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STABILIZATION AND ENZYMATIC TREATMENT OF RICE BRAN TO IMPROVE OIL YIELD

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ABSTRACT

In the present study, the effect of treatment of rice bran with solvent or microwave heating and then storage of the stabilized bran on the production of free fatty acids (FFA) was examined. Also aqueous enzymatic extraction of the stabilized rice bran was thereafter investigated. Results revealed that receiving the bran directly in hexane is an inefficient method because the FFA of the oil reached 10.5% within 30 min after milling. The microwave stabilized rice bran is a very successful method of stabilization as the FFA of the oil reached 4.17% after storage for 12 month. All enzymatic treatments resulted in increase in % extracted oil over the non enzymatic treated rice bran. The oil resulting from protease and macerozyme treated bran showed FFA less than 5%. Unfortunately, α-amylase treatment resulted in oil with high FFA between 15-17%. Conditions recommended for enzyme treatment are : treatment with protease and macerozyme under the following conditions: 2% enzyme concentration, 1:10 rice bran : water ratio at 1 and 3 hrs of incubation. These conditions yielded ca. 31-32% extracted oil compared to 19.54% extracted oil from untreated rice bran (control). Whereas, the FFA% of oils resulting from these treatments with protease and macerozyme reached 3.33, 3.25% and 3.48, 3.52%, respectively, compared to 2.33% FFA for untreated oil (control).

Keywords: Rice bran, stabilization, microwave heating, enzymatic treatment, protease, macerozyme.

INTRODUCTION

World rice production is about 550 million tons per year and is the staple food for a large part of the population. What people need to know is that 65% of the nutrition, as well as phytochemicals from the rice kernel are actually locked away in the rice bran. The bran is the outer 10% of the brown rice kernel that is removed during the conversion of brown rice to white rice (this process is known as milling). These precious nutrients and phytochemicals in the rice bran (RB) are usually discarded as a wasted food resource, ending up as animal feed or dumped in landfills or as fuel, due to the presence of an enzyme that renders the bran rancid within hours of milling. In order to benefit from the valuable constituents of the rice bran, the enzyme must be deactivated through a process known as stabilization (U.N. Report, 1985). RB constitutes: a) 12-23 % oil, with a fatty acid composition of 19% saturates {mainly palmitic}, 41% monounsaturates {primarily oleic acid}, and 36% polyunsaturates {mainly linoleic acid}. RBO is good edible oil as well as a good frying and cooking oil because of its high smoke point (which prevents fatty acid breakdown at high temperatures), also due to the low viscosity of the oil, it retains less oil on the fried material reducing overall calorie intake, and it is also a good salad oil. It has a long shelf life due to the

presence of the antioxidants in it. b) Rice bran oil (RBO) contains high levels of unsaponifiable matter 4.2%, a very rich source of phytochemicals which imparts many health benefits. c) Rice bran wax (RBW) is a main unsaponifiable component in rice bran oil extracts. RBW is a rich source of policosanol with well documented health benefits. d) Defatted rice bran constitutes 14-16% protein. e) RB contains minerals such as iron, potassium, calcium, chlorine, magnesium and manganese. f) RB contains 20-30%dietary fiber which is mostly insoluble (Juliano, 1994).

Unfortunately, rice bran is an underutilized source, mainly because of the presence of a group of lipase enzymes present in the outer layer of the bran. Upon milling the brown rice, the rice bran is broken into small particles meanwhile the lipases are freed and mixed with the oil hydrolyzing it to free fatty acids (FFA) rendering it unpalatable. This happens so quickly that in hours the oil becomes degraded. Scientists have been working on several methods to inactivate the enzymes irreversibly (stabilization) so as to prevent the oil from deteriorating. Many methods of stabilization have been investigated including treatment with chemicals (Prabhakar and Venkatesh, 1986 and Champagne et al., 1992). Heat treatment proved to be the most practical methods for stabilization (Desikachar,1974). There are different types of heat stabilization procedures: retained moisture heating (Lin and Carter, 1973), added moisture heating (Saunders, 1985), dry heating in atmospheric pressure (Loeb et al., 1949), extrusion cooking (Sayre et al., 1982), γ-radiation (Hafez et al.,1985) ohmic and microwave heating (Tao, 1989; Malekian, 1992; Lakkakula et al., 2004) and dielectric heating (Sreenarayanan and Chattopadhyay, 1986). Microwave heating is a cheap source of heating and a microwave unit can be installed easily in any oil mill. Since enzymes are proteins that are known to be denaturated by solvents and hence loose their activity, then stabilization of rice bran using solvents can be carried out easily in any rice mill(U.N. Report, 1985). Aqueous enzymatic oil extraction is undoubtedly an emerging technology in the fats and oil 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 as a by-product of an aqueous enzymatic treatment of rice bran previous to hexane extraction was reported (Sengupta and Bhattacharyya, 1996). 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 (Dominquez et al.,1994). Reviews reporting on the advantages of aqueous enzymatic extraction of oil over the conventional methods were reported by (Dominquez et al.,1994 and Rosental et al., 1996).

