

## Effect of Enzyme Assisted Aqueous Extraction and Ohmic Heating Treatment on Rice Bran Oil Recovery

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### ABSTRACT

Rice bran oil is the alternate edible oil source that realizes potential revenues from health-promoting compounds such as oryzanol, tocopherol, and tocotrienol. The present experiment was conducted to explore the possibility of oil extraction from rice bran oil using Enzyme assisted extraction coupled with ohmic heating, a "green" alternative source from hexane extraction. A crude form of cellulase enzyme,  $\alpha$ -amylase, and protease are used for oil extraction. After enzymatic treatment, the slurry was treated with ohmic heating by varying process parameters such as endpoint temperature, time, and electric field strength. The optimum conditions were 54°C 5 min endpoint temperature, and 179 V/Cm electric field strength. The predicted critical value was experimentally verified, where the experimental yield of rice bran oil was 80.2%, which was close to the predicted value (79.81%). The quality parameter of the extracted oil was compared with the conventional method. Fatty acid profile, protein, peroxide value, acid value, protein content was higher in Enzyme assisted ohmic heating treatment, and antioxidant (tocopherol) was significant at 210nm, suggesting the possibility of using this method for oil extraction to improve the quality of residue and extracted oil.

**Keywords:** Rice bran oil, Crude enzyme, Tocopherol, Free fatty acid, Oil recovery.

### INTRODUCTION

Rice is a staple food in many countries. As an increasing world population, ~101 million tons of rice production needed in 2015 (Malte F Stuecker, 2018). In 2017 rice production was

approximately 680 million tonnes produced around the world. The human consumption of antioxidants from rice is lesser due to the consumption of polished rice.

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Intake of antioxidants from rice can be increased by utilizing rice bran, obtained as a by-product of the rice milling industries. In 2015 the global rice bran oil market was estimated at over 1 million tons. Production is most concentrated in countries such as India, china, japan, Thailand, and Vietnam. The rice bran oil market is anticipated to increase at a significant compound annual growth rate of 5.14% from 2017 to 2021.

The most common methods used for oil extraction are hydraulic pressing, solvent extraction, expeller pressing (v c Kalia, 2001), supercritical Co<sub>2</sub>, Cold percolation. But due to several problems during extraction, such as low extraction yield, environmental issues, the toxic compound produced, need an alternate method of oil extraction.

The aqueous oil extraction (AOE) process was started in the 1950s. This method was widely used in those days, as it looked cheaper, less dangerous method than solvent, and it does not release any volatile organic compound. The oil recovery was less, as lipid bodies which are bounded by a biological membrane. Thus to increase the oil recovery combined method of Enzyme assisted aqueous extraction (AEOE) can be used to rupture the biological membrane and hydrolysis the protein (H.Dominguez, 1995).

Aqueous enzymatic extraction of oil is used as a novel technology to extract canola oil (Robert hagenmaier, 1971). In this method, several parameters are involved, such as to regulate pH extraction buffer, and it produces a range of higher quality of protein and oil. The advantages of this technique are complete avoidance of solvent, and the physicochemical quality of the oil is not affected (Shao Bing Zhang, 2007). The extracted oil was analyzed had higher quality compared with the conventional technique. The parameter analyzed are refractive index, specific gravity, iodine value, unsaponification value, and saponification value of canola was not affected. Rice bran oil produced from the AEOE method with pre-treated rice bran had the lowest value of FFA, the highest level of  $\gamma$ -oryzanol, and antioxidant activity. In spite of

the advantages of this method, the lower yield was obtained due to the emulsion layer. Thus downstream processes such as de-emulsification and ohmic heating treatment can be carried out to break the emulsion layer was reported by (Loypimai et al., 2001).

Ohmic heating is called joules heating, in which the heat occurs in the form of internal energy generation in the product. The Alternating current is passed through both sides of the electrode, which is in contact with the food, internal heat generation takes place, and helps increase oil recovery. The ohmic heating accelerates the seed's cell wall by electroporation techniques, which cause an increase in oil yield. The heat supplied to the product is uniform; thus, fouling does not occur (Kautkar Sheshrao Sakharam, 2016).

