Ultrasound assisted solvent extraction oil from dried rice bran soapstock

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Abstract. In this study, neutral oil was recovered from dried rice bran soapstock using ultrasound assisted solvent extraction (UASE). Response surface methodology (RSM) was employed to optimize the UASE parameters to obtain the maximum yield of neutral oil using face centered composite design (FCCD). The parameters included ultrasound power (0.5-4.5W/g), process temperature (35-55°C), and sonication time (4-26 min). The quadratic response models were generated and statistical analysis was performed to validate the models. The results showed that the optimum extraction conditions were ultrasound power of 3.75 W/g, process temperature of 49.71°C, and sonication time of 22.68 min. Ultrasound power and temperature were the most significant parameters for the extraction process. Under the optimal condition, the predicted neutral oil recovery was 98.10%, while corresponding experimental result was 98.21%, suggesting good agreement between the predicted and experimental data and high predictive ability of the models.

1. Introduction
Rice bran soapstock is a byproduct from chemical neutralization process of rice bran oil production. It is obtained after de-acidification by alkali treatment of crude rice bran oil. Rice bran soapstock is not soluble in water and contains both of hydrophobic and hydrophilic region in their molecular [1]. In chemical neutralization, approximately 14.20-14.42% of neutral oil [1] and 90% of gamma oryzanol [2] are lost in soapstock. At the present, most soapstock is used as animal feed, soap and detergent. Meanwhile, rice bran oil soapstock has been known as high unsaturated fatty acids (UFAs), e.g., oleic, linoleic, and linolenic acid, which help lower cardiovascular risks [3]. Therefore, the utilization of rice bran soapstock as noted can help adding value to agricultural products and also reducing contamination from rice bran oil industrial waste.

Most researches are focused on converting soapstock to acid oil by using enzymatic hydrolysis or acidulation with sulfuric acid followed by ammonia neutralization. This acid oil is mainly used for biodiesel synthesis or chemical use with low price. However, there are no studies for the recovery of neutral oil from rice bran soapstock.

Solvent extractions (hexane, petroleum ether, methanol, and dichloromethane) are widely used for oil extraction because of easy, lower boiling point (63–75°C) and excellent solubilizing ability [4]. However, solvent extraction method is time-consuming, difficult automatic, and often consuming toxic solvent. Furthermore, these solvents are not suitable for extraction oil from soapstock because of emulsion formation and dissolution of the soapstock. In this study, mixture of ethyl acetate and ethanol was used for extraction because it can create moderate non-polar solvent, and prevent...
emulsion formation and dissolution of the soapstock. Besides, ethyl acetate and ethanol are accepted for use in food as well as cosmetic product [5].

Ultrasound assisted extraction technique was used to improve the extraction efficiency and reduce the extraction time. High intensity ultrasound application induces the growth of bubble inside liquids, causing of the occurrence of the cavitation phenomenon [6]. Thus, the diffusion and the solubility of target compounds increased in solvent during extraction.

This study, thus, investigated the effect of variables of ultrasound assisted solvent extraction parameters on the oil recovery from dried rice bran soapstock (DRBS) using response surface methodology (RSM) with face centered composite design (FCCD). The ultrasound assisted solvent extraction parameters included ultrasound power, process temperature, and sonication time which were varied between 0.5, 2.5, and 4.5 W/g; 35, 45, and 55°C; and 4, 15, and 26 min, respectively.

2. Materials and methods

2.1. Materials

The saponifiable components were removed from chemical neutralization of rice bran being termed wet rice bran soapstock (WRBS). The WRBS was heated to 80°C in water bath, and then saponified by adding 1.25%wt NaOH solution with constant stirring for 30 min. The solid was decanted and vacuum evaporated at 100°C, 550 mmHg for 2-3 h. The final moisture of dried rice bran soapstock (DRBS) was 4.0-4.5%, and used as the starting material for the extraction.

Methanol (Mallinckrodt), ethyl acetate, n-hexane, ethanol, chloroform, and, BF₃-methanol absolute value (< 98.5%) and NaOH anhydrous pellet (98%) were from Sigma-Aldrich (Darmstadt, Germany). Standard fatty acid methyl esters (37-component FAME Mix of Supercool, USA) were used for quantifications.