The aim of this work was to study the effect of microwave heating as well as solvent stabilization on the FFA content of the rice bran as well as the storage stability of the stabilized oil over a period of 48 weeks. The stabilized rice bran was then subjected to aqueous enzymatic treatment prior to oil extraction in order to investigate the increase in extracted oil over solvent extraction.

MATERIALS AND METHODS

Rice bran: Short grain rice (Giza 178 variety) was brought from a Rice Technology Training Centre at Sakha in the state of Kafr El-Sheikh. The rice was dehusked and milled using a Satake milling machine (Japanese type farmer mill) at Rice Technology Training Centre at Alexandria, where the rice bran was collected and directly sieved through a 20 mesh sieve to remove brokens and husks.

Enzymes: The enzymes used in this work were: protease (from *Bacillus lichneformis,* ≥2.4 U/g) a product of Novozyme Corp.

Macerozyme R-10(mix. Of celllulase, hemicellulase and pectinase, >2500U/g) obtained from Phytotechnology Laboratories.

α- amylase(from *hog pancreas,* ~50 U/mg) a product of Biochemika, Fluka.

Stabilization by Solvent: 10g lots of the sieved rice bran were immediately received on n-hexane in 500 ml glass beakers and stirred with an electric stirrer. The hexane was evaporated and the oils dried tested for their FFA content. FFA % was determined on oils after 10, 20, 30, 60, and 120 min. This was carried out in the laboratory of the rice mill.

Microwave Heat Stabilization: One hundred gram per batch of rice bran samples, after sieving were adjusted to a moisture content of 21%, then heated in a microwave oven (Sharp Electronic Corp.) at 800 W and 2450MHz.The microwave chamber was preheated at 100% power for 3 min. Each sample of the raw rice bran was placed in plastic zipper top-bag and was spread out evenly, the bag was then sealed. The sample was heated at 100% power for 3 min. the temperature of sample after heating in the microwave was 105 ± 2°C. The sample was allowed to cool to room temperature (~ 25°C), and then placed in plastic zipper top-bags. The samples were placed in ice boxes to keep the bran cold. The samples were transferred to the laboratory and stored at 0°C (deep freezer) until further work.

Enzymatic treatment: Enzymes were used in this study. Protease, Macerozyme, and α- amylase. For each experiment 50g of stabilized rice bran was suspended in distilled water at ratios of 1:5, 1:7 and 1:10 (w/v). The suspension was stirred using a magnetic stirrer with heating to the appropriate temperature of each enzyme. Enzyme was added at concentrations of 1, 2, and 3% (w/w or v/w) of rice bran. After adjusting the pH for each enzyme (as recommended by the manufacturer), the mixture was transferred to a shaking water bath at 100 rpm for 1, 2, or 3 hrs. Incubating pH was fixed at the optimum range for each enzyme using 1N NaOH and 6N HCl. Mixture containing protease was incubated at a pH ~ 7.5, 37°C, the mixture involving Macerozyme R-10 was incubated at pH ~ 4.5, 50°C while that for α- amylase was incubated at pH ~ 6.9, 53°C, 0.02% $CaCl_2$. After 3hr incubation time, the pH was shifted to a value of (pH ~2) then the temperature was raised to ~80°C for 5 min to assure complete inactivation of the enzyme.

Solid –liquid separation: After inactivation of the enzyme, the mixture was centrifuged at 4500 rpm for 10 min at 10°C in a Heraeus centrifuge. Two phases – liquid and meal phase - were obtained. The liquid phase was drained and the meal was mixed and dried overnight in a draught air oven at 60°C. The dry meal was ground and analyzed for oil.
Analytical methods: Oil content, moisture content and free fatty acid% were determined according to (AOCS,1998) methods of analysis.

