Food Research 7 (5) : 219 - 225 (October 2023)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

The optimization of oil extraction by surfactant-assisted aqueous extraction process of rice bran (*Oryza sativa* L.) using Box-Behnken design

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Article history:
Received: 1 December 2021
Received in revised form: 24 January 2022
Accepted: 21 May 2022
Available Online: 8 October 2023

Keywords:
Rice bran oil,
Extraction,
Surfactant-assisted aqueous extraction process,
Extracted oil,
Response Surface Method,
Box-Behnken Design

DOI:
https://doi.org/10.26656/fr.2017.7(5).968

Abstract

Surfactant-assisted aqueous extraction process (SAAEP) is used as an environmentally friendly alternative method for oil extraction. Rice (*Oryza sativa* L.) is one of the most widely produced and consumed foods, and it produces bran as a by-product. Therefore, this study aims to determine the optimum conditions for SAAEP from rice bran. The response surface methodology (RSM) technique was used to determine the optimum conditions. Furthermore, the Box-Behnken design (BBD) was selected as the study design with three factors and three levels. The independent variables used were surfactant and oil ratio (SOR) (3, 3.5, and 4), ultrasonication amplitude (60%, 70%, and 80%), and ultrasonication contact time (15, 20, and 25 mins. Meanwhile, the dependent variable used was oil content. The extraction was carried out based on SAEEP. Also, the rice bran used was the IR 32 SR variety which passed the stabilization stage with a moisture content of 4.25%, extracted oil of 20.64%, and 2.04 meqO_2/kg FFA levels. The optimal point was predicted to produce an extracted oil of 19.326%. Furthermore, the prediction results were evaluated by experimental extraction at the optimal level. The results showed an extracted oil of 18.52±0.04%, which is a difference of about 0.81% from the predicted values. It was concluded that the rice bran oil content can be successfully optimized using the RSM technique. Therefore, the optimum conditions for extracting rice bran oil are within the specified range of factors.

1. Introduction

Rice bran is a major co-product of the rice milling process accounting for 5–8% of milled rice (Pradeep *et al*., 2014; Puniah *et al*., 2021). It is a natural source of protein (14–16%), fat (12–23%), crude fibre (8–10%), carbohydrates, vitamins, minerals, essential unsaturated fatty acids and phenolics (Garba *et al*., 2017). Rice bran oil is extracted from rice bran, the outer layer of the rice grain. It is commonly used as a cooking oil in many Asian countries, including Japan, India, and China. As a byproduct of rice milling, rice bran is usually used as animal feed or discarded as waste. Yet, it has recently gained attention for its potential health benefits as an oil. RBO is known as a wonder oil for its numerous health benefits. It has a number of advantages over other edible oils because of the presence of a unique antioxidant known as oryzanol (Puniah *et al*., 2021; Lerma-Garcia *et al*., 2009). It is also a good source of nutritionally important compounds such as γ-oryzanol, tocopherols, tocotrienols and sterols (Garofalo *et al*., 2021). The crude RBO contains about 1.1-2.6% of γ-oryzanol, 0.2% of tocols, 70% of which is tocotrienol, and 3-5% of phytosterols and fatty acids sterol esters. After the milling process to extract the RBO, the rice bran needs to undergo a process called stabilization to inactivate the enzymes and inhibit lipid oxidation. This process is essential to prevent the deterioration of fat and valuable bioactive compounds in the bran (Garba *et al*., 2017; Bodie *et al*., 2018)

Surfactant-assisted aqueous extraction process (SAAEP) has been used as an environmentally friendly alternative method for effective oil extraction from passion fruit seed waste generated from the juice extraction industry (Ghadave *et al*., 2014; Surlehan *et al*., 2019). The surfactant is used to reduce the interfacial tension of the oil and water, therefore allowing it to be efficiently released into the aqueous extraction medium. Previous studies have used an extended type of surfactant that lowers the interfacial tension for efficient oil release. However, the extended surfactants are not food grade and their toxicity to humans is questionable, hence the extracted oil is used mainly for non-food

