Enzyme-Assisted Aqueous Extraction of Rice Bran Oil

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ABSTRACT: In the present study, rice bran oil was extracted by enzyme-assisted aqueous extraction under optimized aqueous extraction conditions using mixtures of Protizyme™ (protease; Jaysons Agritech Pvt. Ltd., Mysore, India), Palkodex™ (α-amylase; Maps India Ltd., Ahmedabad, India), and cellulase (crude cellulase; Central Drug House, Delhi, India). The optimal conditions used were: mixtures of amylase (80 U), protease (368 U), and cellulase (380 U), with 10 g of rice bran in 40 mL distilled water, pH 7.0, temperature 65°C, extraction time 18 h with constant shaking at 80 rpm. Centrifugation of the mixture at 10,000 × *g* for 20 min yielded a 77% recovery of the oil.

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KEY WORDS: Amylase, cellulase, enzyme-assisted aqueous extraction, protease, rice bran oil.

Refined rice bran oil is a high-quality cooking oil, whereas crude bran oil has been used for soap manufacture and for the production of industrial fatty acids (1). In humans, rice bran oil is reported to improve plasma lipid and lipoprotein profiles (2). The occurrence of γ-oryzanol and tocotrienols is presumed to be responsible for its hypocholesterolemic effect (3). Oil extraction from rice bran is conventionally done by solvent extraction (4). Enzyme-assisted aqueous oil extraction has emerged as an ecofriendly process for oil extraction (5–7). The addition of specific enzymes during extraction enhances the oil recovery by breaking the cell wall and lipid bodies (8,9). The many advantages of this approach and its economics have been reviewed (8). Recently, amylase has been employed to facilitate the extraction of rice bran oil. However, this approach yielded only a 5% increase in oil recovery (10). In this work we show that appropriate choice of enzyme(s) and optimization of extraction conditions make it possible to improve the oil recovery from rice bran by enzyme-assisted aqueous oil extraction.

A main feature of oilseed cotyledon cells is the existence of discrete cellular organelles called lipid and protein bodies that contain, respectively, most of the oil and protein in the grain. The cell wall is composed of cellulose, hemicellulose, lignin, and pectin, whereas lipid bodies are enveloped in a lipoprotein layer. Hydrolases such as cellulases, hemicellulases, and pectinases break down the cell wall, while proteases permeabilize the liposome membrane and facilitate oil release from the oil bodies (8,11). Thus, in enzyme-assisted aqueous extraction, the soluble components diffuse into water. The released oil forms a separate liquid phase leading to better oil recovery (8,11). However, the difference in oilseed oil composition determines the choice of enzyme to be used for each oil seed (12). Enzyme-based aqueous extraction has been successfully employed to recover oil from coconut by using polygalacturonases, amylases, and proteases (5), from soybean by using proteases (6), and from corn germ oil by using protease, cellulase, and hemicellulase (7), giving oil recovery in the range of 90–98% and good-quality protein meal. In the case of rice bran, aqueous enzymatic extraction alone has not resulted in reasonable oil yields to date. Recently, a procedure involving enzymatic treatment prior to solvent extraction or pressing has been described (10): A thermal treatment of rice bran was first applied to deactivate lipase, as well as to gelatinize starch prior to reaction with amylase. Amylase treatment was 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, could be subjected to protease treatment for protein extraction or directly treated with solvent to obtain bran oil. Under the defined extraction conditions, using hexane, yields of oil were 5% higher when rice bran was previously treated with amylase (10). The present work explores the use of proteases, amylase, and cellulase, individually and in combination, for rice bran extraction.

MATERIALS AND METHODS

Rice bran was purchased from the local market. Protizyme™ [from *Aspergillus flavus*, 75,000 units protease activity/g, consisting of mostly acidic (pH 3–4), neutral (pH 5–7), and alkaline proteases (pH 7–10)] was obtained from Jaysons Agritech Pvt. Ltd. (Mysore, India). Palkodex™ (from *A. niger*, 300 units/mL of α-amylase activity, optimum pH 4.0–5.0, optimum temperature 60–65°C) was purchased from Maps India Ltd. (Ahmedabad, India). A crude cellulase preparation (from *A. niger*, 0.5–1.0 units/mg solid) was obtained from Central Drug House (Delhi, India). Glucoamylase (from *A. niger*, 30–60 units per mg protein) was procured from Sigma Chemical Co. (St. Louis, MO). All of these enzyme preparations were used without any further purification. All other reagents used were of analytical grade.

