Enzymatic Process for Extracting Oil and Protein from Rice Bran

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ABSTRACT: Enzymatic extraction of oil and protein from rice bran, using a commercial protease (Alcalase), was investigated and evaluated by response surface methodology. The effect of enzyme concentration was most significant on oil and protein extraction yields, whereas incubation time and temperature had no significant effect. The maximal extraction yields of oil and protein were 79 and 68%, respectively. Further, the quality of oil recovered from the process in terms of free fatty acid, iodine value, and saponification value was comparable with solvent-extracted oil and commercial rice bran oil, but the peroxide value was higher.

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KEY WORDS: Alcalase, enzymatic extraction, oil extraction, response surface analysis, rice bran, rice bran oil, rice bran protein.

The utilization of enzymes in food processing has long been recognized, with a view to achieve high product yields, reduce by-products, and avoid severe operational conditions. The uses of enzymes in the extraction of oil, protein, and other components from oil-containing seeds/fruits have been reviewed recently (1–3). Such processes involve the treatment of oil-containing materials with cell wall-degrading enzymes in order to extract oil and other components under milder processing conditions; for example, the extraction temperature is lower, explosive solvent is not required, and harmful wastes are not produced (1,3). This technology has been developed to extract oil from many materials: avocado (4), coconut (5–8), corn germ (9), rapeseed (10), soybean (11,12), and sunflower (11,13–15).

Rice (*Oryza sativa*) bran is a by-product of milling in rice-processing countries. The bran derived from rice grain during the whitening process is rich in protein, oil, and carbohydrate. It is normally used for extracting oil and as animal feed and a food ingredient. In commercial production of rice bran oil, *n*-hexane is generally used as an extractant. However, *n*-hexane has been identified as an air pollutant (3). It was therefore thought desirable to investigate oil extraction by using an aqueous and enzymatic process that might eliminate some problems associated with the use of solvent.

The application of an enzymatic process for extracting rice bran has been reported recently (16,17). Oil yields were high when rice bran was treated with cellulase and pectinase and then extracted with *n*-hexane (16). However, the use of enzymatic treatment alone did not result in high yields (17). Some papers have reported on the use of enzymes for extracting oil from rice bran. The present work investigates the effect of operational parameters including enzyme concentration, incubation time, and temperature on oil and protein extraction yield. The quality of oil recovered in terms of free fatty acid content, iodine value, peroxide value, and saponification value was also analyzed and compared with *n*-hexane-extracted oil and a commercial rice bran oil.

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MATERIALS AND METHODS

Materials. Full-fat rice bran used for all experiments was obtained from Tilda Ltd. (Essex, United Kingdom). The rice bran was screened to pass a 16-mesh sieve (1,000 μm aperture size), placed in polypropylene bags, and stored at −18°C. It was thawed at 4°C on the day before use. The rice bran contained 19.97% oil, 14.12% protein, 18.22% total dietary fiber, 22.04% starch, 8.81% ash, 8.71% moisture, and 8.13% other components (deduced by difference). The commercial sample of rice bran oil (CHIM^{TM}) was produced by Amorn Chai Ltd. (Ayuthaya, Thailand). The enzyme used in the experiments was Alcalase 0.6L. It was supplied as a brownish liquid preparation by Novo Nordisk A/S (Bagsvaerd, Denmark) and had a density of 1.26 g/mL at 20°C. It is a microbial endopeptidase from *Bacillus licheniformis*. Its proteolytic activity was reported to be 0.6 Anson Units (AU)/g .

Experimental design. Response surface methodology (RSM) was employed with Box-Behnken design (18) to investigate the effect of enzyme treatment on (i) oil extraction yield, (ii) protein extraction yield, and (iii) free fatty acid content in the oil recovered. Three independent parameters, enzyme concentration, incubation times and temperature, at three different levels each, were employed. The parameters chosen and their levels were based on preliminary experiments carried out in our laboratories. The experimental plan was designed and the results obtained were analyzed using Design-Expert version 5.0 (Stat-Ease Inc., Minneapolis, MN) software to build and evaluate models and to plot the three-dimensional response surface curves.