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eISSN: 2550-2166 / © 2023 The Authors.
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purposes such as biodiesel or lubricants (Do *et al*., 2010; Gadhave and Waghmare, 2014; Ramly *et al*., 2016). Special surfactants with unique structures have polarity groups between hydrophilic and lipophilic. They also act like a bridge that provides stronger interactions between the hydrophobic groups and the hydrophobic oil. This strong interaction contributes to the reduction of interfacial tension (IFT) to an ultralow level. However, the health aspects and effectiveness of extended surfactants in food processing applications cannot be ascertained. Tween 80 is a non-ionic surfactant commonly applied to commercial food that has relatively low toxicity, and low irritation potential and is widely used in pharmaceutical microemulsion preparations (Ramly *et al*., 2016). Its structure consists of polyoxyethylene (20) and sorbitan monooleate. Meanwhile, compared to Tween 20 and 40, Tween 80 is less viscous and has a lower critical misel concentration (CMC), which means the system will use less surfactant to achieve minimum IFT (Sabatini *et al*., 2011).

Response surface methodology (RSM) is a set of mathematical and statistical techniques to analyze problems where several independent variables affect the response variable. The goal is to optimize the response, shorten the research run, and save costs and time. It consists of two methods, namely central composite for designs with two independent variables, and the Box-Behnken for those with three or more (Umesh *et al*., 2015). The Box–Behnken design (BBD), an RSM tool, has been widely used by investigators for the optimization of experimental trials (Ahmad *et al*., 2015; Umesh *et al*., 2015; Wang *et al*., 2020). The Box–Behnken design is advantageous because it does not contain any points at the extremes of the cubic region created by the two-level factorial level combinations that are prohibitively expensive or impossible to test because of physical constraints in experimentation. The BBD has been widely used in pharmaceuticals, bioprocessing, food engineering, agrochemicals, and other industries to extract biological active compounds, such as polysaccharides, phenolic compounds, and proteins from various sources, intended for human use (Gu *et al*., 2016; Liu *et al*., 2018).

This study aimed to determine the optimum conditions for extracting rice bran oil using the SAAEP.

2. Materials and methods

2.1 Materials

The rice bran sample was purchased at a rice mill in Sragen, Central Java, Indonesia, and it was an IR 32 SR variety. The resulting bran oil is stored in the freezer without exposure to light. The other materials were surfactant Tween 80 (Merck), distilled water, and reagents.

2.2 Surfactant-assisted aqueous extraction process

The surfactant Tween 80 was dissolved in 100 mL of distilled water and macerated on a hot plate magnetic stirrer with the system temperature maintained at 85°C for 15 mins Subsequently, 20 g of rice bran was added with a maceration speed of 400 rpm for 60 mins and ultrasonicated at 100°C The treatment variations as independent variables were the ratio of surfactant to oil (SOR), ultrasonication amplitude, and ultrasonication contact time. The slurry was then centrifuged using a swing bucket rotor at 3000 rpm for 20 mins until gravity separated them into dregs, water, and oil. The upper oil phase was transparent and clear. To optimize the separation, it was placed in an incubator for 24 hrs at 50°C to remove the water portion, hence the oil could be separated and blown with nitrogen gas to ensure removal of the aqueous portion. Therefore, the rice bran oil was calculated in terms of (%w/w).

2.3 Experimental design

The optimization of the SAAEP was carried out using RSM. The RSM level of the independent variable for the previous oil content was determined based on preliminary experiments. Also, a total of 15 experiments were carried out with three independent variables, and three levels were developed according to the BBD design as shown in Table 1. BBD has been widely used to experiment with designs that evaluate the non-linear relationship between response values and factors. Compared to other designs, it has the advantage of reducing the number of trials, (Liu *et al*., 2018). In this study, the independent variables were the ratio of surfactant to oil (SOR) (X1), ultrasonication amplitude (X2, %), and ultrasonication contact time (X3, mins), while the dependent variable was the bran extracted oil.

The independent variables or factors used in the extraction process are:

1. The ratio of SOR (X3 = 3, 3.5 and 4)
2. Ultrasonication amplitude (X2 = 60%, 70%, and 80%)
3. Ultrasonication time (X1 = 15, 20, and 25 mins)

While the dependent variable or the observed response was extracted oil (%).

2.4 Statistical analysis

The data were analyzed using the Design Expert 11 (Stat Ease Inc., MN, USA) statistical software. Furthermore, the experimental data were applied to the

https://doi.org/10.26656/fr.2017.7(5).968

quadratic polynomial model through which the regression coefficients were obtained. The generalized quadratic polynomial model used for the response surface analysis is shown by the following equation (1):

$$Y = \beta_0 + \sum_{I=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \qquad (1)$$

where Xi and Xj values are the independent variables that influence the responses Y; β0, βi, βii, and βij values are the coefficients of the regression model for intercept, linear, quadratic, and interaction terms, respectively; and k is the number of variables.