Aqueous extraction of rice bran oil. Rice bran (10 g) was dispersed in 40 mL distilled water to make a slurry and was stirred on a magnetic stirrer at 20 rpm (revolutions per minute) for 30 min. The pH of the slurry was adjusted to the desired value with 0.1 N NaOH or 0.1 N HCl, and the mixture was incubated at 65°C with constant shaking (80 rpm)

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for 18 h. The upper oil phase was collected after centrifugation at 10,000 × *g* for 20 min and weighed. Oil recovery was calculated as the percentage oil (w/w) obtained with respect to total oil present in rice bran:

$$\% \text{ oil recovery} = \frac{\text{weight of oil extracted} \times 100}{\text{total weight of oil estimated by Soxhlet method}} \quad [1]$$

The total amount of rice bran oil was determined by solvent extraction using *n*-hexane in a Soxhlet apparatus following the standard AOAC procedure (13) and was found to be 16.7 g/100 g of rice bran.

Enzyme-assisted aqueous extraction of rice bran oil. The enzyme-assisted aqueous extraction of oil was carried out as described above except that the mixtures of amylase, protease, and cellulase were added before the pH of the slurry was adjusted to 7.0. The oil recovery was similarly calculated as described above. All experiments were done in triplicate, and variations were in the range of ±2%.

RESULTS AND DISCUSSION

Table 1 shows the effects of various enzymes on the amounts of oil extracted by aqueous extraction at pH 5.0 and 40°C temperature. Rice bran (10 g) was dispersed in 40 mL distilled water to make a slurry and stirred at 20 rpm on a magnetic stirrer. The pH of the dispersed solution was adjusted to 5.0 with 0.1 N HCl. Enzymes were then added in various combinations, and the samples were kept at 40°C for 18 h. The oil was recovered as an upper layer after centrifugation at 10,000 × *g* for 20 min. The upper oil layer was separated and weighed. Oil recovery was expressed relative to that obtained by Soxhlet extraction with hexane. It can be seen that the synergistic effects of amylase, cellulase, and protease are required in order to achieve significant oil recovery. Aqueous extraction in the absence of added enzyme did not give any oil recovery. In order to assess the effect of pH on oil recovery, the triple enzyme pretreatment was carried out at different pH values. The temperature of extraction was kept at 65°C since the enzyme preparations used are reported to be stable up to this temperature (14,15). Figure 1 shows that maximum oil recovery (76%) was obtained at pH 7.0. Hence, further experiments were conducted at pH 7.0.

TABLE 1
Enzyme-Assisted Aqueous Extraction of Rice Bran Oil

Enzyme	Oil recovery (%) (w/w)
Protizyme (368 U)	6.0
Cellulase (380 U)	4.0
Protizyme + cellulase (368 U + 380 U)	11
Amylase + cellulase + Protizyme (80 U + 380 U + 368 U)	67
Cellulase + amylase (380 U + 80 U)	10
Amylase + Protizyme (80 U + 368 U)	15

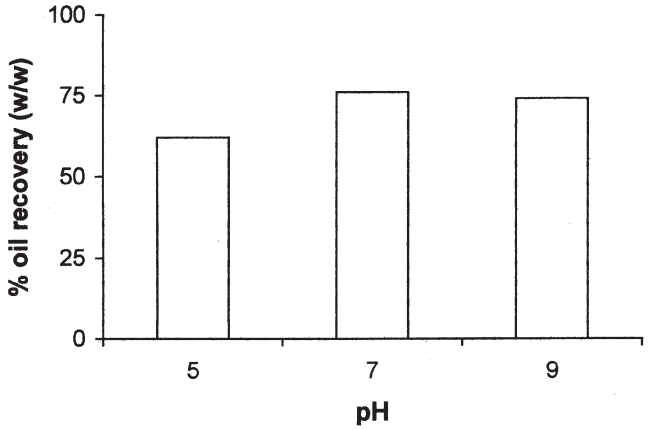

FIG. 1. Effect of pH on oil recovery by enzyme-assisted aqueous extraction of rice bran oil. Dispersions of rice bran in distilled water were adjusted to pH 5.0, 7.0, or 9.0 with 0.1 N NaOH or 0.1 N HCl. To these mixtures were added amylase, protease, and cellulase: 80, 368, and 380 U, respectively. The oil was then extracted as described in the Materials and Methods section.

Figure 2 shows that the minimum incubation time to achieve maximum oil recovery by enzyme-assisted aqueous extraction was *ca.* 18 h. Table 2 shows that the agitation rate during the enzyme step also plays a critical role. Rice bran (10 g) was dispersed in 40 mL distilled water to make slurry and was stirred with low-speed stirrer set at 20 rpm for 30 min. The pH of the mixture was adjusted to 7.0 (by 0.1 N NaOH) followed by addition of amylase, protease, and cellulase: 80, 368, and 380 U, respectively. The oil was extracted by following the procedure described in the Materials and Methods section. Increasing the speed to 100 rpm and beyond led to progressive decreases in oil recovery. It was observed that oil obtained at 80 rpm was clear, whereas shaking at 100 rpm and beyond led to formation of a clearly visible emulsion in the oil layer.