Enzymatic treatment and solid–liquid separation. Experiments were carried out in a 1.0 L reactor. For each experiment, 100 g of rice bran was mixed with 500 mL buffer (0.05 M boric acid-NaOH, pH 9.0) giving a mixture of rice bran

and buffer at a 1:5 (wt/vol) ratio and then heated to 90°C for 15 min to inactivate enzymes (16). After that, it was cooled and placed in a waterbath set at a desired temperature and was stirred at 1,000 rpm by a high-shear mixer (Silverson SL2T, Silverson Machines Ltd., Chesham, Buckinghamshire, United Kingdom). The mixture pH was adjusted to 9.0 by adding 1 N NaOH, followed by addition of Alcalase. Enzyme concentration, incubation time, and temperature were varied from 0–2 g/100 g bran, 1–3 h, and 40–60°C, respectively. After enzyme treatment, the mixture was centrifuged at $16{,}800 \times g$ at 20°C for 30 min to separate solid (or meal) and liquid phase. The cream floating atop the liquid phase and adhering to the wall of the centrifuge bottles was collected and de-emulsified. The wet meal was mixed, weighed, and sampled for determining moisture content and total dry meal. The remaining wet meal was dried overnight in a hot-air oven at 85–90°C. The dry meal was ground and analyzed for residual oil and protein. All experiments were replicated twice.

Oil recovery. De-emulsification of cream was carried out by boiling; further details are described by Hanmoungjai *et al.* (19). The oil recovered thus is defined as enzyme-extracted oil.

n-*Hexane-extracted oil.* Crude oil in full-fat rice bran was extracted with *n*-hexane in a Soxhlet extractor for 4 h, evaporated in a rotary evaporator, and dried in a hot-air oven at 100°C for 30 min to eliminate residual *n*-hexane.

Analytical methods. The oil and protein content of the full-fat rice bran and the meal were determined with *n*-hexane by the Soxhlet method and by the Kjeldahl method (N·5.95), respectively (20). The ash content was analyzed by burning in a furnace at 600°C for 2 h (20). The moisture content was determined as the loss of weight at 103°C in 3 h (21). The rice bran and the meal were also analyzed for starch and total dietary fiber contents using the methods recommended by AOAC (20). The quality of all oils in terms of free fatty acid content, iodine value, peroxide value, and saponification value were also analyzed by the methods recommended by AOAC (20). Oil color was characterized spectrophotometrically by measuring absorbance in the range 400 to 700 nm (21). γ-Oryzanol content was determined by measuring optical density in diethyl ether solution at 315 nm (22). The reference standard for γ-oryzanol was supplied by A & E Connock (Perfumery & Cosmetics) Ltd. (Fordingbridge, United Kingdom). The oil was also analyzed for acetone-insoluble material using the standard procedure recommended by IUPAC (21). Tocopherols content was determined by the Emerie-Engel method using (+)-α-tocopherol (Sigma, Dorset, United Kingdom) as standard (21). Fatty acid composition was analyzed using gas chromatography (PerkinElmer 8500 Chromatograph, Beaconsfield, Buckinghamshire, United Kingdom), following the method described by Long *et al.* (23).

Extraction yield. The oil extraction yield and protein extraction yield were expressed as follows:

$$\text{oil extraction yield, \% } (Y_1) = \frac{[\text{total oil in rice bran}] - [\text{residual oil in meal}]}{[\text{total oil in rice bran}]} \cdot 100 \quad [1]$$

$$\text{protein extraction yield,\% } (Y_2) = \frac{[\text{total protein (in rice bran + enzyme)}] - [\text{residual protein in meal}]}{[\text{total protein (in rice bran + enzyme)}]} \cdot 100 \quad [2]$$

RESULTS AND DISCUSSION

The experimental plan and the results of oil extraction yield, protein extraction yield, and free fatty acid in oil obtained are