2.5 Verification of model

To verify the predictive value of the model, the optimum conditions with the maximum yield for extracted oil were determined and used in the extraction test. Furthermore, the precision of the fitted model was verified by comparing the predicted experimental value obtained from the three replicates.

3. Results and discussion

3.1 Model fitting

The optimization of the extraction conditions was carried out using the Box-Behnken Design (BBD) method with 15 experiments. Also, the SOR, ultrasonication amplitude (%), and ultrasonication contact time (mins) were selected as three variables that could potentially affect extracted oil, as shown in Table 1. Furthermore, the analysis of variance (ANOVA) was statistically significant ($P < 0\ 0.05$), which showed the variables in the model can explain the experimental variation of extracted oil (%w/w). The coefficient of determination (R^2), the adjusted value of R^2 (adj R^2), and the discrepancy are shown in Table 1. The results showed the model is suitable for the experimental data.

Table 1 shows the effect of the extraction condition on the yield of extracted oil. Extracted oil of rice bran is in the range of 4.49%-18.52%. Furthermore, the varieties of IR 32 SR used in this study have extracted oil was 20.65% (Soxhlet method). This means it achieves an efficiency of 89.62%. The SAAEP reaches its optimum after an experiment of 15 runs. Naksuk *et al*. (2009) and Sabatini *et al*. (2011) added a deionized water wash step as a second extraction process on 150 g of peanut and canola seeds. The oil extraction efficiency was 87.1% for peanuts and 85.6% for canola seeds. This showed that the SAAEP extraction has high effectiveness. Meanwhile, in this study, the effective extracted oil of rice bran was obtained from a combination of factors and levels at running 1, 2, 9, 12, and 14, which were 15.72%, 18.52%, 14.32%, 16.46%, and 16.86%, respectively. and means that it has an extraction efficiency of 69.71% - 89.90%. Based on preliminary research the actual oil content in the Ir 32 Sr variety is 20.6%. The difference in response in each running is probably closely related to the interaction between factors with a certain level.

Table 2 shows the interaction between the SOR ratio with ultrasonication amplitude and contact time significantly affecting the extracted oil response ($p < 0.05$). Furthermore, the high F-value in the two interactions showed a high interaction effect between factors on the response, as stated by (Jiao *et al*., 2014).

Table 1. Experimental design and dependent variable responses to extraction conditions.

Standard Order^a	Run Order^b	Coded Variables			Independent Variables			Dependent Variables
		X1	X2	X3	SOR	Amplitude ultrasonication (%)	Contact Time ultrasonication (min)	Extracted Oil (%)
6	1	1	0	-1	4	70	15	15.72
4	2	1	1	0	4	80	20	18.52
2	3	1	-1	0	4	60	20	12.88
1	4	-1	-1	0	3	60	20	4.49
5	5	-1	0	-1	3	70	15	5.13
3	6	-1	1	0	3	80	20	11.86
11	7	0	-1	1	3,5	60	25	10.86
14	8	0	0	0	3,5	70	20	14.15
15	9	0	0	0	3,5	70	20	14.306
9	10	0	-1	-1	3,5	60	15	10.41
13	11	0	0	0	3,5	70	20	13.72
8	12	1	0	1	4	70	25	16.46
10	13	0	1	-1	3,5	80	15	10.23
12	14	0	1	1	3,5	80	25	16.86
7	15	-1	0	1	3	70	25	5.37

^{a}No randomized, ^{b}Randomized

Table 2. The analysis of variance for the response surface quadratic model.

