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Enzymatic Pretreatment of Stabilized Rice Bran with Mixed Enzymes: Evaluation of Oil

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ABSTRACT

The goal of the present investigation was to study the effect of a mixed enzyme preparation composed of Macerozyme and Protease (M and P) on the oil extractability from rice bran. Mixtures of P and M at different enzyme:enzyme ratios, different enzyme concentrations, different bran:water ratios and different time of hydrolysis were investigated. The sequence of the addition of the enzymes proved that one step addition of the enzyme mixture at the beginning of hydrolysis was the most appropriate method. Experiments with mixed enzymes at the different conditions proved that highest % increase in oil extractability (38%) over the control were achieved under the following conditions of hydrolysis: 2% enzyme concentration (1:1 and 2:1 M:P ratio) at 1:10 bran:water ratio and 3 and 6 h hydrolysis and 1.5% enzyme concentration (1:1 M:P ratio) at 1:10 bran:water ratio for 6 h hydrolysis. Hydraulic pressing after enzymatic pretreatment and miscella treatment gave slightly improved oil qualities.

Key words: Rice bran, enzymatic hydrolysis, oil extraction, hydraulic pressing, miscella treatment, oil quality

INTRODUCTION

Rice bran constitutes the outer 10% of the brown rice that is removed during the conversion of brown rice to white rice. The definition of rice bran as suggested by FAO is "a by-product of polishing brown rice, comprising the pericarp, aleurone layer, germ and some endosperm" (Abdul-Hamid *et al*., 2007). Most people think that the polished white rice they eat is nutritious. What they really need to know is that 65% of the nutrition as well as the phytochemicals are actually locked up in the rice bran. Rice bran contains about 20% oil, 15% protein and approximately 50% carbohydrate, of which starch is the main component. Rice bran is a rich source of dietary fiber. Oryzanol and vitamin E, potent antioxidants, are present in rice bran oil. Rice bran is also rich in B-complex vitamins (Saunders, 1985). Rice bran oil is notable for its very high smoke point of 490°F (254°C) and its mild flavour, making it suitable for high-temperature cooking such as stir frying and deep frying (Tophe Rice Bran Oil, 2007). The fatty acid composition of rice bran oil is in the range of 16.5-21.8% palmitic, 1.8-2.9% stearic, 35-41% oleic, 34.4-42.6% linoleic, 0.3-2.9% linolenic, 0.7-1.1% arachidic and 0.7% behenic (Sayre and Saunders, 1990; Gopalay *et al*., 2006).

Unfortunately, rice bran is an underutilized nutritious source and the precious nutrients and phytochemicals in the rice bran are discarded as a wasted food resource, ending up as animal feed, in the production of fertilizers, fuels or dumped in landfills. The main reason why all those precious

nutrients are not effectively utilized is the presence of a group of lipase enzymes found in the outer layer of the bran. Upon milling of the brown rice the enzymes are freed and instantly act upon the oil in the rice bran hydrolyzing it to free fatty acids, thus render the bran rancid within hours of milling (FAO/WHO, 1985). For effective utilization of rice bran, several methods have been developed for stabilization or inactivation of lipolytic enzymes in freshly milled rice bran.

Aqueous enzymatic oil extraction is undoubtedly an emerging technology in the fats and oils industry since it offers many advantages compared to conventional extraction. For instance, it eliminates solvent consumption which reportedly may also lower investment costs. In a recent publication, the extraction of higher-quality oil and the production of a protein concentrate or hydrolyzate as a by-product of an aqueous enzymatic treatment of rice bran, rapeseed and sunflower, previous to hexane extraction was reported (Sengupta and Bhattacharyya, 1996; Zhang *et al*., 2007; Latif and Anwar, 2009). Enzymatic treatment of oilseeds prior to oil extraction has been reported to increase the quantity of extracted oil due to the degradation of cell walls by specific enzymes as reported in reviews by Dominguez *et al*. (1994) and Rosenthal *et al*. (1996).

In our previous publication (Mourad *et al*., 2009) rice bran has been stabilized by a microwave treatment which gave oils that were stable for 48 weeks. The stabilized rice bran was enzymatically hydrolyzed using protease, macerozyme and α-amylase enzymes. The three enzymes resulted in an appreciable increase in the extracted oil yield. Protease and macerozyme gave oils with acceptable acidity while α-amylase gave oils with high acidity (15-19% free fatty acids).

The aim of the present work was to investigate the effect of the pretreatment of stabilized rice bran with enzyme mixtures formulated from protease and macerozyme at different conditions on the oil yield, followed by the study of the effect of different oil extraction methods after the pretreatment on the oil characteristics. The use of protease/macerozyme mixture on rice bran meal followed by hydraulic pressing has not been studied before. Enzymatic treatment of rice bran in miscella is rather a new investigated approach.

MATERIALS AND METHODS

Materials

Rice bran: The rice (*Oryza sativa*) variety (Giza 178) was obtained from Rice Technology Training Centre at Sakha, Egypt, a crop of year 2010. The rice was dehusked and milled using a Japanese type farmer mill at Rice Technology Training Centre at Alexandria, to yield rice bran and polished rice. The rice bran was collected and directly sieved to pass a 20 mesh sieve to remove brokens and husks. The rice bran was immediately subjected to microwave stabilization as described by Mourad *et al*. (2009). The sample was allowed to cool to room temperature (~25°C), placed in plastic zipper top-bags, after which the samples were transferred to the laboratory (Fats and oils department-NRC) and stored at 0°C (deep freezer) until further work.

Enzymes: The enzymes used in this work were: Protease (from *Bacillus licheniformis,* = 2.4 U g^{-1}) a product of Novozyme. Macerozyme R-10 (mixture of cellulase, hemicellulase and pectinase, >2500 U g^{-1}) obtained from Phytotechnology Laboratories.

Methods: Moisture, oil, protein, ash, crude fiber, Peroxide Value (PV), Free Fatty Acid (FFA), Iodine Value (IV), Saponification Value (SV) and unsaponifiable matter (USM) were determined according to AOCS (2009). Fatty acid methyl esters of rice bran oil were determined by gas-liquid chromatographic technique (GLC) according to Ludde *et al*. (1960). A set of standard methyl esters

of 14:0, 16:0, 16:1, 18:0, 18:1, 18:2 and 18:3 fatty acids were used as authentic samples to enable the characterization of the unknown fatty acids by reference to their retention times. Peaks identification and quantification were performed by using Philips PU 4810 computing integrator.

Determination of oxidative stability of oil: Hundred gram oil samples were stored in 200 mL open beakers in a draught air oven at 60°C in the dark for 10 days. The oil samples were analyzed every two days to determine the progress in the formation of thiobarbituric acid reactive substances (TBARS) which was carried out based on the method of Egar *et al.* (1981).