This study's main objective is to increase oil yield using crude Enzyme and effective demulsification technique (ohmic heating treatment), which results in the higher quality parameter in both oil and residue (defatted rice bran cake).

## MATERIALS AND METHODS

Freshly milled Rice bran sample milled from Ponni parboiled paddy was procured from Bharath rice mill, Thanjavur, Tamilnadu. Rice bran was sieved using a 500  $\mu$ m sieve to remove the husk and broken from rice bran. Then it is packed in a polythene bag and sealed and stored in a refrigerator at 4°C till used for experimentation.

### Enzyme Preparation

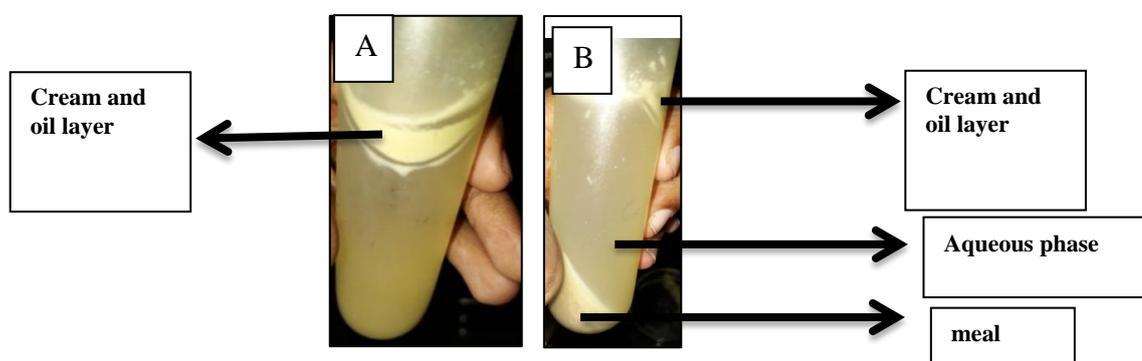
The crude cellulase,  $\alpha$ -amylase, protease, is produced in the IIFPT laboratory, Thanjavur. The crude form of Enzyme was produced by solid-state fermentation involving *Aspergillus niger* and *Aspergillus flavus*. The produced Enzyme was stored in a refrigerated condition.

### Enzyme treatment and ohmic heating treatment

Extraction of rice bran oil with the application of enzymatic hydrolysis and ohmic heating involves various unit operations such as stabilization of rice bran, enzymatic treatment -I, enzymatic treatment-II, and downstream processing. Rice bran was steam stabilized. In

enzymatic treatment-I, rice bran slurry was mixed at 1000 rpm, and pH was adjusted to 4.75 using 0.1N HCL. 4.0 ml of crude cellulase was inoculated and incubated at 37°C for three h in a shaking incubator at 80 rpm. After Treatment-I, pH was adjusted to 7.0 with 0.1N NaOH, crude  $\alpha$  amylase and protease was added and incubated at 40°C for 18 h in a shaking incubator at 80 rpm. Then ohmic heating is done to improve oil recovery and inactivate Enzyme. During ohmic heating treatment, the effect of electric field strength, temperature, and holding time was varied and

optimized using statistical software. There are many statistical approaches for assessment of the effect of these factors. In this study, RSM (design expert software) was used. The effect of ohmic heating of enzymatically treated rice bran slurry was determined at three levels (100, /140, /180 v/cm) with electric field strength at three levels of time (5,10,15 min) and temperature (50,/60 and 70°C) and optimized by design expert software using response variable(oil recovery and quality parameter).



**Fig. 1: Phase separation of the enzyme-treated rice bran cream after Centrifugation at 7,168 g for 15 min at 4°C**

### Downstream Processing

After ohmic heating treatment, downstream processes such as centrifugation, demulsification, and separation of oil and drying are carried out. Rice bran slurry was centrifuged at 7,168 g for 20 min at 4°C, and it results in cream and oil layer, aqueous phase, and residual meal. The cream and oil layer was separated using a micropipette, and the demulsification (freeze-thaw technique) was done. Oil was separated using a separating funnel and dried in the hot-air oven to remove the remaining moisture from oil.