RESULTS AND DISCUSSION

Rice bran is a rich source of essential nutrients, vitamins, minerals, and phytochemicals. Effective utilization of rice bran necessitates its stabilization as soon as the rice is milled. Stabilization is the process by which the lipases and oxidase enzymes present in the bran are deactivated so as to prevent the triglycerides from the rapid hydrolysis into free fatty acids. The free fatty acids increase bran acidity and reduce pH, thus an off-flavor and a soapy taste are produced, and functional properties change. Two stabilization methods were investigated in this work: stabilization by hexane and microwave heat stabilization.

1. Effect of stabilization by hexane

Enzymes are protein in nature which are known to be denatured by solvents (U.N. Report, 1985). In this work hexane was chosen to be investigated as both the extracting and stabilizing solvent for rice bran because it is already the most commonly used solvent in the oilseed industry. If hexane was found effective it can act as a vehicle to transfer the rice bran from the mill to the oil factory. Table 1. shows the free fatty acid % of the oil at different time intervals after milling. The oil extracted from the rice bran directly after milling contained 2.3 % FFA. While the FFA was raised to 5.6, 7.2, 10.5, 13.6, and 18.9 % after 10, 20, 30, 60, and 120 min, respectively, from milling, while being soaked in hexane. These results prove that hexane can not be used for stabilizing rice bran.

Table 1: FFA% of rice bran oil stabilized by hexane

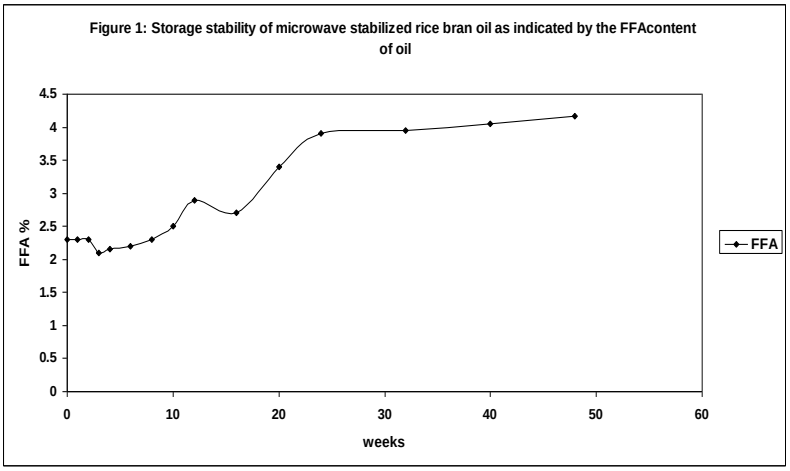
Bran sample	FFA%
directly after milling	2.3 ±0.52
after 10min	5.6 ± 0.61
after 20min	7.2 ± 0.49
after 30min	10.5 ± 0.71
after 60min	13.6 ± 0.59
after 120min	18.9 ± 0.78

The rate of FFA formation in bran or brown rice flour is high. Approximately 30% of the oil can be hydrolyzed to FFA within a week under conditions of high humidity and temperature (Champagne and Hron, 1992).Champagne et al. (1990, 1991, 1993) Champagne and Hron (1992a, 1992b, 1993) developed processes for stabilizing brown rice kernels and their flours to lipolytic hydrolysis using liquid ethanol and ethanol vapors and reported stability of oil up to six months. Isopropanol extracted rice bran gave oils stable to heat induced oxidation more than hexane extracted oils, probably because the antioxidants are extracted more readily by the isopropanol (Proctor and Bowen, 1996).

2. Effect of stabilization by microwave heating

Microwave heating is considered as one of the most energy-efficient and a rapid method for heating food items (Yoshida et al., 1991).This method of cooking or processing saves time and energy. Microwave heating for bran stabilization has a significant advantage. It causes internal heating of particles within the microwave cavity, providing distribution of heat within the bran similar to conventional heating. The dipolar water molecules in the bran are excited by the electromagnetic waves and the water molecules are made to spin. The resulting enhanced kinetic energy, along with the friction produces the heat that results in the even distribution of heat (Roman, 1989). Since water molecules play an important role in this process, the initial moisture content is a critical factor in the microwave stabilization of rice bran.

Figure1: is a diagrammatic presentation of the oxidative stability of microwave stabilized rice bran oil as indicated by the FFA content of the oils. Directly after milling the brown rice, the rice bran's moisture was adjusted to 21% moisture packed in zipper-top bags, subjected to microwave heating for 3 min, and then stored at 0°C for 48 weeks.



