

Enzymatic Treatment of Rice Bran to Improve Processing

N. Hernandez, M.E. Rodriguez-Alegría, F. Gonzalez, and A. Lopez-Munguia*

Instituto de Biotecnología, UNAM, Cuernavaca, Morelos 62271, Mexico

ABSTRACT: A modification of the process of oil extraction from rice bran is proposed, introducing one or two enzymatic reactions previous to solvent extraction. Although a total aqueous enzymatic extraction process did not result in reasonable oil extraction yields, an interesting alternative results from enzymatic reactions previous to solvent extraction or pressing. A thermal treatment of rice bran is first applied to deactivate lipase, but also to gelatinize starch previous to reaction with α -amylase. This is followed by a saccharifying step with glucoamylase to produce glucose (28 g/100 g of rice bran treated), while the residual paste, 66.7% of the original bran, may be subjected to a proteolytic process for protein extraction or directly treated with the solvent to obtain bran oil. Finally, under the defined extraction conditions using hexane, yields of oil are 5% higher when rice bran has been previously treated with α -amylase.

Paper no. J9159 in *JAOCS* 77, 177–180 (February 2000).

KEY WORDS: α -Amylase, glucoamylase, glucose, protease, rice bran oil, rice bran oil extraction.

Rice is produced in more than 100 countries, accounting for about 25% of all cereal grain production (1). More than 534 million tons were produced in 1994. Rice is not a common source of edible oil compared to other traditional cereal or seed sources such as corn, cotton, sunflower, or soybean among others. However recent research has demonstrated that rice bran oil can be recommended for the replacement of saturated fat in the diet, as unsaponifiable ingredients such as oryzanol, a mixture of ferulic acid esters of sitosterol and cycloartenol, which are unique to rice bran oil, have the ability to reduce blood cholesterol levels, particularly low density lipoprotein (LDL)-cholesterol (2). Rice bran is the brownish portion of rice taken out in powder form during dehusking and milling of paddy. The hull accounts for 20% of the rice seed, while about 6% is obtained as bran, including the pericarp, the seed coat, the nucellus, the aleurone layer, and part of the exterior layers of the endosperm. Although rice bran contains about 20% oil, 15% protein, and approximately 50% carbohydrate, of which starch is the main component, it has never been proposed as a source of sugars. In developing countries, production is dispersed among several rice mills that usually lack processing facilities for by-products. In some cases, it is used as cattle feed, but for the reasons al-

*To whom correspondence should be addressed at Instituto de Biotecnología, UNAM, Apartado Postal 510-3, Cuernavaca, Morelos 62271, Mexico. E-mail: agustin@ibt.unam.mx

ready mentioned, there is a growing interest in recovering its components, particularly oil and its soluble components.

Oil extraction has been traditionally carried out with hexane. In a typical process, stabilized rice bran is treated with hexane to obtain crude oil and defatted bran. Several methods exist to stabilize the bran by thermally inactivating rice lipase, which otherwise affects the quality of oil, increasing its fatty acid content (3). Hexane extraction conditions have been optimized particularly in terms of extraction temperature (4), as it is related to the final wax and gum content of the extracted crude oil.

Enzymes have been used in vegetable oil extraction processes to facilitate oil release and in some cases to eliminate the requirements of hexane in aqueous extraction processes (5). After hydrolytic reaction of exogenous enzymes with ground seeds or other oily products, the oil is obtained either by direct centrifugation of an emulsified phase when sufficient water is used, or by pressing the resulting paste; e.g., coconut or avocado (6,7). However, in many cases, the extraction of oil is only facilitated by the enzymatic treatment as the oil is obtained by the usual solvent extraction method from the dewatered cake after the aqueous reaction (8). One advantage of the aqueous extraction processes is that during the partial degradation of the cell wall components, by-products may be obtained, particularly protein concentrates in the aqueous phase. Currently, about 450,000 metric tons of rice oil are produced worldwide (9). However, there are few reports concerning the use of enzymes as an alternative for rice bran processing. Nevertheless, in a recent publication, the extraction of a higher-quality oil and the production of a protein concentrate as a by-product of an aqueous enzymatic treatment of rice bran previous to hexane extraction was reported (10). In the present paper, we report an alternative method for processing rice bran.