### Quality parameters of stabilized rice bran and extracted oil

The proximate analysis, such as moisture content, fat, and protein of stabilized rice bran, was analyzed according to the standard method AOAC (1995), free fatty acid of extracted oil was estimated according to (AOAC 1995). Protein content, peroxide value and an acid value of extracted oil were also estimated according to (AOAC 1995). The

antioxidant of extracted oil(Tocopherol) was analyzed by high-performance liquid chromatography (HPLC) consisting of an LC-10ATVp pump, SCL 10A system controller, and variable Shimadzu SPD 10AVp UV VIS detector and loop injector with a loop size of 10  $\mu$ l. The peak area was calculated with CLASSVP software. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250 4.6 mm, i.e., the particle size of 5 $\mu$  C-18; Phenomenex, Torrance, CA, USA) at 25°C. The wavelength used was 210 nm. Oryzanol standard was employed for peak identification and quantification. For antioxidant determination, AOAC (1995) method was employed.

### Statistical analysis

Stat graphic software (Design expert 6.08) was used to analyze data. Analysis of variance was done to determine the effect of treatment factors on FFA and oil yield values. The

surface response plot was also made to show the interactive effects of factors.

field strength, holding time, and endpoint temperature. The efficiency of ohmic heating treatment of rice bran slurry was quantified in terms of oil recovery and free fatty acid.

## RESULTS AND DISCUSSION

Ohmic heating treatment on oil recovery is affected by a number of factors such as electric

**Table 1 Analysis of variance for Response Surface Quadratic Model**

Source	Sum of squares	Design of Freedom	Mean square	F-value	Prob> F
Oil Extraction yield					
Model	169.37	9	18.82	7.34	0.0077
Residual	17.95	7	2.56		
Lack of fit	8.74	3	2.91	1.27	0.3987
Pure error	9.21	4	2.30		
Total	187.31	16			
Coefficient of variation= 0.77%, R <sup>2</sup> value= 0.9042					
Free fatty acid(%)					
Model	2.67	9	0.30	8.01	0.0060
Residual	0.26	7	0.037		
Lack of fit	0.13	3	0.043	1.29	0.3926
Pure error	0.13	4	0.033		
Total	2.93	16			
Coefficient of variation = 6.01% ,R <sup>2</sup> value= 0.9115					

Prob < 0.05 indicates statistical significance.

Response surface analysis was used for optimizing the process parameter of ohmic heating treatment on Enzyme treated rice bran slurry. Both a linear model and second-order model were tested using F-test at a 95%

confidence level. The oil recovery and free fatty acid were explained satisfactorily using second-order models with no significant lack of fit (Table 1).

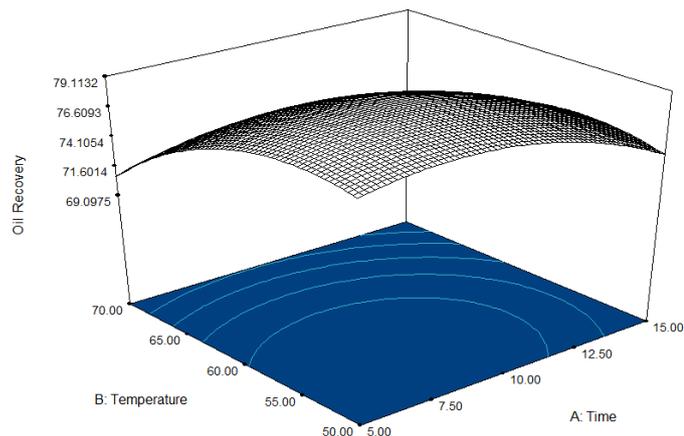
$$\text{Oil recovery} = 78.12 - 1.33A - 2.93B + 0.90C - 2.31A^2 - 2.96B^2 - 0.51C^2 + 0.50AB - 1.56A - 1.05BC \quad [1]$$

$$\text{FFA} = 3.04 + 0.19A + 0.33B - 0.20C - 0.072A^2 + 0.28B^2 + 0.11C^2 - 0.39AB - 0.075AC + 0.17BC \quad [2]$$