Enzymatic hydrolysis of rice bran: The enzymes used in this work were: Protease (P) and macerozyme R-10 (M). In the basic hydrolysis experiment, 50 g of stabilized rice bran were suspended in distilled water at different bran:water ratios of 1:5, 1:7, 1:10 (w/w) and were each stirred on a magnetic stirrer while heating to the appropriate temperature for each enzyme. The enzyme was added at concentrations of 1, 1.5 and 2% (w/w) of the rice bran weight. After adjusting the suitable pH for each enzyme mixture, the mixture was transferred to a shaking water bath at 100×g for 1, 2, or 3 h. Incubation pH was fixed at the optimum range for each enzyme using 1 N NaOH and 6 N HCl. At the end of the hydrolysis experiment the pH was shifted to a value of (pH~2) the temperature was then raised to ~80°C for 5 min to assure complete inactivation of the enzyme. After enzyme inactivation, the hydrolyzed mixture was centrifuged at 4500×g for 10 m, with temperature of centrifuge adjusted to 10°C. Two phases, a liquid and a meal phase were obtained. The liquid phase was drained and the cake was mixed and dried overnight in a draught hot air oven at 60°C. The dry cake was ground and its oil content was determined.

Sequence of addition of enzyme mixture: In this experiment the sequence of the addition of the enzyme mixture (M and P) at 1% enzyme concentration and 1:1, M:P ratio w/w was studied and the effect on oil yield and FFA% of the oil was determined. Two step addition and a one step addition of the enzymes were investigated:

Two step addition of enzyme mixture: First the pH and temperature were adjusted for maximum activity of Macerozyme (M) (pH 4.5-5, 50°C, 1 h), the enzyme was then inactivated then pH and temperature shifted to those for maximum activity of protease (P) (pH 7.5, 37°C, 1 h). This was designated Experiment A.

One step addition of enzymes: Here both P and M were added together at the beginning of then hydrolysis followed at:

- pH 6.0 and temperature 40°C, for 2 h. This was designated Experiment B
- pH 6.5 and temperature 40°C for 2 h. This was designated Experiment C
- Here the two enzymes were added together at the beginning of the experiment. The conditions were adjusted to pH 4.5 and temperature 50°C and experiment carried for 1 h, then pH shifted to 7.5 and temperature lowered to 37°C for another hour. This was designated Experiment D

The enzymes were deactivated at the end of each of the experiments A-D, as described in the enzymatic hydrolysis of rice bran, basic experiment (above).

Table 1: Parameters investigated during the enzymatic pretreatment of rice bran with enzyme mixture M and P

Enzyme concentration (%)	M:P (ratio)	W:B (ratio)	Time (h)
			1
1	1:1	5:1	3
			6
			1
1.5	1:1	7:1	3
			6
			1
2	1:1	10:1	3
			6
			1
1	2:1	5:1	3
			6
			1
1.5	2:1	7:1	3
			6
			1
2	2:1	10:1	3
			6
			1
1	1:2	5:1	3
			6
			1
1.5	1:2	7:1	3
			6
			1
2	1:2	10:1	3
			6

M: Macerozyme, W: Water, P: Protease, B: Bran

Determination of optimum conditions for enzyme mixtures: In this experiment one step addition of enzyme mixture (M and P) was applied in all experiments. Several parameters were investigated including: enzyme concentration, ratio of enzymes in the mixture M:P, w/w, bran:water ratio (w/w) and time of the reaction according to Table 1. After the enzyme hydrolysis the oil was extracted in a Soxhlet extractor with n-hexane, to determine the effect of different enzyme treatments on oil yield.

Effect of oil extraction method on oil characteristics: Three different methods for oil extraction following enzymatic hydrolysis were examined with the aim to compare their effect on oil characteristics.

Enzymatic hydrolysis of stabilized rice bran (SRB) followed by solvent extraction of oil: Fifty gram of stabilized rice bran were suspended in 500 mL of distilled water. Enzyme mixture (P and M, 1:1, w/w) was added at a concentration of 1.5% of rice bran weight. The mixture was stirred using a magnetic stirrer while heating to 40°C. The pH of the mixture was adjusted to 6.5 with 1 N-NaOH and 6 N-HCl. Then, the mixture was transferred to a shaking water bath at 100×g for 1 h. Incubation temperature and pH were maintained constant during the time of the

experiment. At the end, the enzyme mixture was inactivated as previously described. The mixture was centrifuged at 4500×g for 10 min. at 10°C. Two phases-liquid and a cake phase were obtained. The liquid phase was drained and the cake was dried overnight in a hot air oven at 60°C. The dry cake was ground and extracted with n-hexane using a Soxhlet extractor. This method yielded Oil Sample (OS).

Enzymatic hydrolysis of stabilized rice bran followed by hydraulic pressing: The enzymatic pretreatment of rice bran was carried out as in the above experiment. Then the dried cake was subjected to hydraulic pressing on the cold to obtain the oil. Pressure was applied at 1600 bar; the hydraulic press was locally made in National Research Centre. This method yielded oil sample (OH).

Enzymatic hydrolysis of stabilized rice bran in the presence of hexane (miscella): Fifty gram of stabilized rice bran were suspended in 500 mL of distilled water. Enzyme mixture was added at concentrations of 1.5% P and M (1:1) of rice bran weight. The mixture was stirred on a magnetic stirrer while heating to 40°C. The pH of the mixture was adjusted to 6.5 with 1 N NaOH and 6 N HCl. Then, 1.5 volume of hexane based on the weight of rice bran was added, the mixture was transferred to a shaking water bath at 100 rpm for 1 h. Conditions were kept constant throughout the reaction. Enzymes were deactivated as previously mentioned. 30% (w/v) ammonium sulphate concentration was then added to the slurry and vortexed gently. This was subsequently incubated at 37°C for 1 h for the three phase formation. The three phases formed were separated by centrifugation at 2000 xg for 10 min (Gaur *et al*., 2007). The upper organic layer was collected then dried over anhydrous sodium sulphate and evaporated on a rotary evaporator under reduced pressure to obtain the oil extracted in this phase. The amounts of oil recovered were calculated as percentages of total oil present in rice bran.

OC the control samples that have been treated under the same conditions but without the addition of enzymes as OS, OH and OM.

Statistical analysis: The data were analyzed statistically by ANOVA and Duncan's multiple range test (Walter and Duncan 1969) using commercially available software packages SPSS Inc., Chicago, IL, A $p<0.05$ was considered significant.

Correlation studies were carried out on a HP home computer, using excel program.

RESULTS AND DISCUSSION

Proximate composition of rice bran: Analysis of rice bran (Table 2) revealed 19.42% oil, 13.99% protein, 10.23% ash, 19.68% crude fiber and 36.68% nitrogen free extract. These results were close to the values reported by Mihara *et al.* (1974), Anderson and Guraya (2001) and Sereewatthanawut *et al.* (2008).