2.2. Methods

2.2.1. Soxhlet extraction (SE). The mixture of ethanol in ethyl acetate (EE) (85% ethyl acetate and 15% ethanol, v/v) was used in extraction neutral oil from DRBS based on preliminary investigation showing a suitable extraction solvent. The extraction was experimented at 90°C for 4 hours with the solvent to solid ratio of 10:1 (Kumar et al., 2009). The neutral oil fraction was centrifuged at 10,000 rpm, 10°C for 15 min to remove solid, and then solvents were evaporated by using vacuum evaporator at 500 mmHg, 55°C to dryness before further analysis. The neutral oil yield was expressed as the neutral oil extracted by SE and the weight of DRBS (equation(1)). The EE technique yielded 14.90±0.59 g of neutral oil per 100 g DRBS, which was set up 100% oil yield.

\[
\text{Neutral oil yield} = \frac{\text{Weight of oil extracted}}{\text{Weight of DRBS}} \times 100
\]  

(1)

2.2.2. Ultrasound assisted solvent extraction (UASE). The extraction was performed using 40 kHz ultrasound advice (VCX750 Vibrancell; Sonic & Materials, Inc., Newtown, CT, USA) with a 3.0 mm flat tip probe. Briefly, a 20 g DRBS was dispersed in 200 mL EE in flask. The ultrasound treatment was carried out at 20 kHz using an ultrasonic generator. The ultrasound power and process temperature were varied between 0.5, 2.5, and 4.5 W/g; and 35, 45, and 55°C. These mixtures were sonicated at different sonication time (4, 15, and 26 min) with pulse durations of 5 s. The rice bran oil fraction was then centrifuged and concentrated by vacuum evaporation. The recovery was expressed as the proportion of the neutral oil extracted by UASE and the initial oil as determined by EE.

2.2.3. Experimental design. In this study, experimental design included three factors, ultrasound powder (X₁), process temperature (X₂), and sonication time (X₃). Three levels of three factors were designed using Modde 5.0 (Umea, Sweden) (table 1). Response surface methodology (RSM) with a face centered composite (FCC) was employed, and the design of experiment of 17 runs, and three replicates. A second order polynomial model was generated to predict the response variables of the oil recovery (Y).
\[ f(Y) = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ij} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \]  
(2)

where \( Y \) is the predicted response; \( \beta_0 \) is a constant; \( X_i \) and \( X_j \) are independent variables, \( \beta_i \) and \( \beta_{ij} \) are the linear coefficients, and cross coefficients; \( \beta_{ij} \) is the quadratic coefficients.

The coefficient of determination (\( R^2 \)), lack of fit (LOF), and F-test were employed to determine the adequacy of the model. The robustness and predictive models developed were evaluated based on \( R^2 \), adjusted \( R^2 \), and model predictive ability (\( Q^2 \)).

**Table 1.** Experimental and predicted rice bran oil recovery \((Y)\) under variable of ultrasound power \((X_3)\), process temperature \((X_2)\) and sonication time \((X_1)\).

| Run | Code variable | Experimental value | Predicted value |
|-----|---------------|--------------------|-----------------|
| 1   | -1 -1 -1      | 0.5 35 4           | 72.35±1.98      | 72.19±1.45 |
| 2   | 1 -1 -1       | 4.5 35 4           | 82.68±1.73      | 82.22±1.68 |
| 3   | -1 1 -1       | 0.5 55 4           | 85.84±2.01      | 85.59±2.10 |
| 4   | 1 -1 -1       | 4.5 55 4           | 91.95±2.05      | 91.83±2.09 |
| 5   | -1 -1 1       | 0.5 35 26          | 86.24±1.90      | 85.93±1.86 |
| 6   | 1 -1 -1       | 4.5 35 26          | 90.81±2.01      | 90.62±1.05 |
| 7   | -1 -1 1       | 0.5 55 26          | 95.24±1.31      | 95.27±1.21 |
| 8   | 1 -1 1        | 4.5 55 26          | 96.45±1.05      | 96.17±1.34 |
| 9   | -1 0 0        | 0.5 45 15          | 90.20±1.82      | 90.89±1.01 |
| 10  | 1 0 -1        | 4.5 45 15          | 95.30±1.09      | 96.35±1.15 |
| 11  | 0 0 -1        | 2.5 35 15          | 85.17±2.17      | 86.30±1.34 |
| 12  | 0 0 1         | 2.5 55 15          | 95.17±1.75      | 95.76±2.06 |
| 13  | 0 0 0 -1      | 2.5 45 4           | 86.51±2.01      | 87.52±2.11 |
| 14  | 0 0 0 1       | 2.5 45 26          | 95.84±1.73      | 96.57±1.63 |
| 15  | 0 0 0        | 2.5 45 15          | 95.30±1.18      | 94.62±1.59 |
| 16  | 0 0 0        | 2.5 45 14          | 95.30±1.23      | 94.62±1.85 |
| 17  | 0 0 0        | 2.5 45 15          | 96.71±1.05      | 94.62±2.01 |

2.2.4. **Physicochemical properties.** The quality of oil extracted from rice bran soapstock was measured by its chemical properties in term of peroxide value (Cd 8b-90, AOCS-1997), acid value (Ca 5a-40, AOCS-1997), iodine value (Cd 1d-92, AOCS-1997) [8].