MATERIALS AND METHODS

Rice bran was provided by the Buenavista rice mill (Cuautla, Morelos, Mexico). Its average original composition is shown in Table 1. Enzymes were kindly provided by Novo Nordisk through Biotec SA (Thermamyl® 120L α -amylase, Viscozyme and Celluclast; Mexico City, Mexico); ENMEX (Diazyme glucoamylase and HT Proteolytic; Mexico City, Mexico); and Enzimas y Productos Químicos SA de CV (Protease 2A; Mexico City, Mexico).

Usually, enzymatic reactions were carried out in 150-mL agitated glass reaction vessels at 200 rpm using a jacket for temperature regulation. Batch reactions were performed with

TABLE 1
Changes in Composition of Rice Bran During Different Steps of the Enzymatic Process

	Rice bran		Rice bran after α -amylase treatment		Rice bran after hexane extraction	
	%	g	%	g	%	g
Moisture	9.0	0.9	5	0.33	7.1	0.39
Protein	13.4	1.34	5.8	1.05	19.2	1.06
Total fiber	6.5	0.65	10.1	0.67	10.1	0.56
Ash	7.6	0.76	8.5	0.57	9.9	0.55
Oil	15.2	1.52	20	1.33	2.8	0.15
Carbohydrates	48.3	4.83	40.6	2.7	50.8	2.79
Total mass	—	10	—	6.66	—	5.51

10 g of rice bran in 50 mL of water. The usual enzyme load was 1% wt/vol at the indicated temperature and pH. After the enzymatic reaction, the residual bran meal was recovered by centrifugation at 8,000 rpm for 15 min.

Analysis was done using AOAC methods (11): oil was measured by Soxhlet method (945.48); total protein by Kjeldahl (955.04); fiber by method 962.09; ash by method 945.46; and total carbohydrates were determined by difference. Soluble protein was measured by the method of Lowry *et al.* (12). Sugars also were analyzed by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). For TLC, 10 cm \times 10 cm \times 200 μ m Whatman silica gel 60 plates (Maidstone, United Kingdom) were used, with butanol/ethanol/water (3:5:2) as solvent. The plates were developed by spraying α -naphthol and sulfuric acid in ethanol and heating at 100°C (13) for 3 min. HPLC analysis of the products was carried out using Waters-Millipore (Milford, MA) chromatography equipment with a refractive index detector and a Nova-Pak (Milford, MA) aminated (4.6 \times 250 mm) column using acetonitrile/H₂O (70:30) as eluant at a flow rate of 1.4 mL/min. Maltodextrins and methyl-glucoside (Sigma, St. Louis, MO) were used as standards.

Statistics. All experiments were conducted in triplicate. Data are reported as means \pm SD.

RESULTS AND DISCUSSION

Enzymatic reactions involving rice bran in water with common macerating enzymes such as glucanases present in commercial products like Viscozyme or Celluclast, resulted in low extraction yields when a 1:5 (w/w) bran/water ratio was used. Less than 20% of the total oil content (Table 1) was recovered with the oil included in oil bodies on the water surface after centrifugation, and stabilized by lipophilic proteins. No improvements were obtained by changing parameters such as reaction temperature (up to 50°C), enzyme concentration (up to 3% wt/vol), and centrifugation speed (15 min at 12,000 rpm). Also, when evaluating the extraction yields with hexane, no improvements in yield were obtained.

Rice bran is particularly unstable due to the presence of lipase, which is activated once the cell structure is disrupted during milling, requiring a thermal treatment to avoid hydrolysis of triglycerides. Considering the important amount of starch present in rice bran, we decided to extend the heat treatment to 15

min at 120°C, instead of 15 min at 90°C or 1 min at 120°C, which previously was proposed (1,10), in order not only to deactivate the lipase but also to gelatinize starch, and to carry out a liquefaction treatment with α -amylase (Thermamyl). The first enzymatic treatment was carried out at 90°C, pH 6. After 2 h of reaction, the whole medium was centrifuged and the supernatant containing the solubilized starch hydrolysate adjusted to pH 4.2 followed by the addition of 1% wt/vol glucoamylase (Diazyme) at 60°C. Reducing sugars and glucose production were followed during the reaction as shown in Figure 1, where it may be observed that after 20 h of reaction, a glucose concentration of 70 g/L in the reaction medium was obtained, equivalent to 28% of the initial rice bran. An HPLC chromatogram of the reaction medium before and after the saccharification treatment is shown in Figure 2. It may be observed that, as expected, the main product obtained was glucose, with a small amount of released sucrose. A mass balance and proximate analysis of the residual bran are given in Table 1; 84% of the solubilized carbohydrates was recovered as glucose, 5% as sucrose, and the remaining 10% probably corresponded to pectin.

