Enzymatic Extraction of Mustard Seed and Rice Bran

R. Sengupta and D.K. Bhattacharyya*

Department of Chemical Technology, University Colleges of Science and Technology, Calcutta - 700 009, India

ABSTRACT: Aqueous enzymatic extraction was investigated for recovery of oil from mustard seed and rice bran. The extraction process was reproducible based on statistical analysis of extraction data under different extraction conditions. The most significant factors for extraction were the time of digestion with enzymes, seed or bran concentration in water, volume of hexane added before recovery, and amount of enzyme(s) used. The pretreatment steps of each material before enzyme digestion influenced oil yield.

Quality of enzyme-extracted mustard oil was better with respect to color and odor than commercial expeller-extracted and Soxhlet-extracted oils. Most of the characteristics of rice bran oil were identical to those of commercial solvent-extracted oils, but rice bran oil had a lower content of colored substances and higher acidity (free fatty acid). Enzymatic extraction led to recovery of a protein concentrate with increased protein and reduced fiber and ash contents in the mustard and rice bran meals.

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KEY WORDS: Cellulase, enzymatic extraction, mustard seed, pectinase, protein, rice bran.

Enzymes from microbial sources are gaining importance in the processing of oil-bearing materials to recover oils and nonoil constituents. Certain microorganisms are unique in character, with enzyme systems that are capable of actions on other constituents of oil-bearing materials. Such enzyme activities of microorganisms can be useful in treating oil-bearing materials to release oil globules readily by their action on proteins, cellulose, and hemicellulose in a controlled manner.

Among the microorganisms with such enzyme activities, two organisms, namely, *Bacillus subtilis* and *Aspergillus niger*, have the desired enzymes and have already been examined for the extraction of oilseeds, such as soybean and rapeseed (1). Reports are available on enzymatic extraction of oils from olive (2–4), rapeseed (1,5,6), soybean (1,7), sunflower (5), coconut (8,9), avocado (10), cottonseed (7), groundnut (5), melonseed (1), canola (11,12), palm fruit (13), shea fat (14,15), and corn germ (16,17). An enzymatic process for the extraction of rapeseed oil with the aid of enzyme preparation Sp 311 from a selected strain of *Aspergillus niger* has been developed (6). Among the advantages claimed for the use of enzymes in processing oilseeds are low energy consumption and solvent usage, higher nutritive value of the protein, and extremely good quality of the oil, requiring no refining after extraction.

Mustard seed is a major oilseed crop in some Asian countries, and rice bran is a major by-product in Asia and other rice-producing countries. The oils from these two source materials have some quality problems. Mustard oil has color and odor problems, and rice bran oil has a high content of free fatty acid (FFA), wax, and unsaponifiable matter. On the basis of previous reports involving other oil-bearing materials, it is expected that enzyme extraction will yield oils and meals of better quality.

The present work investigates enzymatic extraction for recovering oils and meals from mustard seed (*Brassica juncea*) and rice bran (*Oryza sativa*) with the aid of microbial enzymes by examining the extraction parameters, such as temperature, seed and enzyme concentrations, and time of extraction, to obtain basic technological information for the enzymatic extraction process. A control experiment was done without any enzyme and also without hexane; instead an aqueous medium with enzyme was used.

The quality of oils in terms of color, free fatty acid and unsaponifiable matter content, peroxide, saponification, and iodine values has also been evaluated. The quality of the two oils recovered by the enzymatic process has been compared with the quality of those recovered by other processes, including Soxhlet extraction. The specifications of edible-quality mustard oil and rice bran oil, as specified by the Indian Standard Institution, have also been compared. Commercial mustard oil is extracted by the expeller method, and rice bran oil by solvent extraction of precooked and pelletized bran with *n*-hexane.