Effect of pretreatment of microwave stabilized rice bran with enzyme mixtures on yield and FFA of the oil: In view of our previous results Mourad *et al.* (2009), treatment of stabilized rice bran with single enzymes protease P and macerozyme M resulted in (26%) increase in oil extractability over untreated rice bran. Therefore, enzyme mixtures from both enzymes were chosen to be formulated and tested. α-amylase enzyme was previously studied in (Mourad *et al.*,

Table 2: Proximate composition of stabilized rice bran

Composition	Rice bran
Oil (%)	19.42
Protein (%)	13.99
Ash (%)	10.23
Crude fiber (%)	19.68
Nitrogen free extract (%)	36.68

Values are given on moisture free basis

Table 3: Effect of the sequence of addition of enzyme mixture macerozyme and protease (M and P) on % extracted oil and FFA of the oil*

Experiment	Extracted oil (%)	FFA (%)
A	30.47^{c}	3.42^{c}
B	30.92^{b}	3.51^{b}
C	31.26^{a}	3.66^{a}
D	30.91^{b}	3.43^{c}
Control	19.42^{e}	2.36^{d}

*All experiments were carried out at 1% concentration of enzyme mixture M and P, 1:1 M:P ratio and 1:10 bran:water ratio. A: Two step enzyme addition, first (M) at pH 4.5-5, temperature 50°C, 1 h, followed by the addition of (P) at pH 7.5, temperature 37°C, for another 1 h, B: Addition of (M and P) at the same time at pH 6.0, temperature 40°C, for 2 h, C: Addition of (M and P) at the same time at pH 6.5, temperature 40°C, for 2 h, D: Addition of (M and P at the same time at pH 4.5-5, temperature 50°C for 1 h, then change pH to 7.5, temperature 37°C, for another 1 h. Significantly different values at $p<0.05$ are indicated by different letters in the same column

2009) but was excluded in this study because, it resulted in improved oil yield, although the free fatty acid FFA of the resulting oil was very high (15-19% FFA), It is reported that bran oil with an excess of 10% FFA and bran with more than 5% are considered unsuitable for human consumption (Tao *et al*., 1993). Thus, the enzyme mixtures under investigation included mixtures of protease and macerozyme (P and M).

Several conditions for the addition of the enzyme mixtures to the aqueous bran slurry had to be investigated in order to elucidate the optimum conditions of the experiment.

Sequence of addition of enzyme mixture: In these experiments 1% mixture of the P and M (1:1.w/w), using bran:water ratio (1:10; w/w) was investigated as to their effects on the oil yield and its FFA%. The sequence of the addition of the enzyme mixture was studied:

- **Two step addition of enzymes:** As in experiment A in materials and methods section
- **One step addition of enzymes:** Here, both P and M were added at the same time as in experiments B, C and D, in materials and methods section

Results of the above investigation (Table 3) revealed that highest oil yield was achieved under conditions of experiment C, where 31.26% oil was extracted, compared to 19.42% oil extracted from control. Experiments A, B and D resulted in extraction of ~30.47, 30.92 and 30.91% oil, respectively. All experiments were significantly different with control. Experiments B and D were not significantly different with each other but were significantly different with experiments A and C. The FFA% of the oils resulting from all experiments proved to be within a permissible level but they increased from 2.36 FFA% of control oil to between 3.4-3.6 FFA%, for oils from A, B, C and D.

The coming set of experiments were carried out under conditions of experiment C, where M and P mixture was added at the beginning of the experiment at the following conditions: pH 6.5 and temperature 40°C for 2 h.

Table 4: Effect of treatment of microwave stabilized rice bran with enzyme mixture macerozyme and protease (M and P) at different concentrations and ratios on oil and free fatty acids (FFA%) at 1:5 bran:water (B:W) ratio

	Time (h)					
	1		3		6	
Enzyme mixture	----------%----------		----------%----------		----------%----------	
(% and M:P ratio)	Oil	FFA	Oil	FFA	Oil	FFA
Non	23.76±0.108^{g}	3.14±0.035^{h}	24.13±0.130^{i}	3.25±0.040^{g}	24.70±0.255^{e}	3.41±0.0917^{c}
1, 0.5:0.5	26.17±0.166^{f}	3.75±0.040^{e}	27.05±0.061^{h}	3.79±0.030^{f}	27.94±0.100^{d}	4.35±0.0400^{b}
1, 0.67:0.33	32.68±0.127^{b}	3.84±0.046^{d}	32.82±0.180^{b}	4.00±0.075^{e}	33.20±0.210^{a}	4.37±0.0100^{b}
1, 0.33:0.76	30.09±0.111^{e}	3.97±0.015^{c}	30.34±0.206^{f}	4.26±0.017^{c}	30.53±0.387^{c}	4.55±0.0100^{b}
1.5, 0.75:0.75	31.09±0.100^{d}	3.48±0.042^{g}	31.54±0.141^{d}	4.21±0.026^{cd}	31.75±0.140^{b}	4.48±0.0305^{b}
1.5, 1:0.5	32.84±0.243^{ab}	4.13±0.020^{b}	33.05±0.091^{ab}	4.36±0.025^{b}	33.15±0.755^{a}	4.80±0.0500^{a}
1.5, 0.5:1	29.92±0.080^{e}	3.95±0.040^{c}	30.07±0.1059^{g}	4.15±0.040^{d}	30.39±0.428^{c}	4.82±0.0150^{a}
2, 1:1	31.83±0.069^{c}	3.63±0.050^{f}	31.83±0.206^{c}	4.03±0.051^{e}	31.88±0.130^{b}	4.78±0.0350^{a}
2, 1.33:0.67	33.03±0.163^{a}	3.92±0.070^{c}	33.12±0.1464^{a}	4.35±0.035^{b}	33.33±0.550^{a}	4.78±0.0210^{a}
2, 0.67:1.33	30.91±0.171^{d}	4.77±0.032^{a}	30.94±0.111^{e}	4.77±0.015^{a}	31.04±0.045^{c}	4.84±0.3060^{a}

M: Macerozyme, P: Protease. Significantly different values ($p<0.05$) of extracted oil (%) and FFA (%) in the same columns are indicated by different letters

Determination of optimum conditions for the pretreatment of microwave stabilized rice bran with enzyme mixtures for highest yield and low FFA of the oil: In this experiment one step addition of enzyme mixture (M and P) at same conditions of pH and temperature as in experiment C were applied in all experiments. The parameters investigated included: enzyme concentrations 1, 1.5 and 2%; ratio of enzymes in the mixture 1:1, 2:1 and 1:2 M:P; bran:water ratios 1:5, 1:7 and 1:10 (w/w); and time of the reaction 1, 3 and 6 h. After the enzymatic hydrolysis the oil was extracted by a Soxhlet apparatus using n-hexane as solvent.

Results in Table 4 indicated the % oil extracted and % FFA of the oil resulting from hydrolysis of rice bran under different enzyme concentrations; different M:P ratios; different hydrolysis time; and at 1:5 bran:water ratio. At hydrolysis time 1 h and 1, 1.5 and 2% enzyme concentration, there was a significant difference between treatments (different M:P ratios) and the control and in between the treatments. Highest % extracted oil 33.03, 32.84 and 32.68% was achieved under the following conditions 2% at (2:1 M:P ratio), 1.5% at (2:1 M:P ratio) and 1% at (2:1 M:P ratio). Other statistical analysis of the results can be easily depicted from the table.