2.2.5. **Gas Chromatography with Flame-Ionization Detection (GC-FID) analysis.** Fatty acid composition of oil was analyzed by GC-FID (Agilent 7890C axis detector, England). Fatty acid was trans-esterified to fatty acid methyl esters (FAMEs) in BF₃-methanol following Stanisavljević et al. [8] procedure. The temperature program was set up as follows: The oven temperature started from 50°C to 250°C at a rate of 40°C/min, the inject temperature of 180°C, and the ion source temperature of 230°C. The volume of injected oil was 0.2 µl.

2.3. **Statistical analysis**

Tukey (HSD) test, using a Statgraphic centurion XV software package (Ver. 10.2., Statsoft Inc., USA) was performed to determine if there were significant differences in oil recovery at various ultrasound powers, treatment temperatures, and treatment times during UAE. The mean values were given that the significant differences at \( p<0.05 \) were observed.

3 **Results and discussion**

3.1 **Fitting model**

Table 1 tabulates the experimental and predicted values of the oil recovery. Specifically, the oil recovery from the experiments were 72.35-96.71%. The statistical significance of quadratic model terms was determined by ANOVA, and the significance of regression coefficients by F-value, p-value, and lack of fit (LOF). The high F-values (49.75) and very low probability (\( p<0.05 \)) of the regression model for response of oil recovery indicated model terms were significant. Moreover, the p-value of
the lack of fit in model was more than 0.283 and non-significant, which illustrated the model satisfactorily fitted to the experimental data. In addition, the high R² value (0.985) and adjusted R² (0.965) of the model indicates that oil recovery model suggested good agreement between the experimental and predicted data. Q² value of 0.941 indicated that the variability of responses was well explained by the models with small prediction error. The model of response surface was generated based on the fitted second-order polynomial equation is given below:

\[ Y = 14.097 + 0.407X_1 + 0.706X_2 + 0.674X_3 - 0.199X_1X_2 - 0.533X_1^2 - 0.383X_2^2 \] (3)

**Table 2.** ANOVA results for the response surface quadratic model on the recovery of rice bran oil.

| FFA          | Degree of freedom | Sum of squares | Mean square | F-value | p-value |
|--------------|-------------------|----------------|-------------|---------|---------|
| Total Corrected | 16               | 16             | 15.38       |         |         |
| Regression   | 9                 | 9              | 15.14       | 49.75   | 0.000   |
| Residual     | 7                 | 7              | 0.237       |         |         |
| Lack of Fit  | 5                 | 0.207          | 0.042       | 0.280   | 0.283   |
| Pure Error   | 2                 | 0.029          | 0.015       |         |         |

R² = 0.985; Adjusted R² = 0.965; Q²=0.941, p< 0.05 indicates statistical significance.

3.2 **Effect of process variables on rice bran oil recovery**

As shown in table 3, the linear term of X₁ (ultrasound power), X₂ (process temperature), and X₃ (sonication time), cross-product term of X₁X₂, and quadratic of X₁², X₂² had highly significant effects on the recovery of oil. Among of these parameters, X₂ had the greatest effect on the recovery compared with other independent variables (X₁ and X₃). The interaction of X₁X₂ on the model significantly affected (p> 0.05) than X₁X₃, and X₁X₁. Meanwhile, the second-order term of X₁² and cross-product of X₁X₂ and X₁X₃ had no significant effects on the rice bran oil extraction efficiency.

Figure 1a shows the interaction between X₁ and X₂ on the oil recovery. An increase of X₁ from 0.5 to 4.5 W/g and X₂ from 35 to 55 °C, given X₃ of 15 min accelerated an increase in the oil recovery. The oil recovery achieved an equilibrium and non-significant difference when X₁ and X₂ increased to 4.5 W/g and 55 °C. The maximum oil recovery was achieved under ultrasonic power (X₁) of 3.71 W/g and temperature (X₂) of 45 °C.