MATERIALS AND METHODS

In the present work, aqueous enzymatic extraction was investigated for mustard seed (Ultadanga Oil Mill, Calcutta, India) and rice bran (Sethia Oil Mills, Burdwan, West Bengal, India). The enzymes were supplied by Novo Nordisk (Bagsveard, Denmark). Mustard seed and rice bran were also extracted in a laboratory Soxhlet extractor with food-grade *n*-hexane. The oils and meals obtained from this process were analyzed compared as control samples with the oils and meals

*To whom correspondence should be addressed at Department of Chemical Technology, University Colleges of Science and Technology, 92, Acharya Prafulla Candra Road, Calcutta - 700 009, India.

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obtained by aqueous enzymatic extraction. Soxhlet extraction was considered ideal for comparison in view of its minimum effect on the quality of oil and meal. Quality of the oils from the above two processes was also compared with the standard specifications of the two oils as established by the Indian Standard Institution.

Enzyme-aided aqueous extraction involved the following steps: (i) Pretreatment of the seed material; (ii) extraction with enzymes; (iii) collection of the oil and other fractions, such as protein, molasses and hull.

(i) The seed/bran was finely pulverized (–20 mesh) and then mixed with water and heated at 90°C for 15 min to inactivate myrosinase and lipase activity in the mustard seed and rice bran, respectively. In another set of experiments, rice bran was cooked at 120°C for 1 min, instead of water heating, to obtain better recovery of oil with enzyme.

(ii) The mixture of water and ground seed, pretreated by the above process, was then cooled, and the pH of the mixture was adjusted to 4.5 with 1:1 HCl. The mixture was then treated with the enzymes pectinase (Pectinex Ultra SPL) and cellulase (Celluclast R) at a definite temperature for a definite period. The enzymatic reaction was carried out in a temperature-controlled glass reactor by stirring the seed-water mixture with a low-speed stirrer at 100 rpm. The pH of the mixture was kept constant throughout the reaction period. The temperature, water and seed/bran ratio, enzyme amount, and reaction time were independently varied, keeping other parameters fixed, so as to obtain the best conditions for extraction.

(iii) After enzymatic reaction for a definite period, the temperature of the seed/bran–water mixture was raised and maintained at 80°C for 5 min to destroy the activity of cellulase and pectinase. Then, 1.5 volumes of *n*-hexane (optimum value) based on the weight of seed/bran was added, and the mixture was stirred for 15 min to de-emulsify the oil–water emulsion. It was then centrifuged at 15,000 cpm (27,000 × *g*) for 10 min. The liquid phase after centrifugation consisted of two layers. The upper layer was oil in hexane (miscella), and the aqueous layer was syrup of dissolved carbohydrates (molasses). The miscella in the upper layer was separated by means of a separatory funnel and, after distillation of hexane, yielded the crude oil. The solid phase consisted of an upper layer of sedimented proteins and a lower layer of cell debris or hull. The different fractions were dried and analyzed. All experiments were replicated twice.

Analysis of the samples. The oils, obtained by either enzyme-aided aqueous extraction or Soxhlet extraction, were analyzed for fatty acid composition (18), saponification value (19), iodine value (20), peroxide value (21), unsaponifiable matter (22), free fatty acid content (23), and color (24). Wax content (25) of rice bran oil and amount of allyl isothiocyanate in mustard oil (26) were also analyzed. All data represent means of two duplicate determinations. The analysis of meals included nitrogen (27), ash (28), and fiber contents (29) to evaluate their quality.

Statistical treatment of results. To determine the reproducibility of the experiment, the results were subjected to the standard response surface method (RSM) analysis (30) with a second-degree polymonial of the factors (or covariates). The numerical work was performed on a PC with the software package SAS (SAS Institute, Cary, NC), which has a procedure for RSM analysis.

RESULTS AND DISCUSSION

The aqueous enzymatic extraction process for recovery of oil from mustard seed and rice bran shows that it depends on temperature, time of reaction with enzymes, amount of enzymes, and concentration of seed or bran in water. The amount of hexane is also an important parameter in achieving maximum oil recovery.

