

## Study on the Preparation Process of Rice Bran Oil by the Ultrasonic Enzymatic Extraction

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**Abstract:** The influence of oil rate and quality of rice bran oil by enzymatic extraction ultrasonic assisted were studied through designed experiment. The optimum enzymatic hydrolysis conditions were determined by the way of the orthogonal test. The results showed: after lipase enzyme in the rice bran (40 mesh sieve) was killed by the hot steam, the solution compounding material liquid ratio was 1:5 (W/V), 60°C, power and time of ultrasonic treatment were 120 W and 55 min, cellulose 1.2%, protease 0.6% and amylase 0.3%, pH 4.5, temperature 55°C, enzyme hydrolysis 5.5 h, the oil rate would reach to 92.63%.

**Keywords:** Cellulose, enzymatic extraction, rice bran oil, ultrasonic

### INTRODUCTION

Rice bran is the by-product of rice processing, accounted for 5-8% in grain weight. Rice bran contains grease (12-24%), protein (12-15%), cellulose (9.8-10%), vitamins (VB1, VB2, VP) and mineral elements. Rice bran oil, extracted from the rice bran, contains the unsaturated fatty acids, accounted for 38-42% oleic acid and 32-35% linoleic acid. The linoleic acid is widely recognized as an essential fatty acid and decreased the blood cholesterol, prevents atherosclerosis and other health effects, so the rice bran oil is a kind of ideal edible vegetable oil (Wang, 2006; Marlene *et al.*, 2005). High efficiency and maximize extracted oil components from rice bran become research hotspots in oil industry (Liu, 2002; Ghosh, 2007; Xing *et al.*, 2011). Preparation methods of rice bran oil include the mainly mechanical press method, solvent extraction, enzymatic extraction and supercritical CO<sub>2</sub> extraction method (Wang *et al.*, 2008; Zhang *et al.*, 2011, 2009a; Guo *et al.*, 2008; Song *et al.*, 2007). Compared with other preparation process, enzymatic extraction can destroy cell wall of rice bran by the hydrolysis of cellulase, protease and amylase, so that fat within the cell wall release as possible, mild reaction condition, simple equipment, high rate and quality of rice bran oil (Ma *et al.*, 2005; Aparna *et al.*, 2001). In recent years, ultrasonic aided technology has become an effective method for edible oils and fats from natural product extraction (Lin *et al.*, 2008; Wu *et al.*, 2011; Zhang *et al.*, 2009b; Chen *et al.*, 2010). In this study, raw material pretreatment and enzymatic hydrolysis conditions by ultrasonic enzymatic extraction of Timmy

bran oil were studied and the extraction process parameters were optimized through the orthogonal experiment.

### MATERIALS AND METHODS

**Chemicals and enzyme:** Rice bran was provided by Xiang Sheng Industrial Company Limited in Changsha; cellulase (40000 U/g) and protease (50000 u/g) were get from the Jarrow bio-enzyme Limited; amylase (6000 U/g) Lvweikang Biological Engineering Company Limited in Shenzhen; All the other chemicals are analytical grade.

#### Methods:

**Rice bran pretreatment:** The rice bran crushing and sieving and steam heating method for lipase inactivation was mixed evenly by adding water. At proper temperature the solution was treated with ultrasonic according to treatment time, frequency and temperature. The test results could be discussed based on the impact of oil yield. So a suitable size (40 mesh sieve) of rice bran and solid-liquid ratio (1:5 W/V) were selected for the follow-up test.

**Rice bran oil extraction:** According to the (Guo *et al.*, 2008) method, the leaching liquor, completed the enzymatic hydrolysis reaction, was heated to 85°C, holding 15 min, so as to lose activity of amylase, protease and cellulase. Then, according to ratio of the rice bran and isopropyl alcohol (W/V) for 1:2 joining the isopropyl alcohol, stirring evenly, the temperature was maintained 60°C, soaking 2 h. Mixed oil was

separated by the centrifugal, distilled to obtain the rice bran oil.

**Technological process:** Rice bran → crushing sieving → heat treatment → ultrasonic treatment → cooling → liquid preparation → enzymatic hydrolysis → thermal inactivation → solvent extraction → centrifugal separation → solvent evaporation → rice bran crude oil.