At hydrolysis time 3 h all hydrolysis conditions gave results that were significantly different with control and in between one another except for treatments 1% at (2:1 M:P ratio) and 1.5% at 2:1 M:P ratio and 2% at 2:1 M:P ratio that pointed to no significant difference in between them. The latter treatments yielded the highest % extracted oil ~33%. When enzymatic hydrolysis of rice bran using the mixed enzyme M and P was prolonged to 6 h, highest % extracted oil from the bran was attained at same conditions of hydrolysis as at 1 and 3 h which also reached 33% extracted oil. At 6 h, all treatments were significantly different with the control. No significant difference was observed between 1, 1.5, 2% at 1:2, M:P ratio, nor between treatments 1, 1.5, 2% at 2:1, M:P ratio.

Thus, at 1:5 bran:water ratio highest extracted oil was achieved at 1, 1.5, 2% enzyme concentration and 2:1 M:P ratio of enzyme mixture when hydrolysis was carried for 1, 3 and 6 h.

Table 4 also illustrated that the % FFA of the oils extracted after the conditions pointed out in the table. Naturally FFA of all oils resulting from the enzymatic hydrolysis increased over control (~3% FFA). Hydrolysis for 1 and 3 h increased the FFA% of the extracted oils to ~4% except for

Table 5: Effect of treatment of microwave stabilized rice bran with enzyme mixture macerozyme and protease (M and P) at different concentrations and ratios on oil and free fatty acids (FFA%) at 1:7 bran:water (B:W) ratio

	Time (h)					
	1		3		6	
Enzyme mixture	%-		%-		%-	
(% and ratio M:P)	Oil	FFA	Oil	FFA	Oil	FFA
Non	24.99±0.097^{f}	3.35±0.032^{f}	25.38±0.148^{d}	3.35±0.032^{e}	25.84±0.145^{e}	3.62±0.026^{g}
1, 0.5:0.5	28.08±0.040^{e}	3.95±0.035^{e}	29.37±0.068^{c}	4.17±0.043^{d}	29.78±0.185^{d}	4.54±0.050^{f}
1, 0.67:0.33	32.82±0.160^{b}	3.87±0.053^{e}	33.03±0.068^{a}	4.35±0.035^{bcd}	33.50±0.405^{a}	4.54±0.015^{f}
1, 0.33:0.76	30.46±0.230^{d}	3.94±0.025^{e}	30.76±0.209^{b}	4.44±0.410^{bcd}	30.72±0.292^{c}	4.63±0.021^{e}
1.5, 0.75:0.75	32.98±0.0802^{b}	4.25±0.045^{b}	33.13±0.051^{a}	4.55±0.025^{bc}	33.29±0.205^{a}	4.71±0.025^{d}
1.5, 1:0.5	33.13±0.119^{ab}	4.10±0.036^{cd}	33.33±0.280^{a}	4.65±0.020^{b}	33.48±0.271^{a}	4.95±0.040^{b}
1.5, 0.5:1	30.52±0.307^{d}	4.15±0.026^{c}	30.89±0.856^{b}	4.35±0.040^{bcd}	31.18±0.192^{b}	4.72±0.015^{d}
2, 1:1	33.37±0.081^{a}	4.05±0.036^{d}	33.33±0.156^{a}	4.23±0.031^{cd}	33.48±0.258^{a}	4.87±0.021^{c}
2, 1.33:0.67	33.11±0.125^{ab}	3.94±0.061^{e}	33.18±0.125^{a}	4.22±0.0251^{cd}	33.36±0.286^{a}	4.88±0.015^{c}
2, 0.67:1.33	31.22±0.163^{c}	4.45±0.032^{a}	31.14±0.161^{b}	4.94±0.035^{a}	31.25±0.238^{b}	5.05±0.040^{a}

M: Macerozyme, P: Protease. Significantly different values ($p<0.05$) of extracted oil (%) and FFA (%) in the same columns are indicated by different letters

treatment 2% enzyme at (1:2 M:P ratio) which reached 4.77% FFA. When hydrolysis was carried out for 6 h the FFA% of oils resulting from most of the treatments increased to ~5%. Significant and non-significant differences between treatments can be deduced from the table.

Table 5 gave the results of enzymatic hydrolysis of rice bran prior to oil extraction when using bran:water ratio of 1:7. Other conditions applied included 1, 1.5 and 2% enzyme concentration; 1:1, 1:2 and 2:1 M:P ratio; and hydrolysis time 1, 3 and 6 h. All hydrolysis treatments resulted in different levels of increase in oil extractability over control (~25%). Highest levels in % extracted oil was attained when using 1% enzyme concentration at (2:1 M:P ratio); 1.5% and 2% enzyme concentration at (1:1 and 2:1 M:P ratio) and hydrolysis time 1, 3 and 6 h. Highest levels of extracted oils were ~33%. Examining the results of statistical analysis (Table 5), the significant and non-significant difference between treatments can be deduced.

Similar to results of 1:5 bran:water ratio, using 1:7 bran:water ratio achieved highest extracted oil at 1, 1.5 and 2% enzyme concentration; and 2:1 M:P ratio of enzyme mixture; when hydrolysis was carried for 1, 3 and 6 h.

Table 5 also indicated the effect of enzymatic hydrolysis of rice bran at 1:7 bran:water ratio, with mixed enzyme M and P under different conditions on the FFA content of the resulting oils. As the hydrolysis progressed FFA of the oils increased, after 1 h, FFA was ~4.0%, after 3 h it was between 4.0-5.0% and after 6 h hydrolysis it reached 5.0%, compared to ~3.35% for control. The FFA of the control was significantly different with all treatments. Other statistical differences are indicated in the also Table 5.

The results obtained when enzymatic hydrolysis of the stabilized rice bran was carried out using the mixed enzyme M and P at different concentrations 1, 1.5 and 2%; M:P ratio 1:1, 1:2; and 2:1. Hydrolysis time was 1, 3 and 6 h; at bran:water ratio 1:10 are shown in Table 6.

Highest % extracted oil reached 34-34.6% compared to ~25% for control. All treatments at 1 h hydrolysis were significantly different with control and with one another. At 3 h hydrolysis all treatments were significantly different with control and with one another except for treatments 1.5% (1:1 M:P ratio) and 2% (1:1 and 2:1 M:P ratio). At 6 h hydrolysis 2% (1:1 M:P ratio) and 2%

Table 6: Effect of treatment of microwave stabilized rice bran with enzyme mixture macerozyme and Protease (M and P) at different concentrations and fatty acids (FFA%) at 1:10 bran:water (B:W) ratio