The composition of mustard seed and rice bran is given in Table 1. The effects of the various parameters on oil yield are listed in Tables 2 and 3. The statistical analysis (Tables 4 and 5) of the process in relation to the parameters shows that the aqueous enzymatic extraction process is reproducible because the pure error is almost zero. Statistical significance of different factors for aqueous enzymatic extraction of mustard seed (Table 6) shows that time of enzyme reaction, water and hexane amounts, and the enzyme pectinase are the most significant factors, and temperature and the amount of cellulase are not as significant. Amount of enzymes, reaction time and amount of hexane are also significantly important parameters in rice bran oil extraction.

The pH of the seed/bran and water mixture was maintained at 4.5. Normal hexane was used while stirring for 15 min after completion of the digestion at 80°C of the seed or bran to break the emulsion formed in the process for achieving maximum oil recovery. In the absence of hexane, the extraction process gives a lower yield of oil (Tables 2 and 3).

TABLE 1
Distribution Pattern of Different Constituents in Mustard Seed (*Brassica juncea*) and Rice Bran (*Oryza sativa*)

	Constituent (wt%)				
Sample	Moisture	Oil[a]	Protein	Ash	Other fractions[b]
Mustard seed	6.5	35.1	28.2	4.6	25.6
Rice bran	7.3	20.7	14.6	13.8	43.6

[a]Soxhlet method.
[b]These include fiber, soluble carbohydrates, starch, etc.

TABLE 2
Effect of Different Variables on the Recovery of Oil by Aqueous Enzymatic Extraction of Mustard Seed at a pH of 4.5

Temperature (°C)	Reaction time (h)	% Enzyme (on the weight of seed)		Water/seed (vol/wt)	Hexane/seed (vol/wt)	Oil yield (wt%)	% Recovery of oil[a]
		Cellulase	Pectinase				
40 ± 1	4	2	2	5:1	2.5:1	30.4	86.6
50 ± 1	4	2	2	5:1	2.5:1	35.0	99.7
60 ± 1	4	2	2	5:1	2.5:1	34.7	98.9
50 ± 1	1	2	2	5:1	2.5:1	11.2	31.9
50 ± 1	2	2	2	5:1	2.5:1	18.9	53.8
50 ± 1	3	2	2	5:1	2.5:1	33.0	94.0
50 ± 1	6	2	2	5:1	2.5:1	35.1	100.0
50 ± 1	4	2	2	5:1	1.5:1	35.0	99.7
50 ± 1	4	2	2	5:1	1:1	29.6	84.3
50 ± 1	4	0	0	5:1	1.5:1	22.0	62.7
50 ± 1	4	1	1	5:1	1.5:1	30.2	86.0
50 ± 1	4	5	5	5:1	1.5:1	34.0	96.8
50 ± 1	4	0	0	5:1	Without hexane	17.0	48.4
50 ± 1	4	2	2	5:1	Without hexane	21.5	61.2
50 ± 1	4	5	2	5:1	Without hexane	21.0	59.8
50 ± 1	4	5	5	5:1	Without hexane	25.0	71.2
50 ± 1	4	2	2	3:1	1.5:1	17.0	48.4
50 ± 1	4	2	2	6:1	1.5:1	34.9	99.4

[a]Based on original oil content, see Table 1.

TABLE 3
Effect of Different Variables on the Recovery of Oil by Aqueous Enzymatic Extraction of Rice Bran at a pH of 4.5

Temperature (°C)	Reaction time (h)	% Enzyme (on the weight of bran)		Water/bran (vol/wt)	Hexane/bran (vol/wt)	Oil yield (wt%)	% Recovery of oil[a]
		Cellulase	Pectinase				
40 ± 1	4	3	3	3:1	1.5:1	16.6	80.2
50 ± 1	4	3	3	3:1	1.5:1	17.3	83.6
60 ± 1	4	3	3	3:1	1.5:1	17.5	84.5
60 ± 1	6	3	3	3:1	1.5:1	18.2	87.9
60 ± 1	20	3	3	3:1	1.5:1	18.7	90.3
50 ± 1	4	3	3	3:1	1.5:1	17.3	83.6
50 ± 1	4	3	3	5:1	1.5:1	17.5	84.5
50 ± 1	4	3	3	6:1	1.5:1	16.8	81.1
50 ± 1	4	0	0	5:1	1.5:1	11.2	54.1
50 ± 1	4	2	2	5:1	1.5:1	17.0	82.1
50 ± 1	4	5	5	5:1	1.5:1	17.7	85.5
50 ± 1	4	2	2	5:1	2.5:1	17.2	83.1
50 ± 1	4	2	2	5:1	1:1	14.2	68.6
50 ± 1	4	2	2	5:1	Without hexane	7.0	33.8
50 ± 1	1	2	2	5:1	2.5:1	8.2	39.6
50 ± 1	2	2	2	5:1	2.5:1	11.6	56.0
50 ± 1	3	2	2	5:1	2.5:1	15.7	75.8
50 ± 1	4	2	2	5:1	2.5:1	17.0	82.1
50 ± 1	6	2	2	5:1	2.5:1	17.2	83.0