**Analysis methods:**

**Determination of main components of rice bran:** Determination of rice bran moisture content: constant weight method at 105°C (GB 5009.3-2010). Determination of rice bran crude fat: Soxhlet extraction method (GB/T 5512-2008). Determination of rice bran protein content: Kjeldahl method (GB/T 5511-2008). Determination of rice bran ash content: high temperature burning method (GB/T 22510-2008). Determination of the cellulose content of rice bran: medium filtration method (GB/T 5515-2008).

**Enzymatic hydrolysis:** pH value of the mixed solution was adjusted with 1:1 HCl. Catalysis system was adopted with the appropriate enzyme mixture (protease 0.3%, amylase 0.6% and cellulase 1.2%) to enzymolysis. A series of single factor test was designed to determine the Variable including the amount of enzyme, pH value, temperature and time in the process of rice bran oil. And then, optimal process conditions from enzymatic extraction rice bran oil could be obtained by the orthogonal test.

**RESULTS AND DISCUSSION**

**Main components of rice bran:** The results, the main components of rice bran, were shown in Table 1.

Effect on oil yield of rice bran by extraction assisted ultrasonic. Ultrasonic is an elastic mechanical vibration wave. Compression and rarefaction of the wave can drive the medium, so that the liquid pressure changes and results from the large number of vacuum bubble. When the bubbles burst and generate strong instantaneous pressure, named as "cavitation effect". Rice bran particles and cells were sheared and crushed. This action was helpful for rice bran oil extraction, thus to improve the rate of oil. The tests were conducted to study the influence of rice bran oil rate on the time, power and temperature of the ultrasonic treatment.

**Time of ultrasonic treatment:** The results showed, in the range of ultrasonic treatment time 30-50 min, rice bran oil rate increase rapidly with the treatment time. The improvement was slow in the 50-60 min and decreased as more than 60 min. Due to the ultrasonic processing, the reaction process was strengthened

Table 1: Principal components of rice bran

Composition	Moisture	Fat	Protein	Ash	Cellulose
Content (%)	10.05	16.30	13.65	10.80	29.55

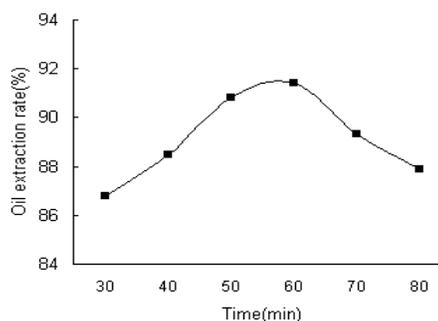


Fig. 1: Effect of rice bran oil rate on ultrasonic treatment time

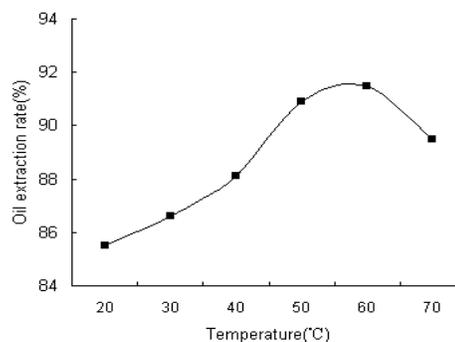


Fig. 2: Effect on rice bran oil rate at ultrasonic treatment temperature

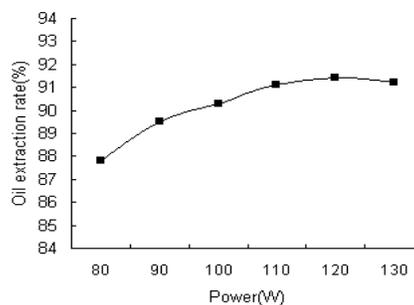


Fig. 3: Effect of rice bran oil rate on ultrasonic treatment power

rapidly in a certain period of time. But while the time was too long, the response would reverse, so that reaction system state to emulsify. "Cavitation effect" would weaken or even disappear at this time, so the rate of oil could reduce. Shown in Fig. 1, oil rate reached 91.5% at the 55 min ultrasonic treatment. The ultrasonic processing time was selected as 55 min.