TABLE 1
Experimental Design and Results[a] Obtained from the Process

	Coded parameter			Actual parameter values			Y_1	Y_2	Y_3
Run	X_1	X_2	X_3	x_1	x_2	x_3	(%)	(%)	(%)
1	–1	0	–1	0	2	40	71.8	49.7	2.5
2	+1	0	–1	2	2	40	74.8	65.8	3.2
3	–1	0	+1	0	2	60	74.5	46.1	1.6
4	+1	0	+1	2	2	60	78.4	66.3	2.4
5	–1	–1	0	0	1	50	70.2	36.2	2.0
6	+1	–1	0	2	1	50	78.1	59.6	3.5
7	–1	+1	0	0	3	50	73.3	48.0	2.7
8	+1	+1	0	2	3	50	76.4	68.2	3.3
9	0	–1	–1	1	1	40	74.9	54.1	2.6
10	0	–1	+1	1	1	60	78.7	65.8	2.1
11	0	+1	–1	1	3	40	76.0	64.9	2.5
12	0	+1	+1	1	3	60	79.1	66.0	2.3
13	0	0	0	1	2	50	77.3	63.8	2.9
14	0	0	0	1	2	50	77.8	67.3	2.9
15	0	0	0	1	2	50	77.9	63.0	2.7
16	0	0	0	1	2	50	77.0	66.9	3.4
17	0	0	0	1	2	50	78.1	67.6	2.6

[a]Values represent the means of two experiments. X_1, X_2, and X_3 represent the coded variables for Alcalase concentration, incubation time, and temperature; x_1, x_2, x_3 represent the actual variables for Alcalase concentration (g/100 g bran), incubation time (h), and temperature (°C); Y_1, Y_2, and Y_3, represent oil and protein extraction and free fatty acid. Y_1 and Y_2 were calculated using Equations 1 and 2, respectively.

TABLE 2
Analysis of Variance for Response Surface Quadratic Model

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	$P > F$[a]
Oil extraction yield[b]					
Model	101.36	9	11.26	26.50	0.0001
Residual	2.98	7	0.43		
Lack of fit	2.15	3	0.72	3.46	0.1309
Pure error	0.83	4	0.21		
Total	104.34	16			
Coefficient of variation = 0.86%, R^2 value = 0.9715					
Protein extraction yield[c]					
Model	1393.19	9	154.80	15.98	0.0007
Residual	67.82	7	9.69		
Lack of fit	49.31	3	16.44	3.55	0.1262
Pure error	18.51	4	4.63		
Total	1461.00	16			
Coefficient of variation = 5.19%, R^2 value = 0.9536					
Free fatty acid (%)					
Model	3.63	9	0.40	4.95	0.0233
Residual	0.57	7	0.081		
Lack of fit	0.19	3	0.063	0.67	0.6151
Pure error	0.38	4	0.095		
Total	4.20	16			
Coefficient of variation = 10.73%, R^2 value = 0.8643					

[a]$P < 0.05$ indicates statistical significance.
[b]Defined by Equation 1.
[c]Defined by Equation 2.

shown in Table 1. Response surface analysis was employed for optimizing the enzymatic process parameters. Both a linear model and a second-order model were tested, using Fisher's *F*-test at 95% confidence level. The following second-order models satisfactorily explained oil extraction yield, protein extraction yield, and free fatty acid with no significant lack of fit (Table 2).

$$Y_{oil}\,(\%) = 77.62 + 2.24X_1 + 0.36X_2 + 1.65X_3 - 2.71X_1^2 - 0.41X_2^2 - 0.035X_3^2 - 1.2X_1X_2 + 0.23X_1X_3 - 0.18X_2X_3 \quad [3]$$

$$Y_{protein}\,(\%) = 65.72 + 9.99X_1 + 3.93X_2 + 1.21X_3 - 9.22X_1^2 - 3.5X_2^2 + 0.48X_3^2 - 0.8X_1X_2 + 1.03X_1X_3 - 2.65X_2X_3 \quad [4]$$

$$Y_{FFA}\,(\%) = 2.90 + 0.45X_1 + 0.075X_2 - 0.3X_3 - 0.012X_1^2 - 0.038X_2^2 - 0.49X_3^2 - 0.23X_1X_2 + 0.025X_1X_3 + 0.075X_2X_3 \quad [5]$$

where Y_{oil}, $Y_{protein}$, and Y_{FFA} are the predicted responses for oil extraction yield (%), protein extraction yield (%), and free fatty acid (%), respectively, and X_1, X_2, and X_3 are the coded parameters for enzyme concentration, incubation time, and temperature, respectively, described in Table 1.