	Time (h)					
	1		3		6	
Enzyme mixture	%-		%-		%-	
(% and ratio M:P)	Oil	FFA	Oil	FFA	Oil	FFA
Non	25.06±0.010^{j}	3.27±0.010^{h}	25.54±0.035^{h}	3.46±0.015^{i}	25.91±0.080^{i}	3.76±0.015^{f}
1, 0.5:0.5	28.44±0.015^{i}	3.69±0.010^{g}	29.84±0.041^{g}	3.97±0.015^{h}	30.34±0.050^{h}	4.44±0.035^{f}
1, 0.67:0.33	32.85±0.025^{e}	4.14±0.010^{b}	33.36±0.060^{c}	4.43±0.020^{b}	33.65±0.061^{d}	4.64±0.035^{e}
1, 0.33:0.76	31.03±0.021^{h}	3.87±0.0153^{e}	31.37±0.036^{f}	4.44±0.015^{b}	31.58±0.075^{g}	4.90±0.010^{c}
1.5, 0.75:0.75	34.32±0.0023^{c}	3.77±0.015^{f}	34.4±0.032^{a}	4.07±0.021^{f}	34.51±0.090^{b}	4.41±0.020^{f}
1.5, 1:0.5	33.62±0.020^{d}	3.92±0.015^{d}	33.95±0.040^{b}	4.02±0.026^{g}	34.08±0.070^{c}	4.85±0.036^{d}
1.5, 0.5:1	31.98±0.031^{g}	3.94±0.006^{d}	32.13±0.055^{e}	4.28±0.015^{d}	32.36±0.061^{f}	4.65±0.037^{e}
2, 1:1	34.48±0.026^{a}	3.97±0.021^{c}	34.54±0.030^{a}	4.14±0.010^{e}	34.67±0.045^{a}	4.96±0.015^{b}
2, 1.33:0.67	34.37±0.015^{b}	4.16±0.015^{b}	34.44±0.030^{a}	4.36±0.015^{c}	34.62±0.120^{a}	4.95±0.040^{b}
2, 0.67:1.33	32.48±0.010^{f}	4.35±0.015^{a}	32.54±0.035^{d}	4.78±0.031^{a}	32.58±0.065^{e}	5.01±0.015^{a}

M: Macerozyme, P: Protease. Significantly different values ($p<0.05$) of extracted oil (%) and FFA (%) in the same columns are indicated by different letters

(2:1 M:P ratio) were not significantly different, apart from these two treatments all other treatments at 6 h were significantly different.

Common with the results of hydrolysis at a bran:water ratio 1:5 and 1:7, it was found that highest % oil extraction was achieved also under the following conditions: 2% enzyme concentration at (2:1 M:P ratio); 1.5 and 2% at (1:1 M:P ratio); and 1.5% at (2:1 M:P ratio).

The FFA of the oils resulting from the enzymatic hydrolysis at 1:10 bran:water ratio and same conditions as the above experiment were given in Table 6. The FFA% of the control oils ranged between 3.27-3.76%. Oils resulting from different treatments had FFA ranging from 3.77-5.01%. FFA% increased with increasing time 1>3>6 h. All treatments yielded oils with FFA that were significantly different with control and in between the treatments. Other statistical differences are clear from the table.

Table 7 gave the calculated % increase in oil extractability as a result of the mixed (M and P) enzymatic treatment of stabilized rice bran at different conditions employed in Table 4-6, before oil extraction. Highest % increase in oil extractability ~38% were achieved under the following conditions of hydrolysis: 2% enzyme concentration (1:1 and 2:1 M:P ratio) at 1:10 bran:water ratio and 3 and 6 h hydrolysis and 1.5% enzyme concentration (1:1 M:P ratio) at 1:10 bran:water ratio for 6 h hydrolysis. This was followed by ~37% increase in oil extractability for treatments 1.5% enzyme concentration (1:1 M:P ratio) and 1:10 bran:water ratio for 1 and 3 h. and 2% enzyme concentration (2:1 M:P ratio), 1:10 bran:water ratio and hydrolysis for 1 h.

Comparing results in Table 7 to those in our previous paper (Mourad *et al*., 2009) using single enzymes (macerozyme and protease), it became obvious that the use of enzyme mixtures has the privilege over single enzymes by achieving a % increase in oil extractability of 38% compared to 26% for single enzyme M and P.

The only literature on the use of enzyme mixtures in the pretreatment of rice bran to improve oil extraction yield has been reported by Sharma *et al*. (2001) and Hanmoungjai *et al*. (2002). Their results were found to be in agreement with our results. Sharma *et al*. (2001) reported on the extraction of rice bran oil by an enzyme-assisted aqueous extraction under optimized aqueous

Table 7: Increase in oil extractability (%) of mixed enzyme (M and P) treated stabilized rice bran over untreated stabilized rice bran

			Bran:water (ratio)		
Enzyme concentration (%)	M:P (ratio)	Time (h)	1:5	1:7	1:10
	1:1	1	4.55	12.18	13.62
1	1:1	3	8.07	17.33	19.21
	1:1	6	11.62	18.97	21.21
	1:2	1	20.21	21.69	23.97
1	1:2	3	21.21	22.89	25.32
	1:2	6	22.01	22.73	26.16
	2:1	1	30.56	31.12	31.24
1	2:1	3	31.12	31.96	33.28
	2:1	6	32.64	33.83	34.43
	1:1	1	24.21	31.76	37.12
1.5	1:1	3	26.00	32.36	37.44
	1:1	6	26.84	33.00	37.87
	1:2	1	19.50	21.93	27.76
1.5	1:2	3	20.13	23.41	28.36
	1:2	6	21.41	24.57	29.28
	2:1	1	31.20	32.36	34.31
1.5	2:1	3	32.04	33.16	34.43
	2:1	6	32.44	33.75	36.15
	1:1	1	27.16	33.32	37.75
2	1:1	3	27.16	33.16	37.99
	1:1	6	27.36	33.75	38.51
	1:2	1	23.49	24.73	29.76
2	1:2	3	23.61	24.41	30.00
	1:2	6	24.01	24.85	30.16
	2:1	1	31.96	32.28	37.31
2	2:1	3	32.32	32.56	37.59
	2:1	6	33.16	33.28	38.31

Values are calculated on average value of control (Table 4-6) = 25.03% oil% increase in oil extractability = enzyme extracted oil-control/control×100

extraction conditions, using mixtures of protease, α-amylase and cellulase. 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 xg for 20 min yielded a 77% recovery of the oil.

Hanmoungjai *et al.* (2002) studied the enzymatic water extraction of oil and protein from rice bran in a laboratory scale set-up. The effects of the following enzymes-Celluclast 1.5 L, hemicellulase, Pectinex Ultra SP-L, Viscozyme L, Alcalase 0.6 L and papain-on oil and protein extraction yields and the level of reducing sugars in the extract were investigated. The results showed that Alcalase was most effective in enhancing oil and protein extraction yields. Papain was found to be superior to all carbohydrase enzymes but it gave lower yields than Alcalase. Celluclast 1.5 L, hemicellulase, Pectinex Ultra SP-L, Viscozyme L did not affect yields significantly but increased the level of reducing sugars in the extract.