**Temperature of ultrasonic:** The experimental results revealed that the oil rate affected more significantly

Table 2: The influence of the quality of rice bran oil by the extraction assisted ultrasonic

Method	Color	Refraction index (n <sup>20</sup> D)	Acid value (mgKOH/g)	Iodine value (g/100 g)	Peroxide value (mol/kg)	Saponification value (mgKOH/g)
Enzyme	Light yellow	1.475	23.50	98.800	5.85	96.350
Ultrasonic enzymatic	Orange	1.472	20.13	106.23	2.35	105.68

Table 3: Orthogonal test results of the oil rate from the rice bran

Test no	A (enzyme dosage %)	B (temp.)	C (pH)	D (time h)	Oil rate (%)
1	1 (1.0)	1 (35)	1 (3.5)	1 (3.5)	83.65
2	1	2 (45)	2 (4.5)	2 (4.5)	86.84
3	1	3 (55)	3 (5.5)	3 (5.5)	90.91
4	2 (1.2)	1	3	2	91.60
5	2	2	1	3	92.35
6	2	3	2	1	90.93
7	3 (1.4)	1	2	3	92.45
8	3	2	3	1	89.65
9	3	3	1	2	90.23
K1	87.13	89.23	88.74	88.41	
K2	91.63	89.61	90.07	89.56	
K3	90.78	90.69	90.72	91.90	
R	4.500	1.460	1.980	3.490	

under different temperature conditions of ultrasonic processing, showed in Fig. 2. When the temperature varied from 20 to 60°C, the oil rate increased from 85.5 to 91.5%. The temperature was higher than 60°C; the oil rated assumed the drop tendency. So the temperature of ultrasonic treatment was decided as 60°C.

**Power of ultrasonic:** As shown in Fig. 3, when power of ultrasonic wave was set up in the range from 90 to 120 W, the effect on oil yield was small. The rate of oil rose slowly with the power increased. When the power was more than 110 W, rate of oil tended to be steady. Rather than 120 W slightly, rate of oil would decreased. So the suitable power was 120 W.

Influence of quality of rice bran oil by extraction assisted ultrasonic. In order to study the influence of quality of rice bran oil by extraction assisted ultrasonic, a series of experiment was designed and the control was accompanied. The test results were shown in Table 2.

Compared with the enzymatic extraction, color of rice bran oil by the extraction assisted ultrasonic was slightly deep, refractive index was similar, acid value and peroxide value were smaller, But iodine value and saponification value were larger. These facts designated that the rice bran oil of ultrasonic assisted extraction can improve the antioxidant capacity and concentration of unsaturated fatty acid. The quality of the oil enhanced by the process of ultrasonic assisted extraction.

**Optimum process condition of the rice bran oil by enzymatic extraction:** According to the rice bran pretreated and the test results, selected over 40 heads of a bran as raw material, heating steam to destroy enzyme inactivation, compound 1:5 (W/V) solution, under the condition of 60°C, ultrasonic power 120 W treatment 55 min, amount of the enzyme (enzyme by adding cellulase, corresponding protease and amylase), temperature, PH, time value range, with L9 (34) orthogonal test to determine the enzymatic extraction

Timmy bran oil the optimum technology condition, the results were showed in Table 3.

The test results showed that, the influence factors order of oil yield were enzyme dosage>time>pH>temperature. The optimum extraction conditions would be getting: the enzyme dosage (A2, cellulase) was 1.2% (protease 0.6% and amylase 0.3%, correspondingly); enzymatic hydrolysis time was 5.5 h; pH 4.5 and temperature 55°C. According to the optimal conditions, using isopropanol extraction and distillation separation, the average of the rice bran oil rate reached 92.63% in 3 times test. The test results of oil rate (approximately 87%) increased by about 5% (Table 2) compared with those without ultrasonic treatment rice bran oil.

## CONCLUSION

Rice bran oil rate by the enzymatic extraction can be increased through the ultrasonic treatment and the quality of rice bran oil can be improved. In a certain test range, more obvious effect factors on the yield of oil are time and temperature of the ultrasonic treatment compared with the influence of ultrasonic power. Ultrasonic-enzymatic extraction rice bran oil, 60°C, ultrasonic power 120 W, after 55 min, the preparation enzymatic extraction system including amylase, cellulose and protease has a synergistic effect. Then the proper material liquid ratio and the ratio of three kinds of enzymes can improve the yield of rice bran oil. Orthogonal test results show that the effect of rice bran oil extraction is enzyme dosage>time>pH>temperature. Optimum extraction technology of the rice bran oil extraction is the enzyme dosage (cellulose 1.2%, protease 0.6% and amylase 0.3%), enzymatic hydrolysis time 5.5 h, pH 4.5, temperature 55°C. In this condition, the rate of rice bran oil is 92.63% and increase in production of 5% above that of the enzyme extraction without ultrasonic treatment. The method has the advantages of simple equipment, mild reaction conditions and high oil yield. The preparation method

of rice bran oil will have a good prospect in the industrial application.

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