The residual bran may then be subjected to a proteolytic treatment in order to obtain a protein concentrate as already pro-

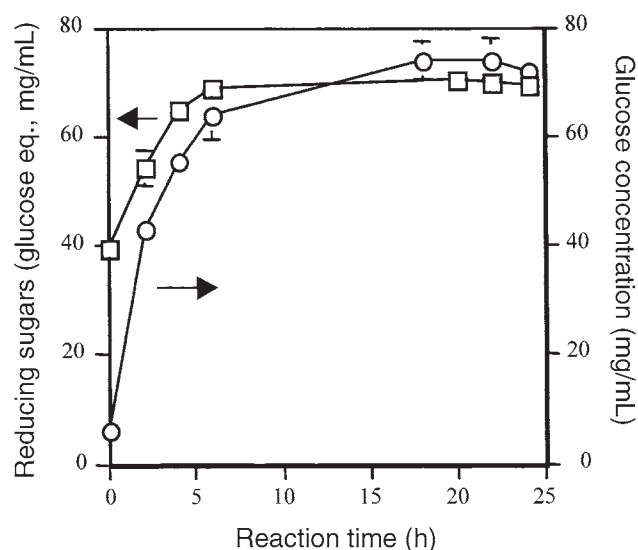


FIG. 1. Evolution of glucose production during saccharification with glucoamylase of rice bran starch (for reaction conditions, see Materials and Methods section). Data are means \pm SD ($n = 3$).

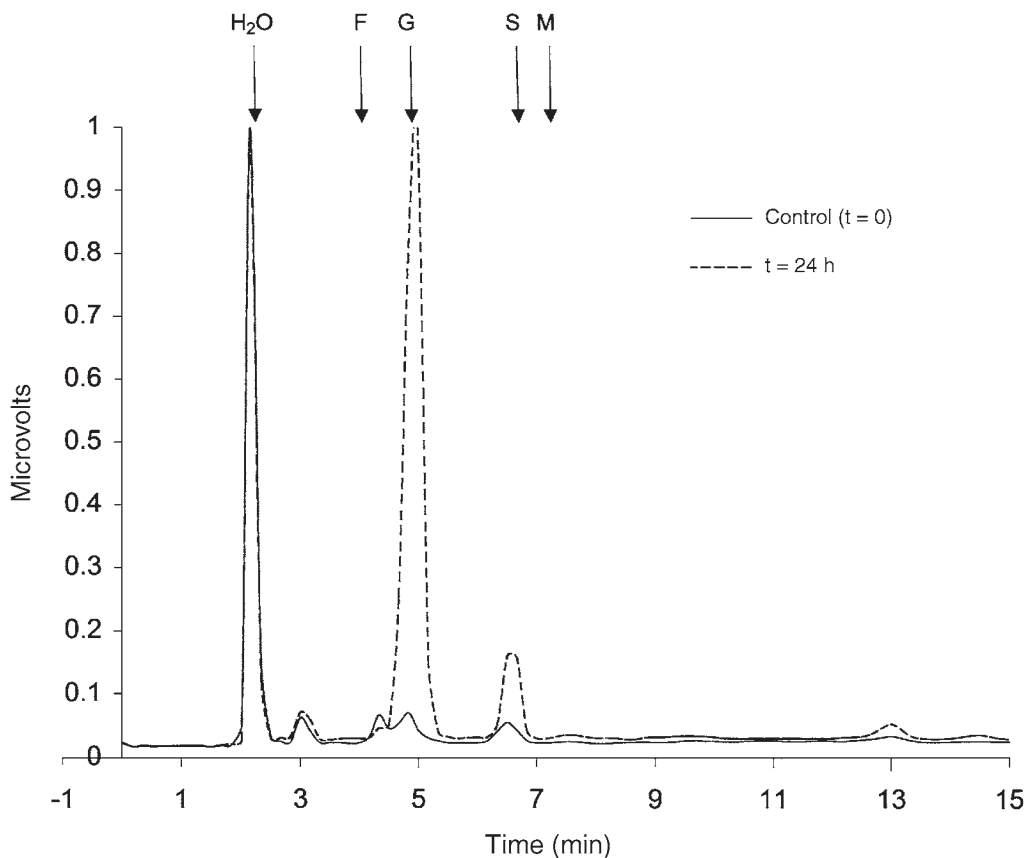


FIG. 2. High-performance liquid chromatogram of the α -amylase (Thermamyl)-solubilized products of rice bran after treatment with glucoamylase (Diazyme). Residence time of standard sugars is indicated (F, fructose; G, glucose; S, sucrose; M, maltose).