Other authors have reported the successful use of enzyme mixtures in the extraction of oil from oil bearing materials other than rice bran (Dominguez *et al.*, 1993, 1995; Sharma *et al.*, 2002; Abdulkarim *et al.*, 2006; Taha and Hassanein, 2007; Latif and Anwar, 2009). All their results

Table 8: Correlation between % increase in oil extractability (OE) and time (t), at several enzyme concentrations (EC), different M:P ratios, different bran:water (B:W) ratios and different hydrolysis time 1, 3 and 6 h

			Range of validity	
OE vs. t	Correlation formula	Correlation coefficient (R)	B:W	H (h)
8a EC (%)				
1	OE = 1.3958t+3.4274	0.993	1:5	1-6
	OE = 1.2939t+11.847	0.919	1:7	1-6
	OE = 1.4508t+13.177	0.928	1:10	1-6
1.5	OE = 0.5066t+23.995	0.949	1:5	1-6
	OE = 0.5066t+23.995	0.995	1:7	1-6
	OE = 0.1495t+36.978	0.999	1:10	1-6
2	OE = 0.0421t+27.086	0.918	1:5	1-6
	OE = 0.0553t^2-0.3013t+33.566	1.000	1:7	1-6
	OE = 0.1537t+37.571	0.995	1:10	1-6
8b				
1	OE = 0.3526t+19.968	0.984	1:5	1-6
	OE =-0.1307 t^2+1.1227t+20.698	1.000	1:7	1-6
	OE = 0.4255t+23.732	0.969	1:10	1-6
1.5	OE = 0.3855t+19.062	0.996	1:5	1-6
	OE = 0.5168t+21.581	0.982	1:7	1-6
	OE = 0.3042t+23.349	1.000	1:10	1-6
2	OE = 0.1063t+23.349	0.982	1:5	1-6
	OE = 0.0613 t^2 +0.04053t+25.074	1.000	1:7	1-6
	OE = 0.0779t+29.714	0.973	1:10	1-6
8c				
1	OE = 0.4232t +30.029	0.989	1:5	1-6
	OE = 0.5484t+30.475	0.994	1:7	1-6
	OE = 0.6179t+30.924	0.961	1:10	1-6
1.5	OE = 0.2389t+31.097	0.950	1:5	1-6
	OE = 0.2716t+32.185	0.979	1:7	1-6
	OE = 0.3842t+33.683	0.939	1:10	1-6
2	OE = 0.2432t = 31.669	0.993	1:5	1-6
	OE = 0.2032t+32.029	0.991	1:7	1-6
	OE = 0.2032t+37.059	0.991	1:10	1-6

8a: 1:1, M: P ratio, 8b: 1:2, M:P ratio, 8c: 2:1, M:P ratio

confirm the fact that the use of enzyme mixtures further improves the extractability of oil from oil bearing materials, over single enzymes.

All enzymatic hydrolysis of rice bran with mixed enzymes (M and P) prior to oil extraction resulted in increasing the quantity of extracted oil over the extracted oil from untreated rice bran. An overall conclusion of this part of the study indicated optimum condition for enzymatic hydrolysis was 1.5% EC (1:1, M:P ratio) at 1:10, bran:water ratio for 1 h. These hydrolysis conditions resulted in 38% increase in oil extractability over control, compared to 26% increase in oil extractability for single enzyme treatment (M alone and P alone).

Regression study: Regression analysis of the results in Table 7 was represented in Table 8-10. Correlation between % increase in oil extractability and time (8a-c) were mostly linear correlations which signified that % increase in oil extractability usually increases with increasing time of

Table 9: Correlation between % increase in oil extractability and bran:water (B:W) ratios, at several enzyme concentrations (EC), different M:P ratios and hydrolysis time(t) 1, 3, 6 h

			Range of validity	
OE vs. B:W	Correlation formula	Correlation coefficient (R)	B:W	H (h)
9a EC (%)				
1	OE = -1E+06 (B:W)^2+17051(B:W)-38.067	1.000	1:5-1:10	1
	OE =-2E+06 (B:W)^2+20541(B:W) -43.341	1.000	1:5-1:10	3
	OE = 2628.8(B:W)-3.49	0.915	1:5-1:10	6
1.5	OE = 3627.7(B:W) -138.6	0.977	1:5-1:10	1
	OE = 3627.7(B:W)-138.6	0.983	1:5-1:10	3
	OE = 3110.4 (B:W)-112.9	0.983	1:5-1:10	6
2	OE = 2977 (B:W)-106.46	0.978	1:5-1:10	1
	OE = 3055.8 (B:W)-110.12	0.984	1:5-1:10	3
	OE = 3138.1 (B:W)-113.53	0.980	1:5-1:10	6
9b				
1	OE = 1083.8(B:W)-28.721	0.999	1:5-1:10	1
	OE = 1182.3 (B:W)-32.144	0.999	1:5-1:10	3
	OE = 1230.8 (B:W)-33.919	0.970	1:5-1:10	6
1.5	OE = 2412(B:W) -89.72	0.993	1:5-1:10	1
	OE =2370.7 (B:W) -86.89	1.000	1:5-1:10	3
	OE = 2266.1 (B:W) -60.69	1.000	1:5-1:10	6
2	1853.8 (B:W) -60.69	0.975	1:5-1:10	1
	OE = 1906.9 (B:W)-63.16	0.957	1:5-1:10	3
	1832.6 (B:W)-59.351	0.960	1:5-1:10	6
9c				
1	OE =-99533 (B:W)^2+9527 (B:W)-196.68	1.000	1:5-1:10	1
	OE = 622.99 (B:W) +2.9895	0.999	1:5-1:10	3
	OE = 497.56 (B:W) +10.368	0.954	1:5-1:10	6
1.5	OE = 898.68 (B:W) -9.4068	0.999	1:5-1:10	1
	OE = 682.11 (B:W) +1.3153	0.996	1:5-1:10	3
	OE = 1075.1 (B:W)-16.156	0.998	1:5-1:10	6
2	OE = 1609.8 (B:W)-41.422	0.937	1:5-1:10	1
	OE = 1588.5 (B:W)-40.123	0.932	1:5-1:10	3
	OE = 1556.7 (B:W)-37.874	0.925	1:5-1:10	6

9a: 1:1, M:P ratio, 9b: 1:2, M:P ratio, 9c: 2:1, M:P ratio, H: Hydrolysis

hydrolysis. Only cases 2% EC (1:1 M:P ratio), 1:7 B:W ratio; 1.5% EC (1:2 M:P ratio), 1:10 B:W ratio; 2% EC (1:2 M:P ratio), 1:7 B:W ratio; and 1%EC (1:2 M:P ratio), 1:7 B:W ratio, revealed curvilinear correlations.

Correlation between % increase in oil extractability and Bran:water ratio (9a-c) also demonstrated a linear correlation for most of the cases. The exception included treatments 1% EC (1:1 M:P ratio) at 1 and 3 h; 1.5% EC (1:2 M:P ratio) at 3 and 6 h and 1% EC (2:1 M:P ratio) at 1 h which pointed out a curvilinear relationship.

