferulic acid ester. The product obtained after an initial crystallization step was the starting material used in a multistage crystallization process using a mixture of alcohol and hydrocarbon solvents. The alcohols were methanol, propanol, or butanol, and the hydrocarbons were hexane, cyclohexane, or toluene. The solvents for recrystallization were recovered by distillation. The authors did not mention the initial raw material used nor the final purity and yield of oryzanol.

Mingzhi and Yanyan (35) reported a four-step procedure for extraction of oryzanol from RBOS. The starting material was a second soapstock (the extra alkali added during the second alkali-refining of RBO results in the formation of another soapstock fraction) of RBO, which was subjected to multiphase fractional crystallization steps. The purity of the oryzanol obtained at the end of this crystallization process was 98% (w/w) with a yield of 1.9% (w/w). The authors did not mention the weight of the second soapstock fraction, hence, the total yield from the process is not known.

Narayan *et al*. (22) used the unsaponifiable material of soapstock (obtained by leaching of pretreated and dehydrated soapstock) as the starting material for the crystallization of oryzanol. Crystallizing solvents used were mixtures of acetone and methanol in different proportions at the reflux temperature. After cooling to room temperature, mucilaginous impurities (i.e., waxes) precipitated out. The mucilaginous impurity was separated, and the eluate was cooled further to 5–10°C overnight for oryzanol crystallization. Oryzanol was obtained at 65% (w/w) purity, and the yield was 70% (w/w). The novelty of this patented process is the selection of the starting material (pretreated and dehydrated RBOS), which had less mass transfer resistance because of the increased surface area and partial removal of interfering impurities such as gums. This patent has the potential for attaining a highly pure oryzanol fraction by incorporating a second recrystallization step after the initial crystallization steps.

Other methods. Other methods for obtaining oryzanol include a combination of methods involving solid-liquid extraction (leaching) and crystallization or LLE and crystallization processing.

Yasuo *et al*. (36) used a four-step procedure for extraction of oryzanol from rice bran acid oil (RBAO). The steps were esterification of RBO to methyl esters, molecular distillation, LLE, and crystallization. FFA were esterified and removed by molecular distillation. Here, esterification of FFA minimizes the heat requirement for distillation because methyl esters of FFA boil at relatively lower temperatures than their corresponding acids. The unsaponified fraction obtained after distillation was subjected to LLE using a mixture of *n*-hexane and THF. The unsaponified fraction initially partitioned into the THF phase, which was then concentrated, thereby increasing the polarity of the solution. The oryzanol was then precipitated by the addition of water and recovered in the aqueous phase. Final crystallization of oryzanol was carried out with hexane. The yield of oryzanol obtained was 32.6% (w/w) with a purity of 98.3% (w/w). This process, however, has the drawback of giving low recovery of oryzanol from the original content in the RBAO.

Tsuchiya and Okubo (37) reported a two-step procedure for extraction of oryzanol from RBAO. The first step involves es-

terification of the FFA to methyl esters and subsequent distillation of the esters to obtain on unsaponified residue. The second step involves subjecting the unsaponified material to column chromatography using a mixture of alcohol and ether as an eluant. The eluant was water-washed and desolventized to obtain oryzanol. This process has the drawback of using column chromatography, which has problems of pressure drop in the column and channeling, leading to uneven flow especially in large-scale applications. The cost of the adsorbent and solvent are high when used on a large scale. The yield of the process also was low, 1.75% (w/w), and the purity of the oryzanol was not reported.

Masao and Yoshizane *et al.* (38) reported a four-step procedure for the extraction of oryzanol from RBOS, including a two-step leaching process and LLE. The objective of the leaching steps was to extract impurities into solvents such as trichloroethylene, benzene, *n*-hexane, or a mixture of benzene and *n*-hexane. The impurities (mainly soap) from the second leaching step were extracted with water while purging the mixture with CO_2 gas for acidification to facilitate leaching. The residual mixture obtained after a second leaching step was subjected to LLE (with the same solvents used in the first leaching step) to extract oryzanol into the organic phase. Finally, oryzanol was obtained in a crystalline form by removing the solvent. The purity of oryzanol obtained was ≥90% (w/w) (% in RBOS). The drawback of this patented method is that it uses chlorinated or aromatic solvents for LLE, which are difficult to remove and require stringent safety methods for use, adding to the cost of processing.