From Figure 1 it can be depicted that the microwave heat stabilized rice bran was very stable to oxidative stability over a period of 48 weeks. Initial FFA of the stabilized oil at day of milling was 2.3% and during

the storage period ranged between 2.3 to 4.17%. 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).

Tao (1989) showed that FFA content of microwave-heated rice bran from a long-grain and a medium-grain varieties increased only slightly during 4 weeks of storage at 25 °C.

Malekian (1992) reported that FFA content in microwave-heated rice bran stored in the refrigerator exhibited very little change for zipper-top bags and vacuum bags types of packaging after 8 weeks of storage. the recommended storage conditions for prevention of hydrolytic rancidity in microwave-heated rice bran are the use of zipper-top bags and a storage temperature of 4-5 °C, for up to at least 16 weeks of storage (Ramezanzadeh et al., 1999).

Our results together with the results of the previous workers prove microwave heating of rice bran to be an efficient method of stabilization. Thus the microwave heat stabilized rice bran was used for further work.

3. Effect of enzymatic treatment of microwave stabilized rice bran on oil yield and FFA of the oil

The use of enzymes in the food industry has long been recognized. The application of enzymes in the field of oil extraction is relatively a new perspective of development in this field. A number of enzymes from cell degrading microorganisms have been used to enhance the extractability of the oil from oilseeds: amylase, glucanase, protease, pectinase as well as cellulolytic and hemicellulolytic enzymes (Fullbrook, 1983). Cell wall degrading enzymes are used to extract the oil by solubilizing the structural cell wall components of the oilseed. Enzymatic treatment offers a high yield and a preservation of valuable extracted components, because of the mild conditions employed (Dominquez et al., 1994 and Rosenthal et al., 1996).

Tables 2, 3, and 4 indicate the effect of treatment of the microwave stabilized rice bran with the enzymes protease, macerozyme (mixture of cellulase, hemicellulase and pectinase), and α-amylase, respectively on the % of extracted oil as well as the FFA% of the extracted oil. Factors which influence the efficiency of the aqueous enzymatic treatment include, Solid: enzyme concentration, water: solid ratio, particle size, pH, time, temperature and degree of agitation. The particle size as well as degree of agitation was the same for all treated samples, the temperature used in all experiments was room temperature. The enzyme concentrations were 1,2, and 3 % (of the weight of bran sample), bran: water ratios investigated were, 1:5, 1:7, 1:10 (w/v), and time examined 1, 2, and 3 hours.

It can be clearly seen from Tables 2, 3, and 4 that enzymatic treatment of stabilized rice bran results in increase of the yield of extracted oil. Highest oil extraction resulted from the treatment with protease and macerozyme under the following conditions: 2% enzyme concentration, 1:10 rice bran ; water ratio, at 1 and 3 hrs of incubation reached ca. 31-32% extracted oil compared to 19.54% extracted oil for untreated rice bran (control). Whereas, the FFA% of oils resulting from these treatments with

protease and macerozyme reached 3.33, 3.25% and 3.48, 3.52%, respectively, compared to 2.33% FFA of untreated oil sample (control). Enzyme α- amylase also resulted in more or less the same results giving rise to ca. 30% highest oil extracted. Unfortunately the FFA of the oils resulting from different conditions with α-amylase treated bran was too high reaching from 15 to 17 FFA% causing the unpalatable oil. The increase in FFA% is presumably due to the activation of α- amylase with $CaCl_2$. It was reported that lipase is activated by calcium (Orthoefer, 2005).

Table 5. demonstrates the % increase in oil extractability from the different treated samples compared to oil extracted from untreated rice bran (control). Percent increase in oil extractability for protease treated samples resulted in ranges from 25.12-62.48% increase, macerozyme treated samples resulted in 23.64 – 62.84% increase and α-amylase treated samples in 50.3 -58.44% increase.

