

Enzymatic Degumming of Rice Bran Oil

Sir:

Annual world production of rice is approximately 500 million metric tons (MMT). Rice bran is a valuable co-product of the rice-milling industry, and its oil content ranges from 12–25% depending on the quality of the bran. The estimated annual production potential for rice bran oil is 3 to 4 MMT, but the actual annual production is far below this at 0.5 MMT, with much of it being of technical grade and mostly used for soap manufacture (1). Owing to inferior processing conditions and lack of cost-effective technologies, the majority of the oil does not go into human consumption. Rice bran oil has a balanced FA profile and contains a host of minor constituents with proven nutritional benefits such as γ -oryzanol, tocotrienols, tocopherols, and squalene. At the same time, rice bran oil differs from other vegetable oils because of its higher FFA content along with its unusually high contents of wax, unsaponifiable constituents, polar lipids (including glycolipids), and pigments (2).

The majority of the nutritional components present in rice bran oil are destroyed or removed during traditional alkali refining. Chemical refining of rice bran oil generally results in losses that are considerably higher than those encountered with other vegetable oils (3,4). These higher losses are attributed to the presence of larger amounts of FFA and nonoily constituents. Refining losses can be considerably reduced using physical refining. The important prerequisite for successful physical refining is to reduce the phosphorus content in the oil to <10 ppm, as phosphorus-containing components cause color fixation in the final oil during exposure to the higher temperatures of physical refining. Water degumming is the simplest method for removing phospholipids (lecithin) from vegetable oils. However, only hydratable phospholipids can be removed during water degumming, leaving 80 to 200 ppm of phosphorus in the oil, depending on the type and the quality of the crude oil and the presence of nonhydratable phospholipids (5).

Many refineries are pretreating rice bran oil with phosphoric acid or an organic acid (such as citric) to remove nonhydratable phospholipids (1). Rice bran oil samples with varying contents of FFA have been degummed with a number of degumming agents, including water, organic acids, inorganic acids, inorganic salts, and surface-active compounds to obtain rice bran oil free of phospholipids (6). However, these methods failed to reduce the phosphorus content to <10 ppm.

Lurgi's Enzymax[®] process (Lurgi AG, Frankfurt am Main, Germany) can also be used to convert nonhydratable phos-

pholipids into water-soluble lysophospholipids, which are then removed by centrifugation, yielding degummed oil low in phosphorus. The Enzymax process employs phospholipase A₂ isolated from porcine pancreas (7). Microbial phospholipase has proven to be superior to porcine pancreatic lecithase and other phospholipases with respect to oil degumming performance and is suitable for degumming oil of different qualities, ranging from water-degummed to crude oil (8). However, enzymatic degumming has only been reported for rapeseed, soybean, and sunflower oils (7,8). In the present study, rice bran oil was degummed with phospholipase A₁ to reduce the phosphorus content to <5 ppm.

Crude rice bran oil (500 g, procured from a local solvent extraction company) was placed into a three-necked round-bottomed flask fitted with a mechanical stirrer and reflux condenser. The oil was heated to about 70°C in an oil bath, and a citric acid solution (45% in water, 0.72 mL) was added. After stirring the contents for 30 min, the temperature of the oil was decreased to 45°C. A sodium hydroxide solution (4% in water, 2.5 mL) was added, and the contents were thoroughly mixed to maintain the pH at 4.5, followed by the addition of lecithase Novo (0.1 mL). Lecithase Novo from Novozymes A/S (Bagsvaerd, Denmark) (produced by submerged fermentation using genetically modified *Aspergillus oryzae*) was used as the source of phospholipase A₁ (4,000 Lecithase Novo units per g). After addition of the enzyme, the oil was subjected to high-shear mixing (10,000 rpm) for 30 min while maintaining the temperature at 45°C. Stirring was continued using a laboratory mechanical stirrer for another 1.5 h, and then the temperature was increased to 70°C to facilitate separation of the oil during centrifugation. The aqueous phase, containing lysolecithin and enzyme, was separated from the oil by centrifugation.

In another set of degumming trials, the enzyme reaction was continued for 4 h. The degummed rice bran oil was then bleached with a mixture of neutral earth and activated carbon (2 and 0.5%, respectively, on the basis of oil) under a pressure of 15–20 mm Hg at 100–105°C and filtered. Enzymatic degumming was also carried out in a similar way with water-degummed rice bran oil. In this case, the crude rice bran oil was initially degummed with 2% water in the traditional way (6), followed by enzymatic degumming as described above.