posed (10). In this particular case, three proteases were added using the same reaction volume (40 mL) in order to study their specificities toward rice bran proteins. The evolution of the proteolysis is shown in Figure 3, where it may be observed that up to 12 g/L of protein was solubilized when the most efficient enzyme (Neutrase) was used in the process. From the original protein in the bran, 21.5% is solubilized in the first reaction during α -amylase treatment. A further 35.6% is obtained as a partially hydrolyzed product in the second reaction with Neutrase. However, after both treatments, more than 95% of the oil remains in the bran and may not be released by further enzymatic reactions (cell wall degradation activities) with high efficiency. The enzymatic process is described in Scheme 1.

After the enzymatic reaction with α -amylase, oil was extracted with hexane with overall yields of 94.5 and 98.3% at 25 and 50°C, with standard deviations of 1.3 and 1.9% respectively. At both temperatures, the yield was about 5% higher than yields obtained by direct extraction from untreated bran. This increase in hexane extraction yield has also been reported for other oil seeds, such as soybean and sunflower (8).

A total mass balance of the extraction process, including the oil extraction stage, is included in Table 1, as results from the composition analysis of the residues in each step of the

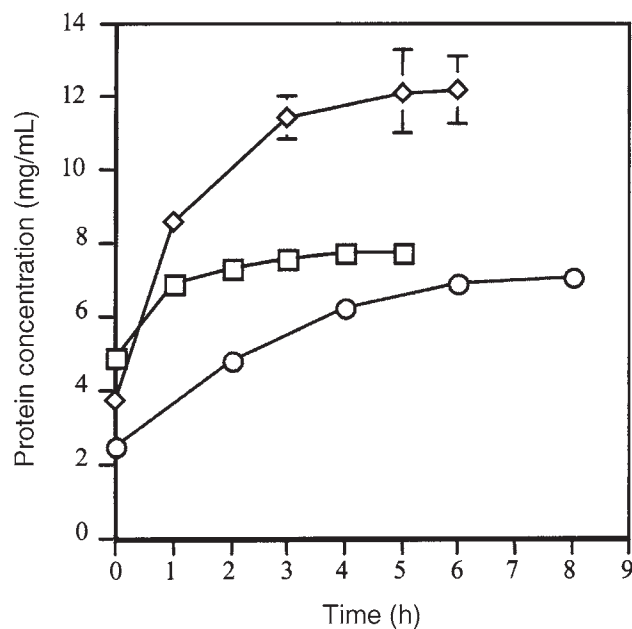
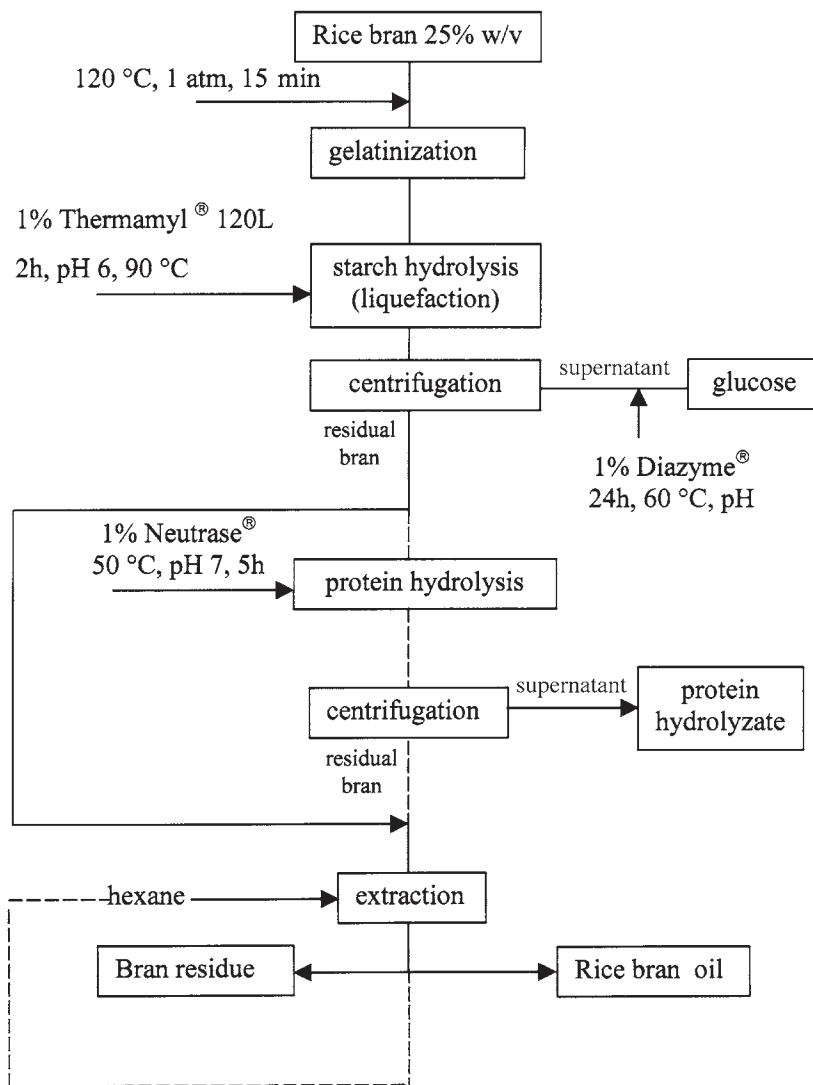