Table 2 : Effect of protease treatment of microwave stabilized rice bran on extracted oil and FFA

Enzyme conc%	Time (hrs)	Bran:water (ratio)	Extracted oil (%)	FFA (%)
1	1	1:5	25.93 ± 0.37	3.68 ± 0.11
1	1	1:7	27.63 ± 0.48	3.76 ± 0.41
1	1	1:10	29.58 ±0.87	3.89 ± 0.45
2	1	1:5	25.36 ± 0.65	3.92 ± 0.26
2	1	1:7	29.22 ±0.49	4.01 ± 0.34
2	1	1:10	31.75 ±0.72	3.33 ± 0.19
3	1	1:5	27.36 ± 0.33	4.21 ± 0.46
3	1	1:7	26.88 ± 0.73	3.54 ± 0.27
3	1	1:10	27.63 ± 0.58	3.89 ± 0.42
1	3	1:5	24.32 ± 0.81	3.62 ± 0.23
1	3	1:7	26.55 ± 0.66	3.94 ± 0.26
1	3	1:10	29.13 ± 0.61	3.36 ± 0.29
2	3	1:5	25.66 ± 0.39	3.69 ± 0.49
2	3	1:7	28.87 ± 0.33	4.02 ± 0.38
2	3	1:10	31.59 ± 0.46	3.25 ± 0.41
3	3	1:5	26.86 ± 0.84	4.32 ± 0.11
3	3	1:7	27.11 ± 0079	4.41 ± 0.39
3	3	1:10	27.45 ± 0.45	4.01 ± 0.44
1	6	1:5	24.45 ± 0.68	4.62 ± 0.46
1	6	1:7	25.63 ± 0.56	4.05 ± 0.17
1	6	1:10	29.65 ± 0.28	3.99 ± 0.13
2	6	1:5	25.15 ± 0.44	3.94 ± 0.46
2	6	1:7	26.58 ± 0.75	4.33 ± 0.38
2	6	1:10	29.54 ± 0.92	4.44 ± 0.15
3	6	1:5	25.87 ± 0.32	4.25 ± 0.32
3	6	1:7	28.24± 0.81	4.36 ± 0.37
3	6	1:10	29.96 ± 0.85	4.51 ± 0.21
Control (stabilized rice bran)			19.54 ± 0.74	2.33 ± 0.44

Scanning electron microscopy analysis has shown that lipid bodies from oilseeds are enmeshed in a kind of cytoplasmic network presumably composed of protein (Wolf and Baker, 1975). The spaces between protein bodies in cotyledon cells are then filled with the lipid body and cytoplasmic network (Young and Schadel, 1990 and Bair and Snyder,1980). Unlike the cytoplasmic features which are characterized by the presence of protein and lipid, the walls which surround the cell are primarily composed of cellulose, hemicellulose and lignin in addition to pectin (Snyder and Kwon, 1987). Thus the role of enzyme macerozyme which is a mixture of cellulase, hemicellulase and pectinase) was to break the structure of cotyledon cell wall. Proteases mainly hydrolyse the proteins in the cell membranes (Bair and Snyder,1980) as well as inside the cytoplasm .The previous results prove the above concept. The previous results are also in agreement with the findings of previous authors.

Table 3 : Effect of Macerozyme treatment of microwave stabilized rice bran on oil recovery and FFA%

Enzyme conc(%)	Time (hrs)	bran :water (ratio)	Extracted oil (%)	FFA (%)
1	1	1:5	26.24 ± 0.51	3.69 ± 0.21
1	1	1:7	24.63 ± 0.26	4.21 ± 0.13
1	1	1:10	26.21 ± 0.44	4.36 ± 0.22
2	1	1:5	28.36 ± 0.32	4.21 ± 0.19
2	1	1:7	27.77 ± 0.36	3.58 ± 0.16
2	1	1:10	31.39 ± 0.43	3.48 ± 0.28
3	1	1:5	25.66 ± 0.22	3.98 ± 0.26
3	1	1:7	27.69 ± 0.46	4.21 ± 0.41
3	1	1:10	29.35 ± 0.52	3.87 ± 0.31
1	3	1:5	27.66 ± 0.37	3.35 ± 0.39
1	3	1:7	26.99 ± 0.42	4.37 ± 0.18
1	3	1:10	29.84 ± 0.12	4.16 ± 0.32
2	3	1:5	28.32 ± 0.36	3.11 ± 0.33
2	3	1:7	29.54 ± 0.29	3.84 ± 0.35
2	3	1:10	31.82 ± 0.49	3.52 ± 0.30
3	3	1:5	24.16 ± 0.15	4.45 ±0.19
3	3	1:7	26.66 ± 0.44	4.23 ± 0.45
3	3	1:10	28.37 ± 0.31	4.1 ± 0.26
1	6	1:5	27.36 ± 0.44	4.36 ± 0.31
1	6	1:7	26.54 ± 0.51	3.99 ± 0.40
1	6	1:10	26.98 ± 0.23	3.86 ± 0.11
2	6	1:5	24.44 ± 0.30	4.16 ± 0.22
2	6	1:7	25.79 ± 0.22	4.25 ± 0.36
2	6	1:10	26.63 ± 0.41	3.66 ± 0.34
3	6	1:5	25.55 ± 0.62	3.87 ± 0.29
3	6	1:7	27.65 ± 0.24	4.21 ± 0.32
3	6	1:10	26.41± 0.28	4.41 ± 0.20
Control (stabilized rice bran)			19.54 ± 0.74	2.33 ± 0.44