Correlation between % increase in oil extractability and Enzyme concentrations (10-c) were in the majority of the cases a curvilinear correlation. This is meant that the increase in enzyme concentration is not necessarily accompanied by increase in oil extractability. Only some cases showed a linear correlation as indicated in the tables. including 1% EC (1:1 M:P ratio) at 1:5 B:W ratio for 1 h; 1, 1.5% EC (2:1 M:P ratio) at 1:7 B:W ratio, for 1 and 3 h; 1.5, 2 % EC (1:2 M:P ratio)

Table 10: Correlation between % increase in oil extractability (OE) and enzyme concentration (EC) at several bran:water (B:W) ratios, different M:P ratios and hydrolysis time (t) 1, 3, 6 h

			Range of validity	
OE vs. EC	Correlation formula	Correlation coefficient (R)	EC (%)	H (h)
10a M:P				
1:1	OE = 22.61EC-15.275	0.919	1.0	1
	OE = -33.54 $(EC)^2$+119.71 (EC)-78.1	1.000	1.5	3
	OE = -29.4$(EC)^2$+103.94(EC)-62.92	1.000	2.0	6
1:2	OE = 9.4$(EC)^2$-24.92(EC) +35.73	1.000	1.0	1
	OE = 9.12$(EC)^2$-24.96 (EC)+37.05	1.000	1.5	3
	OE = 6.4 $(EC)^2$-17.2(EC)+32.81	1.000	2.0	6
2:1	OE = 1.4(EC)+29.14	0.998	1.0	1
	OE = 1.2(EC)+30.27	955.000	1.5	3
	OE = 1.84$(EC)^2$-5(EC)+35.8	1.000	2.0	6
10b				
1:1	OE = -36.04$(EC)^2$+129.26(EC)-81.04	1.000	1.0	1
	OE = -28.46$(EC)^2$+101.21(EC)-55.42	1.000	1.5	3
	OE = -26.56$(EC)^2$+94.46-48.93	1.000	2.0	6
1:2	OE = 5.12$(EC)^2$ -12.32(EC) +28.89	1.000	1.0	1
	OE = 1.52 (EC)+21.29	0.983	1.5	3
	OE = 2.12(EC)+20.87	0.919	2.0	6
2:1	OE = -2.64$(EC)^2$+9.08(EC)+24.68	1.000	1.0	1
	OE = -3.6$(EC)^2$+11.4(EC)+24.16	1.000	1.5	3
	OE = -0.55(EC)+34.445	0.925	2.0	6
10c				
1:1	OE = -45.74$(EC)^2$+161.35(EC)-101.99	1.000	1.0	1
	OE = -35.36$(EC)^2$+124.86(EC)-70.29	1.000	1.5	3
	OE = -32.04$(EC)^2$+113.42(EC)-60.17	1.000	2.0	6
1:2	OE = 5.97(EC)+18.478	0.984	1.0	1
	OE = 4.68(EC) +20.873	0.985	1.5	3
	OE = 4(EC)+22.533	0.951	2.0	6
2:1	OE = 6.07(EC) 25.182	1.000	1.0	1
	OE = 4.31(EC) +28.635	0.965	1.5	3
	OE = 3.88(EC)+30.477	0.997	2.0	6

10a: 1:5, B:W ratio, 10b: 1:7, B:W ratio, 10c: 1:10, B:W ratio; H: Hydrolysis

at 1:7 B:W ratio, for 3 and 6 h; 2% EC (2:1 M:P ratio) at 1:7 B:W ratio, for 6 h; 1,1.5, 2% EC (1:2 M:P ratio) at 1:10 B:W ratio, for 1,3, 6 h.; 1.5, 2% EC (2:1 M:P ratio) at 1:10 B:W ratio, for 3 and 6 h.

Effect of oil extraction methods resulting from stabilized, enzyme treated rice bran on the yield and characteristics of the oil: The enzymatic treatment of the stabilized rice bran resulted in more oil yield than the untreated stabilized rice bran. Optimum oil extraction was achieved at 1.5% mixed enzyme M and P (1:1 M:P ratio), at 1:10 bran:water ratio and hydrolysis for 1 h. The effect of the methods used for oil extraction on the oil characteristics and yield, resulting from the mixed enzyme treated bran were examined. Oil was extracted by three methods, namely, by solvent extraction using n-hexane in a Soxhlet apparatus to give oil (OS); by hydraulic pressing on the cold to give oil (OH) and by enzymatic treatment in miscella to give oil (OM).

Table 11: Chemical characteristics of the oils extracted from stabilized rice bran pretreated with enzyme mixture (M and P)

	Extracted oils			
Oil characteristics	OC	OS	OH	OM
Extracted oils (%)	25.06±0.1193^{b}	33.05±0.0808^{a}	16.50±0.1124^{d}	19.55±0.1258^{c}
Free fatty acid %	2.81±0.0808^{b}	3.17±0.0404^{a}	1.95±0.0305^{d}	2.22±0.0529^{c}
Peroxide value (meq kg^{-1})	10.53±0.0435^{a}	10.46±0.0115	8.17±0.0404	3.37±0.0378
Iodine value (g/100 g)	95.09±1.1290^{d}	95.37±0.2594^{c}	97.45±0.2324^{a}	96.63±0.3957^{b}
Saponification value (%)	183.00±0.5634	176.18±0.4331	189.88±1.1210	186.40±1.0210
Unsaponifiable matter (%)	4.70±0.3785^{b}	4.6±0.28430^{c}	5.11±0.2628^{a}	4.30±0.5732^{d}

OC: Is the oil resulting from stabilized rice bran treated under same conditions of enzymatic treatment but with no enzymes, then solvent extracted, OS: Is the oil resulting after mixed enzyme treatment of stabilized rice bran then solvent extracted, OH: Is the oil resulting after mixed enzyme treatment of stabilized rice bran then subjected to hydraulic pressing, OM: Is the oil resulting after mixed enzyme treatment of stabilized rice bran in hexane (miscella). Values superscripted with dissimilar letters (a, b, c, d etc.) in the same column are significantly different ($p<0.05$)

The control oil (OC) resulted from treating the stabilized rice bran under the same conditions of mixed enzyme treatment but without enzymes then the rice bran was subjected to solvent extraction.

The % oil extracted by the different methods, as well as, the oil characteristics of the different extracted oils were demonstrated in (Table 11). Statistical analysis showed marked significant difference in the % extracted oils by different methods. As expected solvent extraction of the stabilized, mixed enzyme treated rice bran resulted in the highest oil yield 33% oil (OS). Enzymatic treatment of stabilized rice bran in hexane (OM), or miscella treatment resulted in the extraction of 19.55% oil. Hydraulic pressing of the stabilized, rice bran treated with mixed enzyme (OH) gave the least quantity of extracted oil 16.5%. This is probably due to the fact that pressing alone cannot extract all the oil present in the cake and usually pressed meals need further extraction to remove the residual oil in the cake. Stabilized rice bran treated under same conditions of enzyme treatment but without addition of enzymes, then solvent extracted (OC) yielded 25.06 % oil. Control oil (C) is the oil content of stabilized rice bran 19.42% indicated in (Table 2). Statistical analysis of the results of chemical characteristics clearly manifested a significant difference for all characteristics, between the oils resulting from different extraction methods. Oil (OH) had FFA content 1.95% compared to 2.81, 2.22 and 3.17% FFA for OC, OM and OS, respectively. Lowest Peroxide Value (PV) was indicated for oil enzymatically treated in miscella 3.73 meq kg^{-1}. Two other extraction methods and control gave oils OH, OS and OC with PV between 8.17-10.5 meq kg^{-1}. Iodine value of the four extracted oils were rather close ranging from 95.09-97.45 g/100 g. Saponification value was 176.18% for OS and 183.0, 186.4 and 189.88% for OC, OM and OH, respectively. Highest extracted unsaponifiable matter was achieved by hydraulic pressing of the rice bran (OH) after mixed enzymatic treatment reaching 5.1%. Other oil samples OC, OM and OS, contained 4.7, 4.3 and 4.6%, respectively. This means that cold pressing shielded the USM.