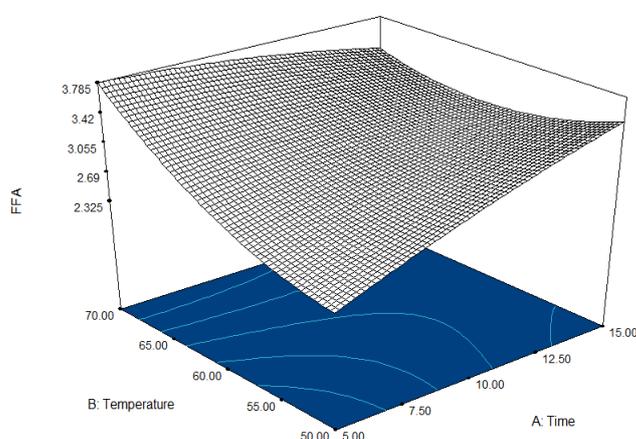
Where oil recovery and FFA have predicted response for oil recovery(%), FFA(%), respectively, and A, B, C are the coded

parameters for time, temperature, and electric field strength.

A)



B)



**Fig. 2: Response surface for oil recovery (A) Free fatty acid (B) as a function of temperature and holding time for 140 v/cm**

Ohmic heating treatment was done after enzymatic treatment on rice bran slurry, for enhancing the oil recovery from rice bran. The oil recovery from a controlled sample (i.e., EAEP coupled with steaming) and EAEP coupled with ohmic heating were varied from 69.9% and 80.2%, respectively. The maximum oil recovery (80%) was obtained when the sample was heated and maintained at 54°C endpoint temperature, 5 min holding time, an electric field strength of 179 v/cm.

In ohmic heating, the heating occurs in the form of internal energy transformation (from electric to thermal) within the bran (i.e., aleurone cell and surrounding surface area) and rapidly penetrate into other surrounding areas. Thus, the combined effect of ohmic heating treatment after the enzymatic

treatment on rice bran slurry increased oil yield.

An increase in temperature reduces the extraction yield, and an increase in time increases the extraction yield. (N. Rao Lakkakula, 2003) stated this was due to enhanced mass transfer effects seen in low-temperature ohmic heating (Fig 2). The increased time that cell wall is exposed to ohmic heating, which allows the cell walls to build up charges and form pores, called electroporation takes place. The oil recovery increased as ohmic heating destabilized the cream formed to release oil in the aqueous medium (Yang Li, 2014). There exist an optimal holding time for extraction, and providing a higher holding time beyond this

optimum does not significantly impact the amount of oil extracted.

Increasing the electric field strength from 100 to 180 v/cm increased the extraction yield. This phenomenon was also observed by (N.Rao Lakkakula, 2003), who found that increase in electric field strength significantly impacted the extraction yield due to the process known as electroporation (Fig 3). Similarly, oil recovery decreased when it reached beyond the optimum level of endpoint temperature. Total heat input loosens the bond holding lipid bodies with proteinaceous bodies in oil-bearing material. This increased the oil fluidity, and oil goes into an aqueous medium (Akash pare, 2012).

The suitability of the model equation for predicting the optimum values was tested using recommended optimal conditions, i.e., at 54°C endpoint temperature for 5 min holding time and 179 v/cm of electric field strength. The experiments were conducted from a set of given predicted optimal conditions using general factorial design. The experimental value (80.2%) was found to agree with the predicted value (79.81%).