Table 4 : Effect of α-amylase treatment of microwave stabilized rice bran on oil recovery and FFA%

Enzyme conc(%)	Time (hrs)	bran :water (ratio)	Extracted oil (%)	FFA (%)
1	1	1:5	30.24 ± 0.81	15.86 ± 0.26
1	1	1:7	31.24 ± 0.62	15.91 ± 0.21
1	1	1:10	30.28 ± 0.53	16.23 ± 0.36
2	1	1:5	29.35 ± 0.84	16.63 ± 0.36
2	1	1:7	30.65 ± 0.42	17.56 ± 0.29
2	1	1:10	30.56 ± 0.61	15.55 ± 0.13
3	1	1:5	30.65 ± 0.71	15.28 ± 0.19
3	1	1:7	30.12 ± 0.76	16.66 ± 0.25
3	1	1:10	30.11 ± 0.46	16.98 ± 0.22
1	3	1:5	29.99 0.41	16.35 ± 0.11
1	3	1:7	30.87 ± 0.62	16.98 ± 0.33
1	3	1:10	30.54 ± 0.53	16.77 ± 0.39
2	3	1:5	30.33 ± 0.79	17.45 ± 0.40
2	3	1:7	30.58 ± 0.59	16.11 ± 0.26
2	3	1:10	30.77 ± 0.61	15.99 ± 0.28
3	3	1:5	30.71 ± 0.55	14.23 ± 0.37
3	3	1:7	29.87 ± 0.49	16.63 ± 0.31
3	3	1:10	30.36 ± 0.52	17.12 ± 0.29
1	6	1:5	30.33 ± 0.68	16.68 ± 0.16
1	6	1:7	29.65 ± 0.88	16.31 ± 0.21
1	6	1:10	29.59 ± 0.81	16.89 ± 0.29
2	6	1:5	30.23 ± 0.74	16.25 ± 0.36
2	6	1:7	30.96 ± 0.59	17.51 ± 0.29
2	6	1:10	30.65 ± 0.70	15.45 ± 0.31
3	6	1:5	30.71 ± 0.83	15.63 ± 0.37
3	6	1:7	30.76 ± 0.69	16.69 ± 0.31
3	6	1:10	29.99 ± 0.61	17.77 ± 0.39
Control (stabilized rice bran)			19.54 ± 0.74	2.33 ± 0.44

Table 5: Increase in oil extractability (%) of enzyme treated stabilized rice bran over stabilized rice bran control

			Increase in oil extractability (%)		
Enzyme conc(%)	Time (hrs)	bran :water (ratio)	Protease*	Macerozyme**	α-amylase***
1	1	1:5	32.7	34.28	54.75
1	1	1:7	41.4	26.04	59.87
1	1	1:10	51.48	34.13	54.96
2	1	1:5	29.78	45.13	50.3
2	1	1:7	49.53	42.12	56.85
2	1	1:10	62.48	60.64	56.39
3	1	1:5	40.02	31.32	56.85
3	1	1:7	37.56	41.7	54.14
3	1	1:10	41.4	50.2	54.09
1	3	1:5	40.02	41.55	53.48
1	3	1:7	36.07	38.12	57.98
1	3	1:10	49.07	52.82	56.29
2	3	1:5	31.32	44.93	55.22
2	3	1:7	47.74	50.97	56.49
2	3	1:10	61.79	62.84	57.47
3	3	1:5	37.46	23.64	57.16
3	3	1:7	38.74	36.43	52.86
3	3	1:10	40.48	45.28	53.27
1	6	1:5	25.12	40.1	55.22
1	6	1:7	31.16	35.89	51.74
1	6	1:10	51.74	38.07	51.43
2	6	1:5	28.71	25.28	54.7
2	6	1:7	36.02	31.98	58.44
2	6	1:10	51.74	36.28	56.85
3	6	1:5	32.39	30.75	57.16
3	6	1:7	44.52	41.5	57.42
3	6	1:10	48.2	35.15	53.48