Figure 3 shows the effect of varying the amount of one component of enzyme mixture while keeping the amount of

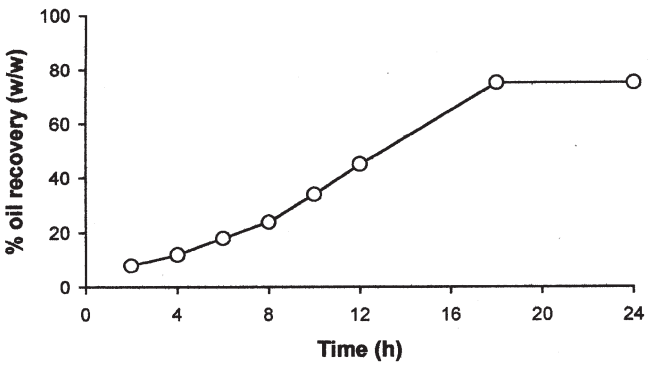

FIG. 2. Effect of incubation time on oil recovery by enzyme-assisted aqueous extraction of rice bran oil. Dispersions of rice bran in distilled water were adjusted to pH 7.0 with 0.1 N NaOH followed by addition of amylase, protease, and cellulase: 80, 368, and 380 U, respectively. Samples were then incubated at 65°C for various times with constant shaking at 80 rpm. The oil was then extracted as described in the Materials and Methods section.

TABLE 2
Effect of Shaking Speed (rpm) on Rice Bran Oil Recovery by Enzyme-Assisted Aqueous Extraction

Shaking speed (rpm)[a]	Oil yield (%) (w/w) without enzyme	Oil yield (%) (w/w) enzyme-assisted
50	13	76
80	14	78
100	8	67
200	6	60

[a]Revolutions per minute.

the other two enzymes constant at the level of experiments corresponding to Figures 1 and 2. It was seen that one could substantially decrease the amounts of cellulase and Protizyme without impairing the oil yield. The above data show that by judicious choice of enzymes and extraction parameters, one can obtain oil from rice bran with a recovery of 76–78% of what is obtainable with Soxhlet extraction.

The economics of enzyme-assisted aqueous oil extraction have been previously compared with solvent-based extraction, which involves a high capital cost to install (8). It was determined that: (i) If market rates for product oil are high, the enzyme-assisted oil extraction process can compete favorably with the conventional approach. (ii) If immobilized (reusable) forms of enzyme are used, the cost can be considerably reduced by recycling enzyme. Such approaches have not yet been explored in the area of rice bran oil recovery. The factors in favor of the enzyme-assisted oil extraction process described here are the ecofriendly and gentle nature of the process. The latter may result in generally better qualities for both the extracted oil and the residual meal.

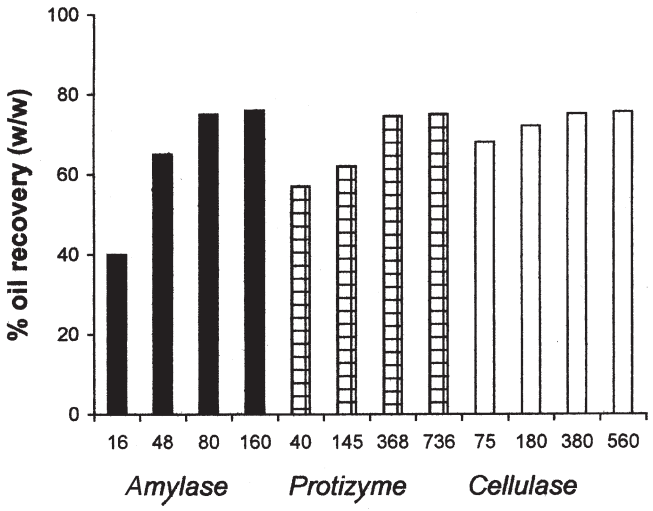

FIG. 3. Effect of varying enzyme units on oil recovery by enzyme-assisted aqueous extraction of rice bran oil. The pH of the dispersions of rice bran in distilled water was adjusted to 7.0 followed by: ■ addition of varying amounts of amylase (16, 48, 80, and 160 U) with fixed amounts of protease and cellulase: 368 U and 380 U, respectively; ⊞ addition of varying amounts of protease (40, 145, 368, and 736 U) with fixed amounts of amylase and cellulase, 80 and 380 U, respectively; □ addition of varying amounts of cellulase (76,185, 380, and 560 U) with fixed amounts of amylase and protease, 80 and 368 U, respectively. The oil was recovered by following the procedure described in the Materials and Methods section.

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