#### Quality Parameters of extracted rice bran oil

The composition of free fatty acid in the extracted rice bran oil was analyzed and

consist of Dodecanoic acid, 10-methyl- methyl ester; hexadecanoic acid; 9,12-octadecadienoic acid; 9-octadecenoic acid; Tridecanoic acid; Methyl 13-methyl-tetra deaconate, Methyl 14-methyl-eicosanoate. Protein extraction (24.4) from Enzyme and ohmic treated defatted bran was higher than the solvent extracted defatted bran (Table 4). The condition that favored protein extraction was alkaline pH, small particle size, and temperature below the level that caused denaturation. (J. M. L. N. de Moura, 2008). The higher oil recovery determined in Enzyme treatment aqueous extraction(79%) is due to effective solubilization and hydrolysis of proteins, which are probably involved in the breakdown of the protein network feature of the cotyledon cells, and in protein(oleosin) based membranes that surround the lipid bodies, thereby liberating more quantity of oil (Sajid Latif, 2009).

The peroxide value of 2.23 was obtained by enzymatic, and ohmic treated oil was a little higher than solvent-extracted oil (refer table 4). FFA value was lesser in Enzyme and ohmic assisted extracted oil. The retention time of tocopherol (3.983) in extracted oil was analyzed (fig 5, table 2).

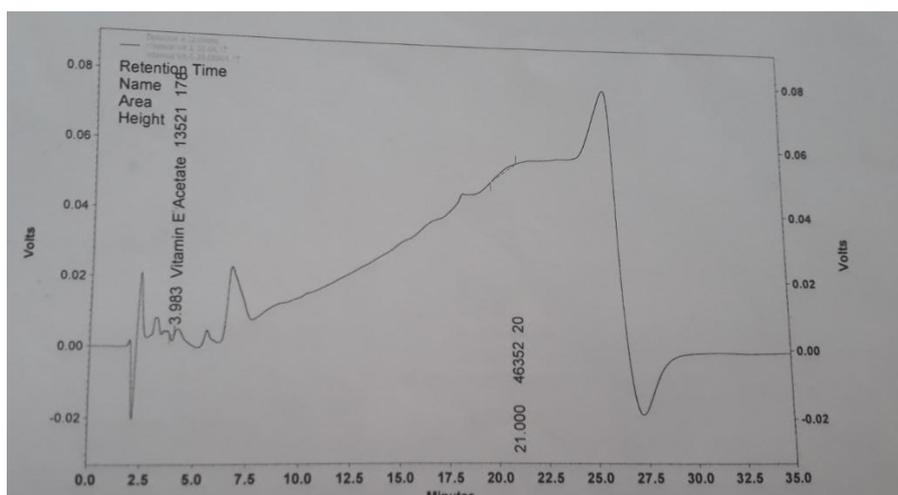


Fig. 3:  $\alpha$ -Tocopherol content of treated rice bran oil

**Table 2: Determination of  $\alpha$ -Tocopherol after ohmic heating and EAEP treated sample**

Detector A(210nm)					
S.no	Retention Time	Area	Height	Concentration(mg/g of sample)	Name of compound
1.	3.983	13521	178	0.035	Vitamin E Acetate

**Table 3 Proximate analysis of stabilized rice bran**

Characteristics	Value
	Stabilized rice bran
Moisture	9.21
Fat(Solvent extracted)	14.8
Ash	9.8

**Table 4 Quality analysis of extracted oil**

Characteristics	Value	
	Rice bran oil(conventional extraction)	Rice bran oil(EAEP)
Free fatty acid	2.5	2.32
Protein(from the meal)	17.5	24.4
Acid value	4.9	5.82
Peroxide value	2.13	1.22

### CONCLUSION

The main aim of this research work was to explore the possibility of oil extraction from ohmic heating and Enzyme assisted aqueous extraction of oil from rice bran. A quadratic polynomial model for predicting the values of the yield of rice bran oil was determined according to the Box-Behnken method. The F-test and p-value indicated that the linear terms of electric field strength, holding time and endpoint temperature, the intermediate-term of electric field strength, and holding time and quadratic term endpoint temperature are significant model terms. The processing parameters were also optimized. A quadratic polynomial model for predicting the values of the yield of rice bran oil was determined according to the Box-Behnken method. The processing parameters were also optimized. The enzyme-assisted aqueous extraction process allows an increase in the oil recovery relative to the control by making this technology as an environment-friendly alternative to conventional hexane oil extraction.

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