FIG. 3. Evolution of the protein solubilization process during treatment of rice bran previously treated with α -amylase and with three commercial proteases. \square , protease 2A; \diamond , Neutrase, \circ , HT Proteolytic. Data are means \pm SD ($n = 3$).



SCHEME 1

process. Finally, a layout of the enzymatic treatment of rice bran is summarized in Scheme 1.

REFERENCES

- Wadsworth, J.I., Rice, in *Encyclopedia of Food Science and Technology*, edited by Y.H. Hui, Vol. IV, John Wiley & Sons, New York, 1992, pp. 264–279.
- Orthofer, F.T., Rice Bran Oil: Healthy Lipid Source, *Food Technol.* 50:62–64 (1996).
- Sayre, R.N., Review of Rice Bran Stabilization Systems with Emphasis on Extrusion Cooking, *Cereal Food World* 27:317–322 (1982).
- Kao, C., and B.S. Luh, in *Rice Utilization*, Vol. 2, edited by B.S. Luh, Aspen Publishers, Inc., Frederick, pp. 295–311 (1991).
- Rosenthal, A., D.L. Pyle, and K. Niranjan, Aqueous and Enzymatic Processes for Edible Oil Extraction, *Enzyme Microb. Technol.* 19:402–420 (1996).
- McGlone, O.C., A. Lopez-Munguia, and J.V. Carter, Coconut Oil Extraction by a New Enzymatic Process, *J. Food Sci.* 51:695–697 (1986).
- Buenrostro, M., and A. Lopez-Munguia, Enzymatic Extraction of Avocado Oil, *Biotechnol. Lett.* 8:505–506 (1988).
- Dominguez, H., M.J. Nuñez, and J. Lema, Enzyme Assisted Hexane Extraction of Soybean Oil, *Food Chem.* 54:223–231 (1995).
- Saunders, R.M., Rice Bran: Composition and Potential Food Uses, *Food Rev. Int.* 415–495 (1986).
- Sengupta, R., and D.K. Bhattacharyya, Enzymatic Extraction of Mustard Seed and Rice Bran, *J. Am. Oil Chem. Soc.* 73:687–692 (1996).
- AOAC, *Official Methods of Analysis of the Association of Official Analytical Chemists*, 16th edn., AOAC, Gaithersburg (1995).
- Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall, Protein Measurement with the Folin Phenol Reagent, *J. Biol. Chem.* 193:265–275 (1951).
- Fried, B., and J. Shelma, *Thin-Layer Chromatography Techniques and Applications*, 2nd edn., Marcel Dekker Inc., New York, 1986, p. 287.

[Received March 2, 1999; accepted October 24, 1999]