Rao *et al*. (39) used a six-step procedure for isolating oryzanol from RBOS. Their procedure includes saponification of the neutral oil present in the soapstock, conversion of the soapstock to an anhydrous material, leaching followed by crystallization, column chromatography, and recrystallization to obtain 90% (w/w) pure oryzanol in an overall yield of 56–70% (w/w). The drawback of the method is that a number of unit operations are involved and that the anhydrous porous RBOS material has considerable mass transfer resistance (due to increased diffusion distance or thickness) during oryzanol extraction. Furthermore, the use of a step involving a Soxhlet extraction process reduces selectivity of leaching at higher temperatures, and a column chromatography step is difficult to scale up. All of these observations indicate the process will be costly and hence may not be commercially feasible.

Seetharamiah and Prabhakar (6) reported a four-step process including LLE, column chromatography, crystallization, and recrystallization to extract oryzanol from RBOS. The solvents used for LLE were a mixture of diethyl ether and methanol. In the latter step, most of the soap could be extracted with methanol. The ether-rich phase was extracted repeatedly with aqueous alkali to isolate most of the oryzanol in the aqueous phase, and the remaining impurities were removed by ether extraction. Finally, the alkaline oryzanol extracts were pooled and neutralized with acetic acid, and oryzanol was extracted back into diethyl ether and the ether phase desolventized to obtain an oryzanol concentrate. This fraction was then subjected to column chromatography on alumina and eluted with a solvent such as hexane and then subsequently with petroleum ether/methanol (9:1 vol/vol) and diethyl ether/methanol (20:1 vol/vol), and the resulting oryzanol product was crystallized. Two crystallizations were done, one with methanol and one with a mixture of methanol/acetone (2:1 vol/vol). At the end of the chromatography step, the yield of oryzanol was 75.7% (w/w) with a purity of 51.4% (w/w). However, the authors of this article did not mention the purity and yield of oryzanol at the end of the recrystallization steps. The limitation of this process is that in the LLE, repeated extraction has to be carried out, which increases the number of extraction steps and decreases the yield of oryzanol. Hence this method may be limited for scaleup production.

ENGINEERING ASPECTS OF ORYZANOL EXTRACTION

The solid handling capacity is defined as the total quantity of dehydrated RBOS that can be processed with a given amount of solvent, which dictates the scale of operation required for the leaching of RBOS and initial investment cost for equipment. Hence it is essential to predetermine the maximal solid handling capacity of the initial processing steps. Leaching RBOS at elevated temperature improves solid handling capacity. However, when organic solvents are used for leaching, high temperatures are not preferred because of solvent losses by evaporation and pressure buildup inside the leaching equipment.

For solid-liquid extraction (leaching) some approaches recommended are: (i) increasing the particle size of broken noodles (a type of dried soapstock produced when wet RBOS is passed through conventional noodle-making equipment such as an extruder) for easy handling and keeping the unwanted material from getting into the extracting phase; (ii) increasing the interfacial area for leaching (pretreated and dehydrated soapstock); and (iii) dispersing the dried soapstock within a compatible solvent environment. The first operation appears promising, but certain disadvantages are noted such as a reduction in the solid-liquid interfacial area available for leaching and an increase in mass transfer resistance, as solvent has to diffuse over a relatively larger distance. In the second operation, the solid-liquid interfacial area is relatively large owing to smaller particle size; however, unwanted material also may be extracted. In the third operation, the increased surface area aids leaching.

Drying operations require considerable attention when selecting the raw material (soapstock) for solid-liquid extraction (leaching). Soapstock contains 70% or more water, hence, its dehydration is important since considerable reduction in scale of subsequent unit operations can be achieved. The form in which water (bound, unbound, entrapped) is present in the soapstock and the sensitivity of dried RBOS to air needs to be addressed. The form of the water present directs the selection of the drying equipment (tray, vacuum, drum dryers).