[a]Based on original oil content, see Table 1.

TABLE 4
Response Surface Analysis[a] for Oil Yield for Mustard Seed and Rice Bran

	Response mean	Root MSE	R^{2b}
Mustard seed	27.875000	2.144629	0.9620
Rice bran	15.638095	1.280682	0.9319

[a]Factors are temperature, enzymes (pectinase and cellulase) time, water and hexane amount.
[b]Indicator of goodness-of-fit.

TABLE 5
Statistical Reproducibility of Oil Recovery of Mustard Seed and Rice Bran by Aqueous Enzymatic Extraction

	Degrees of freedom		Sum of squares		Mean squares	
Residual	Mustard seed	Rice bran	Mustard seed	Rice bran	Mustard seed	Rice bran
Lack of fit	6	5	50.5937	14.7413	8.4322	2.9482
Pure error[a]	5	4	0.0000	0.0200	0.0000	0.0050
Total error	11	9	50.5937	14.7613	4.5994	1.6401

[a]Indicates reproducibility.

TABLE 6
Statistical Significance of Different Factors[a] in Aqueous Enzymatic Extraction of Mustard Seed and Rice Bran

	Factors					
	Temperature (°C)	Cellulase (%)	Pectinase (%)	Time of reaction (h)	Water volume	Hexane volume
Mustard seed	0.1921	0.3463	0.0014	0.0000	0.0001	0.0000
Rice bran	0.8520	—	0.0061	0.0018	0.7755	0.0003

[a]$P < 0.05$ indicates statistical significance.

Quality data of the oils recovered by the enzyme process and by other processes indicate (Tables 7 and 8) that mustard oil and rice bran oil recovered by the enzyme-aided process are better than those obtained by other processes with respect to some characteristics (Table 8). The fatty acid compositions of the two oils are also comparable (Table 7). The free fatty acid content and peroxide value in rice bran oil, obtained by aqueous enzymatic extraction, are higher than those in the oil obtained by Soxhlet extraction. This may be due to the lipase action on the bran initially when it is heated with water. The allyl isothiocyanate content, responsible for the odor of mustard oil, is less in the enzyme-extracted mustard oil.

Recovery of rice bran oil can be achieved almost in full (99.5%) when rice bran is steam-cooked at 120°C for 1 min before enzymatic extraction (Table 9). The oil has a lower free fatty acid content due to deactivation of the bran lipase as a result of cooking. During enzymatic extraction, the nonoil portions of the seed or bran are fractionated into protein, syrup and hull. This is an advantage of enzymatic extraction over the other extraction processes in which the nonoil portion is obtained as a whole. The fractions obtained by enzyme process can be used for different purposes.

Table 10 indicates that the protein content (nitrogen content) in the protein meal fraction of mustard seed and rice bran from the enzyme process is increased, whereas the ash content is reduced in the meals obtained by the solvent process. The high protein and low ash contents of protein meal fractions from the enzyme process indicate their higher biological value for cattle feed or other food uses. The solubilized protein in the syrup fractions is somewhat higher, whereas the hull fraction in mustard seed is almost free from protein but with a high fiber content, indicating satisfactory use as fuel or cattle feed.