***= protease treated stabilized rice bran**
**** = macerozyme treated stabilized rice bran**
***** =α-amylase treated stabilized rice bran**

Sengupta and Bhattacharyya (1996) investigated aqueous enzymatic extraction of mustard seed and rice bran. They reported that most of the characteristics of rice bran oil prepared from the enzymatic method were identical to those of commercial solvent- extracted oil, but had a lower content of colored substances and higher acidity. Sharma et al. (2001) used a mixture of enzymes including a protease, a cellulase and α-amylase in the aqueous extraction of rice bran. The process yielded 77% recovery of oil from rice bran. Hanmoungjai et al. (2001) studied the enzymatic extraction of

oil and protein from rice bran using a commercial protease (alcalase). They reported the effect of enzyme concentration was significant on oil and protein extraction yield. Maximal extraction yields of oil and protein were 79 and 68 %, respectively. Further the quality of oil recovered from the process in terms of FFA, iodine value, and saponification value was comparable with solvent oil and commercial rice bran oil, but the peroxide value was higher. Again Hanmoungjai et al.(2002) studied the effects of the following enzymes- Celluclast 1.5L, 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. Carbohydrases did not affect the yield significantly, but increased the level of reducing sugars in the extract.

CONCLUSION

From the above results it could be easily concluded that the use of microwave stabilization of rice bran freshly milled followed by treatment with protease and macerozyme is highly recommended to obtain a high oil yield with low FFA content. In a continuation of this work, different mixtures of protease and macerozyme at different conditions will be tested.

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تثبيط ومعالجة رجيع الكون بالتحليل الانزيمي لتحسين انتاج الزيت

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في الدراسة الحالية ، تم دراسة تأثير معاملة (تثبيط) رجيع الكون باستخدام المذيب أو التعرض لأشعة الميكروويف ثم تخزين الرجيع المثبط علي تكوين أحماض دهنية حرة في الزيت الناتج. والاستخلاص المائي لرجيع الكون المثبط في وجود انزيمات أيضا.

وقد تم تثبيط الرجيع- الناتج مباشرة من عملية ضرب الأرز- بطريقتين :

الأولي : استقبال الرجيع الناتج من عملية الضرب مباشرة في مذيب الهكسان العادي ومعرفة محتوي الأحماض الدهنية الحرة الموجودة بالزيت علي فترات زمنية .

الثانية : باستخدام فرن الميكروويف .

ثم عمل التحلل المائي للرجيع المثبط بأشعة الميكروويف باستخدام الانزيمات (بروتيز، ماسيروزيم)

وقد أظهرت النتائج أن استقبال الرجيع مباشرة في المذيب عملية غير فعالة حيث وصل محتوي الأحماض الدهنية الحرة الي ١٠,٥% في خلال ٣٠دقيقة بعد عملية الضرب. وعلي العكس من ذلك اوضحت النتائج أيضا أن طريقة التثبيط باستخدام أشعة الميكروويف من أحسن الطرق التي تم التوصل اليها حيث وصلت نسبة الأحماض الدهنية الحرة في الزيت بعد ٤٨ أسبوع الي ٤,١٧%. وقد وجد أن معالجة الرجيع بالانزيمات نتج عنها زيادة في نسبة الزيت عنها في عدم وجودها. كما اتضح أن نسبة الأحماض الدهنية الحرة في الزيت الناتج من المعاملة بالانزيمات (بروتيز، ماسيروزيم) لم تتعدي ٥% في حين أن نسبة الأحماض الدهنية الحرة في الزيت الناتج من المعاملة بانزيم الألفا أميليز تراوحت بين ١٥-١٧%. .

وقد وجد أن الظروف المثلي للمعالجة بالانزيمات هي استخدام انزيمات البروتيز، الماسيروزيم معا عند الظروف التالية:

تركيز الانزيمات ٢%، نسبة الرجيع : الماء ١:١٠،عند ٣,١ ساعة . وقد نتج من هذة المعالجة استخلاص ٣١-٣٢ % من الزيت مقارنة بكمية الزيت المستخلص في عدم استخدام المعالجة (١٩,٥%).