Figure 1 shows the response surfaces of the predicted extraction yields and free fatty acid content with respect to variations in enzyme concentration and incubation time. The results show that enzyme concentration significantly increases oil extraction yield, protein extraction yield, and also free fatty acid content. The maximal extraction yields for oil and protein were determined to be 79 and 68%, respectively. It appears that higher oil extraction yields are accompanied by higher free fatty acid content in the oil. The other variable parameters studied had no significant effect on the yields.

TABLE 3
Quality of Rice Bran Oil Obtained by Different Processes[a]

Analytical characteristic	Industrial specifications[b]	Commercial rice bran oil	*n*-Hexane-extracted oil	Enzyme-extracted oil[d]
Free fatty acid (%)	≤ 0.3	0.1[c]	7.40	2.36
Iodine value	92–115	95.9[c]	95.40	97.18
Peroxide value	≤ 10	5.5[c]	8.20	12.01
Saponification value	180–195	188.3[c]	187.60	188.72
Acetone-insoluble material (%)	—	1.32	10.23	5.45
γ-Oryzanol (%)	—	0.07	2.04	1.76
Tocopherols (%)	—	0.07	0.10	0.09

[a]Values present the means of three determinations.
[b]Thai industrial standards, TIS 44-2516 (1973).
[c]Data from Hanmoungjai *et al.* (19).
[d]Enzymatic extraction conditions: 1.0 g Alcalase/100 g bran at 50°C and pH = 9.0 for 2 h.

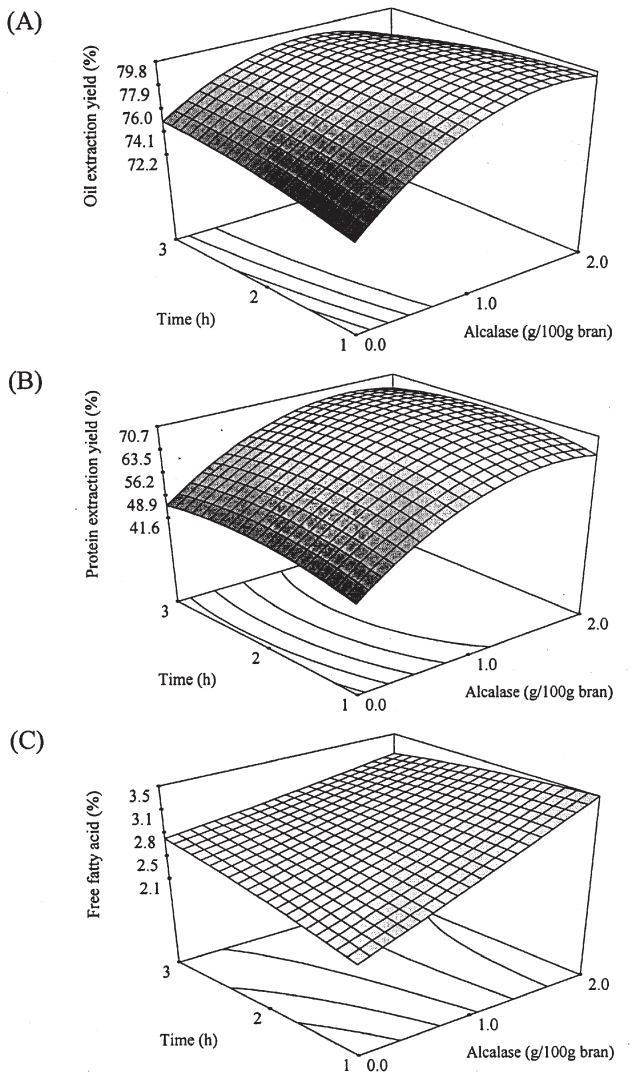

FIG. 1. Response surface for the oil extraction yield (A), protein extraction yield (B), and free fatty acid content (C) as a function of Alcalase concentration and extraction time at 50°C.