The enzymatic extraction process appears effective for achieving almost full recovery of oil from mustard seed and

TABLE 7
Fatty Acid Composition of Mustard Oil and Rice Bran Oil Obtained from Solvent and Enzyme Processes

	Major fatty acids (wt%)								
	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	$C_{22:1}$
Solvent-extracted[a] Mustard oil	—	2.5	1.0	10.4	16.5	12.6	—	7.4	49.2
Enzyme-extracted	—	1.9	0.4	10.2	16.8	12.7	—	6.5	51.3
Commercial (expeller)	—	2–5	0.4–1.2	9–14	14–20	9–17	—	6–8	45–54
Solvent-extracted[a] Rice bran oil	0.3	23.7	1.6	43.4	30.1	0.2	0.6	—	—
Enzyme-extracted	0.3	24.2	1.6	43.1	30.0	0.2	0.6	—	—
Commercial (solvent)	0.3–0.6	16–24	1–3	40–48	30–35	0.2–0.8	0.5–1.0	—	—

[a]Soxhlet method.

TABLE 8
Comparative Characteristics of Crude Mustard Oil and Rice Bran Oil Obtained by Different Processes

	Solvent-extracted oil (Soxhlet)		Standard specifications (ISI) for edible mustard oil[a] and rice bran oil[b]		Enzyme-extracted oil	
Analytical	Mustard	Rice bran	Mustard (expeller process)	Rice bran (solvent process)	Mustard	Rice bran
Free fatty acid (%)	1.1	3.5	1.0–3.0	max 10	1.3	5.9
Saponification value	175.0	184.4	170–176	175–195	174.3	188.4
Iodine value	106.2	89.4	98–108	85–105	106.8	88.9
Peroxide value (meq/1000 g of oil)	1.6	2.0	—	—	2.0	3.9
Unsaponifiable matter (%)	1.2	5.0	1.2 max.	6.0 max.	0.9	4.9
Lovibond color (1-cm cell)	30Y + 1.1R	25Y + 8.2R + 3B	Max. 50(Y + 5R) in 1/4-inch cell	No specified limit	15Y + 0.4R	12Y + 5.3R + 0.2B
Acetone, insoluble (%)	—	4.4	—	—	—	4.0
Odor (as allyl isothiocyanate % by weight)	0.42	—	0.25–0.6	—	0.15	—

[a]IS-546-1963 (Indian Standards).
[b]IS-3448-1984 (Indian Standards).

TABLE 9
Effect of Pretreatment on the Recovery of Oil by Extraction of Rice Bran with Mixture of Enzymes

Soxhlet-extracted oil		Treatment	Enzyme-extracted[b] oil yield	% Recovery	FFA in extracted
Oil (wt%)	FFA (%)	no.[a]	(wt%)	of oil[c]	oil (%)
20.7	3.5	I	18.2	87.9	5.9
		II	20.6	99.5	3.6

[a]Treatment I—water/bran (3:1, vol/wt) heated at 90°C for 15 min. Treatment II—bran steam-cooked at 120°C for 1 min.
[b]Extraction conditions: temperature 60°C, time 6 h, pH 4.5, hexane/bran 1.5:1 (vol/wt), water/bran 3:1 (vol/wt), enzyme (pectinase and cellulase) 3% of each on weight of bran (w/w). FFA, free fatty acid.
[c]Based on original oil content of 20.7%.

TABLE 10
Analysis of Protein, Ash, and Fiber Content in the Nonoil Fractions Isolated from Mustard Seed and Rice Bran by the Enzyme Process

	Nonoil fraction (meal) from solvent process[a]		Nonoil fractions from enzyme process				
			Protein meal		Syrup		Hull
	Mustard	Rice bran	Mustard	Rice bran	Mustard	Rice bran	Mustard
Protein (N × 6.25) (%)	43.3	18.5	55.4	29.4	31.1	11.1	traces
Insoluble fiber (%)	—	—	9.9	15.3	1.2	2.0	57.2
Ash (%)	7.2	17.6	4.7	9.3	8.5	18.5	5.4

[a]Soxhlet process.

rice bran. The two oils are of good quality, and the meals also are satisfactory because of high protein and low ash and fiber contents.

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