In figure 1b, the effect of X₁ and X₃ on the oil recovery appeared as an elliptical shape, given X₂ of 45.0°C. The oil recovery gradually increased with increase in X₁ (from 0.5 to 4.5 W/g) and X₃ (from 4 to 26 min). The maximum oil recovery was under ultrasound power of 3.71 W/g and sonication time of 22.69 min (p< 0.05). This is consistent with Da et al. [2], who documented that high ultrasonic power and extended sonication time increased the oil recovery due to the plant cell wall degradation.

Figure 1c described the effects of X₂ and X₃ on the oil recovery at fixed ultrasound power (X₁) of 2.5 W/g. The extraction efficiency was increased under higher process temperature (X₂) and extended sonication period (X₃). An increase in sonication time needed for the solvent penetration into the sample and raise the diffusion of dissolved oil to outside the solvent. This promoted the extraction efficiency of the oil. However, the high process temperature accelerated solvent evaporation, and to less amount of solvent extraction in subsequently decreased the oil recovery [9]. The maximum recovery was achieved under process temperature of 49.86°C (X₂) and sonication time of 22.68 min (X₃).
3.3 Optimization of the extraction parameters and characteristic of rice bran oil

Based on the FCC design, the optimal UASE conditions that achieved high oil recovery was that of 3.75 W/g for ultrasonic power ($X_1$), 49.86°C for process temperature ($X_2$), and 22.68 min for sonication time ($X_3$). Under optimal condition, the recovery of oil was 98.10% that was in a good agreement with the predicted result (98.21%).

Table 3 showed the physiochemical properties and fatty acid profile of oil extracted from DRBS under the optimal neutralization parameters. As a results, UASE did not affected to the iodine value, FFA, and phosphorus level of recovered oil. However, peroxide value of the oil from SE was slightly higher than that of the oil from UASE due to high process temperature (90°C) and longer extraction time (4 hours). It took only one-seventh the sonication time under UASE (22.68 min) to achieve the comparable neutral oil yield and quality as that under SE (160 min). The quality of oil extracts falls well within the acceptable standard values [10]. The fatty acid profile is an important characteristic of the nutritive value and quality of the oil. From our results, the fatty acid profile is high content of unsaturated fatty acids, i.e. oleic and linoleic. Oleic acid (C18:1) and linoleic acid (C18:2) represented the most predominant fatty acid, followed by palmitic acid (C16:0) in rice bran oil. The high amount of unsaturated fatty acids provides the oil suitable to use as cooking oil for diet enrichment.

Figure 1. The response surface plots of the effects of ultrasound power, process temperature, and sonication time on oil recovery: (a) fixed sonication time at 15 min; (b) process temperature at 45°C; and (c) fixed ultrasound power at 2.5 W/g
quality of neutral oil and fatty acid profile.}

| Quality of neutral oil | SE\(^1\) | UASE\(^2\) |
|-----------------------|---------|-----------|
| **1. Fatty acid profile (%)** |         |           |
| Myristic (C14:0)      | 3.46±0.06 | 3.45±0.09 |
| Palmitic (C16:0)      | 17.02±1.15 | 17.06±1.07 |
| Stearic (C18:0)       | 3.56±0.09  | 3.52±0.08  |
| Oleic acid (C18:1)    | 42.21±1.05 | 42.22±1.55 |
| Linoleic (C18:2)      | 24.27±1.12 | 24.28±1.21 |
| Dihomo γ-linolenic (C20:3) | 4.46±0.10 | 4.43±0.09  |
| Heneicosylic (C21:0)  | 4.05±0.08  | 4.04±0.05  |
| Nevinis (C24:0)       | 0.99±0.02  | 1.0±0.06   |
| **2. Physiochemical properties** |         |           |
| Peroxide value (meq O\(_2\)/kg) | 5.57±0.09 | 5.50±0.03 |
| Iodine value (g I\(_2\)/100g) | 100.15±1.84 | 99.98±0.55 |
| FFA (% oleic acid)    | 0.20±0.12  | 0.19±0.04  |
| Phosphorus concentration (ppm) | 4.50±1.15 | 4.49±1.95  |

\(^{1,2}\) Different letters in row of physicochemical properties denote statistically significant differences between treatments (p< 0.05). The values are the mean of three replications ± standard deviation.

4. Conclusion
The effect of UASE parameters on the oil recovery from the DRBS using RSM with FCCD was investigated. The ANOVA result validated the predictive power of the models, given fitness to experimental data. The optimal UASE was 3.75 W/g, 49.86°C, and 22.68 min. The predicted and experimental results were agreeable in which the predicted oil recovery. Thus, the response model could be deployed for optimization of UASE to enhance the oil yield and quality.

5. References
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