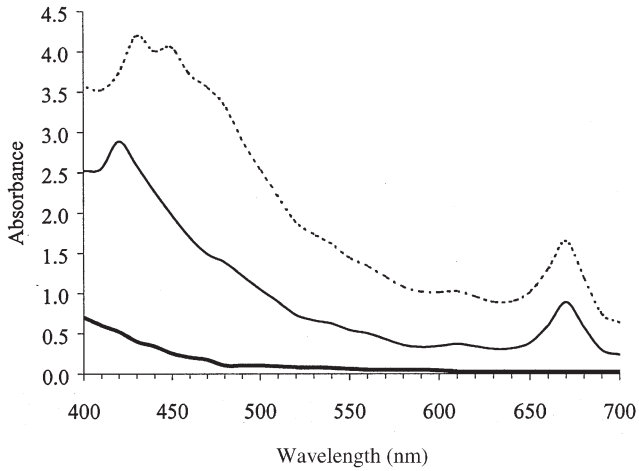

FIG. 2. Visible spectra of commercial rice bran oil (—), enzyme-extracted oil (—), and *n*-hexane-extracted oil (---).

TABLE 4
Fatty Acid Composition of Rice Bran Oil Obtained from Different Processes[a]

Fatty acid composition (%)	Commercial rice bran oil	*n*-Hexane-extracted oil	Enzyme-extracted oil[b]
Myristic acid ($C_{14:0}$)	3.24	2.99	3.22
Palmitic acid ($C_{16:0}$)	13.19	11.88	11.44
Stearic acid ($C_{18:0}$)	1.47	1.40	1.29
Oleic acid ($C_{18:1}$)	26.16	30.13	29.85
Linoleic acid ($C_{18:2}$)	55.06	53.30	53.75
Linolenic acid ($C_{18:3}$)	0.89	0.31	0.45

[a]Values present the means of two determinations.
[b]Enzymatic extraction conditions: 1.0 g Alcalase/100 g bran at 50°C and pH = 9.0 for 2 h.

The qualities of oils obtained by the enzyme-aided process and by other processes are compared in Table 3. It can be seen that the enzyme-extracted oil is comparable to oil obtained by other processes in terms of iodine value and saponification value. However, its free fatty acid value is significantly lower than *n*-hexane-extracted oil. This implies that a lower amount of neutralizing agent is needed in the refining stage. It is also noteworthy that even though the peroxide value of enzyme-extracted oil is higher than *n*-hexane-extracted oil, it only exceeds the industrially specified limit by a small margin. The visible spectra of the oils are shown in Figure 2. It can be seen that enzyme-extracted oil has a lower content of colored substances than *n*-hexane-extracted oil. The fatty acid compositions of the oils obtained by the enzymatic process and other processes are presented in Table 4. It can be seen from the table that the composition of essential fatty acids in enzyme-extracted oil is comparable to commercial rice bran oil and solvent-extracted oil. Palmitic, oleic, and linoleic acids are the major components, which account for 95% of the fatty acids. The meal obtained by the enzymatic process is high in protein and total dietary fiber, which are valuable for animal feed or other foods uses (Table 5).

Thus, the enzymatic process has been shown to be effective for extracting oil and protein from rice bran. The oil re-

TABLE 5
Typical Composition of Meal Obtained from Different Processes[a]

Component	Meal from enzymatic process[b]	Meal from Soxhlet process
Ash (%)	17.14	12.40
Protein (%)	10.27	20.38
Oil (%)	9.88	0.07
Starch (%)	15.23	29.12
Total dietary fiber (%)	42.69	33.89
Other substances[c] (%)	4.79	4.14

[a]Values are expressed on a dry weight basis and represent the means of three determinations.
[b]Enzymatic extraction conditions: 1.0 g Alcalase/100 g bran at 50°C and pH = 9.0 for 2 h.
[c]Deduced by difference.

covered by the enzyme-aided process compares favorably with *n*-hexane-extracted oil and a commercial sample of oil.

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