FFA and phosphorus contents of crude, degummed, and bleached oils were determined by standard AOCS method Ca 5a-40 (9) and standard IUPAC method 2.421 (10), respectively (Table 1). The modest increase in FFA content of the enzyme-degummed oil from 1.4 to 1.7% was due to the FA released during enzymatic hydrolysis of the phospholipids present in the oil. No significant changes in the FFA content were observed when the reaction time was increased from

TABLE 1
Changes in Phosphorus and FFA Contents During Enzymatic Degumming and Bleaching of Rice Bran Oil^a

Substrate	Reaction time (h)	Phosphorus (ppm)			FFA as oleic acid (%)		
		Before enzymatic degumming	After enzymatic degumming	After bleaching	Before enzymatic degumming	After enzymatic degumming	After bleaching
Crude rice bran oil	2	403 ± 3.6	18 ± 0.82	2.4 ± 0.29	7.9 ± 0.25	9.4 ± 0.37	9.4 ± 0.22
Crude rice bran oil	4	403 ± 3.6	16 ± 1.0	2.0 ± 0.08	7.9 ± 0.22	9.6 ± 0.29	9.4 ± 0.37
Water-degummed rice bran oil	2	60 ± 2.9	18 ± 1.41	3.0 ± 0.29	8.0 ± 0.22	9.4 ± 0.22	9.2 ± 0.14
Water-degummed rice bran oil	4	60 ± 2.9	15 ± 0.91	2.4 ± 0.33	8.0 ± 0.34	9.4 ± 0.29	9.2 ± 0.28

^aMean values of four replications ± SD.

2 to 4 h. The phosphorus levels of crude rice bran oil (403 ppm) and water-degummed oil (60 ppm) were reduced to 15–18 ppm after enzymatic degumming. In the case of rapeseed oil, the phosphorus level was reduced to <5 ppm using a similar phospholipase A₁ degumming process (8). In this case, the phosphorus content of the rice bran oil was also reduced to <5 ppm after bleaching the enzyme-degummed oil. The results indicate that phospholipase A₁-mediated degumming followed by bleaching is a very mild protocol for pre-treating rice bran oil to reduce the phosphorus levels to <5 ppm for physical refining.

ACKNOWLEDGMENTS

This work was supported by a research grant from the Technology Mission on Oilseeds, Pulses & Maize, Ministry of Agriculture, and the Council of Scientific & Industrial Research, Government of India (IICT Communication No. 020105).

REFERENCES

- Gingras, L., Refining of Rice Bran Oil, *inform 11*:1196–1203 (2000).
- Kitts, D., Toxicity and Safety of Fats and Oils, in *Bailey's Industrial Oil and Fat Products*, 5th edn., edited by Y.H. Hui, John Wiley & Sons, New York, 1966, Vol. 1, pp. 215–280.
- Cousins, E.R., R. Prachankade, and S. Bhodhiprasart, Ethanolamines and Other Amino- and Hydroxy-Containing Compounds in the Refining of Rice Oil, *J. Am. Oil Chem. Soc.* 32:561–564 (1955).
- Hartman, L., and M.I.J. Dos Reis, A Study on Rice Bran Oil Refining, *Ibid.* 53:149–151 (1976).
- Gibon, V., and A. Tirtiaux, Removal of Gums and Waxes—A Review, *inform 11*:524–535 (2000).
- Bhattacharyya, A.C., and D.K. Bhattacharyya, Degumming of Rice Bran Oil, *J. Oil Tech. Assoc. India* 17:27–30 (1985).
- Buchold, H., Enzymatic Separation of Phosphatides from Vegetable Oils, *Fat Sci. Technol.* 95:300–304 (1993).
- Clausen, K., Enzymatic Oil-Degumming by a Novel Microbial Phospholipase, *Eur. J. Lipid Sci. Technol.* 103:333–340 (2001).
- Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., edited by D. Firestone, AOCS Press, Champaign, 1994, Method Ca 5a-40.
- Standard Methods for the Analysis of Oils, Fats, and Derivatives of the International Union of Pure and Applied Chemistry*, 7th edn., edited by C. Paquot and A. Hautfenne, Blackwell Scientific Publications, Oxford, United Kingdom, 1987, Method 2.421, pp. 183–184.

S.K. Roy, B.V.S.K. Rao, and R.B.N. Prasad*
 Lipid Science & Technology Division
 Indian Institute of Chemical Technology (CSIR)
 Hyderabad, 500 007, India

[Received January 14, 2002; accepted March 28, 2002]

*To whom correspondence should be addressed.
 E-mail: rbnprasad@iict.ap.nic.in