Authors studying the effect of different extraction methods on the quality of oils from different oil bearing plants, reached results close to ours. Tobares *et al.* (2003) evaluating the effect of extraction method and bleaching on the quality of jojoba wax, found that cold pressed wax was noteworthy by its lower peroxide value, higher amounts of tocopherol and total phenol contents. Moreno *et al.* (2003) experimenting on the effect of different extraction methods on the physical and chemical properties of avocado oil, recommended a microwave-squeezing method to yield

Table 12: Fatty acid composition of the oils extracted from stabilized mixed enzyme treated rice bran using different methods of extraction*

	Extracted oils				
Fatty acid composition (%)	OC	OS	OH	OM	Rice bran oil (1, 2)
Myristic acid (C14:0)	0.31	0	0.09	0.3	0.2
Palmitic acid(C16:0)	20.63	20	21.27	26.67	15.0, 16.74
Palmitoleic acid (C16:1)	0.00	0.34	0.13	0	-
Stearic acid (C18:0)	2.06	2.41	2.30	2.48	1.9, 1.9
Oleic acid (C:18:1)	31.97	5.01	24.98	27.3	42.5, 42.8
Linoleic acid(C18:2)	45.02	72.24	51.20	43.25	39.1, 34.7
Linolenic acid (C18:3)	0.01	Trace	0.03	Trace	1.1, 0.19

OC: Is the oil resulting from stabilized rice bran treated under the same conditions, OS: Is the oil resulting after mixed enzyme treatment of stabilized rice bran then solvent extracted, OH: Is the oil resulting after mixed enzyme treatment of stabilized rice bran then subjected to hydraulic pressing, OM: Is the oil resulting after mixed enzyme treatment of rice bran in hexane (miscella), (1): Data from Riceland Foods Inc., (2): Tahira *et al.* (2007) *Average of two analysis

highest amount of oil with the slightest modification of the oil quality. Concha *et al.* (2004) studied the physicochemical properties of oil from *Rosa affinis rubiginosa* seeds after extraction by (1) organic solvent, (2) cold pressing and (3) cold pressing assisted by enzymatic pretreatment using a mixture of enzymes Cellubrix (cellulase and hemicellulase activities) and Olivex (pectinase, cellulase and hemicellulase activities). There were no significant differences in oil quality parameters such as iodine value, refractive index, saponification value, unsaponifiable matter and FA profile, when applying any of the three extraction methods. Hilali *et al.* (2005) prepared and collected samples of argan oil processed by traditional, mechanical and industrial methods. The study clearly demonstrated that press extraction does not alter either the chemical composition of argan oil or its physicochemical characteristics. It also demonstrates that press extraction respects the critical factors reported for traditionally prepared oils and necessary to obtain a beneficial effect on human health. Torres and Maestri (2006) assessed the influence of two extraction methods (two phase centrifugation and pressure) on the oil quality from four olive varieties. Analysis revealed statistically significant differences in some parameters, mainly in FFA, phenol contents and K_{270} values. Pressure extracted oil from variety rich in phenols showed highest oxidative stability.

Fatty acid composition of the oils extracted from enzymatically treated stabilized rice bran by different methods was represented in (Table 12). Fatty acid composition of rice bran oil from the literature is also present in the table, for comparison. All the examined oils contained little myristic acid (0.31-0.09%). Palmitic acid was rather higher than that reported in the literature (20-26.67%) while stearic acid was close to the reported range (2.06-2.48%). Oleic acid was much lower than the reported range (5.01-31.97%); on the other hand linoleic acid was much higher than the reported range (43.25-72.24%).

Tahira *et al.* (2007) analyzed refined rice bran oil and found it to have a refractive index, peroxide value, iodine value and free fatty acid value 1.4792, 0.92 meq g^{-1}, 105 cgl_2 g^{-1} and 0.07% (as oleic acid, respectively. The fatty acid profile showed palmitic acid (16.74%), stearic acid (1.9%), oleic acid (41.79%), linoleic acid (34.55%) and linolenic acid (0.19%) as major fatty acids. Goffman and Bergmann (2003) reported that oleic and linoleic fatty acids constitute more than 80% of the fatty acids of the glycerides. Our results showed the sum of 76.18% for both oleic and linoleic acids present in the hydraulic pressed oil.

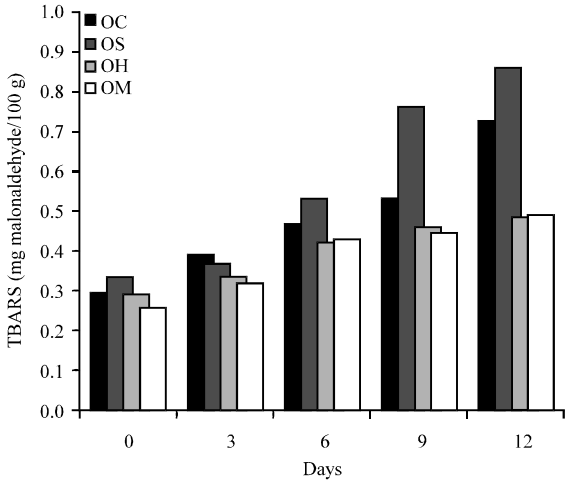

Fig. 1: Oxidative stability of the oils extracted by different methods from enzyme treated rice bran as indicated by TBARS values

Figure 1 illustrated the oxidative stability of the differently extracted oils as represented by the increase in their thiobarbituric acid reactive substances (TBARS) values. It was clear from the figure that both hydraulic pressing and miscella extraction resulted in more stable oils than solvent extraction. Even the control oil treated under same conditions of experiment but without addition of enzyme was more stable than the solvent extracted oil. In accordance to this result (Tobares *et al.*, 2003; Torres and Maestri, 2006) found that hydraulic pressing resulted in oils with more tocopherol and higher phenolic content and is thus more stable. Our results also revealed higher USM for hydraulic pressed oils which indicates why the oil is more resistant to oxidation. When enzymatic pretreatment was carried out in miscella, probably the antioxidants in the oil were shielded from atmospheric oxygen in the surrounding air by the hexane, thus more stable.

Results of oil analysis reveal that both hydraulic pressing after enzyme treatment and enzymatic treatment in miscella are both recommended for good quality oils, over conventional solvent extraction.

CONCLUSION

The present study leads to the conclusion that the use of enzyme mixtures is recommended over the use of single enzymes for the pretreatment of rice bran and probably most oilseeds as it results in an appreciable increase in the quantity of extracted oil, over single enzyme treatment. In view of the shortage of oilseeds in many countries, any improved technology with greater oil yield is a welcome sign. Also hydraulic pressing following the enzymatic treatment and enzymatic treatment in miscella are both recommended for higher quality oils.

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