The major difficulties in isolating oryzanol from soapstock by extraction (either solid-liquid leaching or LLE) are long

phase-separation times and poor partitioning of solute into the extracting phases. As mentioned earlier, RBOS contains significant amounts of surface-active and viscous components, which interfere during the mixing and demixing phases of the process. For instance, during solvent extraction the degree of mixing is crucial, and above a critical solvent level, emulsions can form that are difficult to separate. If the solvent level is below a given level, the extent of mass transfer into the extracting phase is poor owing to the low interfacial area available for mass transfer. As a result, repeated extractions must be done to achieve the desired level of oryzanol separation. Any attempt to destabilize an emulsion by incorporating other solvents, such as alcohol, to hasten emulsion separation leads to problems since the additional solvent partitions unevenly between the phases, hampering the mass transfer of oryzanol into the desired phase thus reducing yields. Although the incorporation of polar solvents results in faster phase separations, the increased processing cost associated with regeneration requires a search for an economic alternative for handling a large amount of solvent during commercial scaleup. The problem can be solved to some extent by recycling the solvent.

During LLE of oryzanol, microemulsion formation (thermodynamically stable biphasic microsize emulsion stabilized by phospholipid, waxes and mono- or diesters of FA or micellar solvation of oryzanol) is often observed. Hence, factors affecting the recovery of oryzanol with this method need to be identified.

During crystallization of oryzanol from the unsaponified matter of RBOS, the precipitation of mucilaginous (waxy-like) impurities often is observed. The precipitation of these impurities takes place when the crystallizing mixture is slowly cooled from 60–70°C to 25–30°C (22). The removal of these impurities should immediately be carried out by decantation of the supernatant. Further lowering of the temperature results in crystallization of oryzanol. The co-precipitation product is a gelatinous-like precipitate, which makes the filtration process difficult owing to its high viscosity. Hence, solid-liquid separation requires proper attention before scaling up the process.

The scaling up of a chromatographic separation step for isolation and purification of oryzanol requires additional engineering inputs. When the column chromatography step is operated in a batch mode, it has problems such as pressure drop, channeling leading to uneven column flow, and the like, which make the scaleup of this step difficult. Alternate operational modes are required in carrying out large-scale chromatography for effective adsorption, washing, and elution operations. One such example is a silica-based continuous chromatography unit (12). This simulated continuous-bed chromatography had a throughput of 9200 kg/d; and the sizes of particles that the stationary phase could handle were >100–300 μm. Thus, large-diameter particles could be used for separation as compared with the batch process. This countercurrent unit resulted in a continuous separation with reduced solvent and absorbent. The amount of solvent used in the countercurrent unit was about 2–5 times less as compared with a batch unit.

Physically refined RBO is a natural oil having positive nutritional characteristics due to the presence of PUFA and to the high levels of natural oxidants such as oryzanol, tocopherol, and tocotrienols (40). It should be noted that presently, physically refined RBO is the main form being marketed (26,41) and that no soapstock is produced from physical refining RBO as compared with chemical refining.

Soapstock from the chemical refining of RBO is the major source of oryzanol. It is reiterated here that conditions of the oil refining process and the seasonal variation of rice bran composition are the two major factors affecting RBO yield variability and the types and amounts of surface-active impurities in the final RBOS. Conventional saponification is carried out at high temperatures and long times (HTLT), which leads to hydrolysis of oryzanol thus reducing its yield. Accordingly, RBO processing conditions need to be optimized to minimize the degradation of oryzanol during processing.

SFE is an emerging technology for extraction of oryzanol from rice bran or RBO, and initial laboratory-scale trials have shown promising results. Production-scale operations, however, require optimized process conditions such as temperature, pressure, and the like. Other limitations of SFE are its high capital costs and low solid material handling capacity as compared with leaching and LLE.

If we consider a plant that processes about 1500 kg of RBOS per day to give a production capacity of approximately 34 kg of oryzanol per day, the total fixed processing cost (the sum of land development and building, plant and machinery and preoperating expenses) will be about US$9.5 million. The total installation cost of plant and machinery will be about $6.5 million while the working capital required will be about $300,000 for 30 days. The variable cost (the sum of processing costs, financial expenses and depreciation per annum, and the total working capital per month) amounts to about $5.9 million. The total cost of production would be about $6.2 million, and the net profit of this plant is estimated to be about $750,000 with a payback period of approximately 1.25 years.

Some of the problems in unit operations such as leaching, LLE, and crystallization after recovery of oryzanol from RBOS once solved can result in a recovery of 50–60% oryzanol with a purity of 60–70% in large-scale operations (22,29). However, the chemical basis for the interference of the surface-active impurities in RBOS during extraction of oryzanol from RBOS is not well understood. The ability to obtain very pure oryzanol cost effectively in high recovery from RBOS still remains a research challenge.

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