Source	Sum of Square	df	Mean square	F-value	p-value	
Model	251.29	9	27.92	8.32	0.0156	significant
A-SOR	168.65	1	168.65	50.26	0.0009	significant
B-Amplitude	44.30	1	44.30	13.20	0.0150	significant
C-Contact time	8.11	1	8.11	2.42	0.1808	
AB	0.7448	1	0.7448	0.2219	0.6574	
AC	0.0625	1	0.0625	0.0186	0.8968	
BC	9.53	1	9.53	2.84	0.1528	
A^2	11.62	1	11.62	3.46	0.1219	
B^2	0.4722	1	0.4722	0.1407	0.7230	
C^2	9.75	1	9.75	2.90	0.1491	
Residual	16.78	5	3.36			
Lack of fit	16.61	3	5.54	66.61	0.0148	significant
Pure error	0.1663	2	0.0831			
Cor total	268.07	14				

Meanwhile, the linear effect of the SOR ratio, amplitude, and contact time of ultrasonication were significant in influencing the extracted oil response ($p > 0.05$).

Statistically, the quadratic model can be used to predict response in the optimization formula, which is indicated by $p < 0.05$. These results are supported by a significant lack of fit ($p < 0.05$), thereby strengthening the quadratic model's suitability in the optimization of the SAAEP. The final equation in terms of coded factors is shown in Table 3.

Table 3. Final equation in terms of coded factors.

Extracted oil	Factor
$^{+}$ 14.7	-
$^{+}$ 4.59	*A
$^{+}$ 2.35	*B
$^{+}$ 1.01	*C
$^{-}$ 0.4315	*AB
$^{+}$ 0.1250	*AC
$^{+}$ 1.54	*BC
$^{-}$ 1.77	*A^2
$^{-}$ 0.3576	*B^2
$^{-}$ 1.62	*C^2

This study obtains a model in the form of a quadratic response by following the first-order equation:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3$$

Where Y is the response (extracted oil), β_0 is the coefficient constant, β_1 is the regression coefficient of the effect of factor 1 (SOR), β_2 is for factor 2 (amplitude), β_3 is for factor 3 (Contact time), β_{12} is the regression coefficient of the interaction effect of factor 1 and 2 (SOR x Amplitude), $\beta 1_3$ is the regression coefficient of the interaction effect of factor 1 and 3 (SOR x Contact time). Furthermore, β_{23} is the regression coefficient of the interaction effect of factors 2 and 3 (Amplitude x Contact time). In addition, the regression coefficient of each factor and the interaction between factors are determined based on the analysis of Design Expert v.11. The results can be presented in the equation:

$$Y = 14.07 + 4.59A + 2.35B + 1.01C - 0.4315AB + 0.1250AC + 1.54BC - 1.77A2 - 0.3756B2 - 1.62C2$$

Figure 1 shows the distribution of the data spread around the horizontal line within the limit of ±3. These results showed the predicted and actual values of the extracted oil in all experiments have residuals of ±3. According to Esmaili *et al*. (2011), the data spread between±3 indicates the fit of the model is high in predicting response. Furthermore, random data around

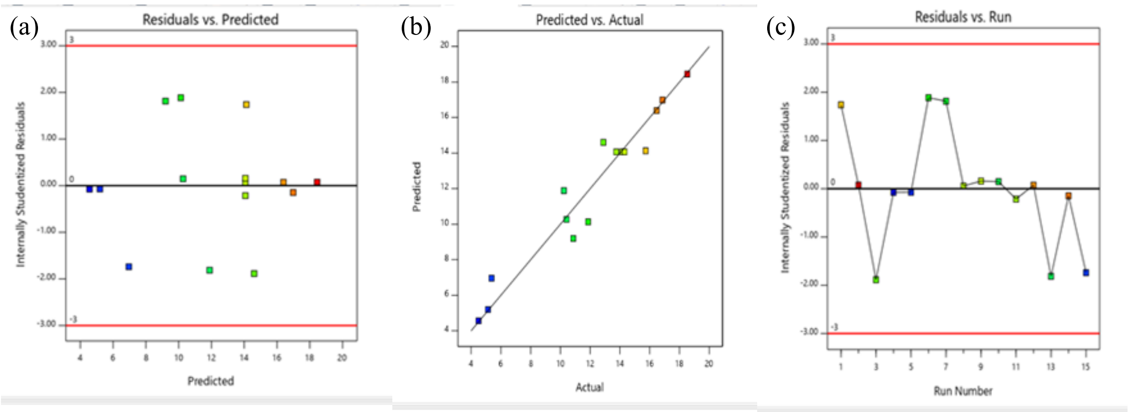

Figure 1. (A) Residual vs Predicted, (B) Residual vs Run, and (C) Predicted vs Run.

https://doi.org/10.26656/fr.2017.7(5).968

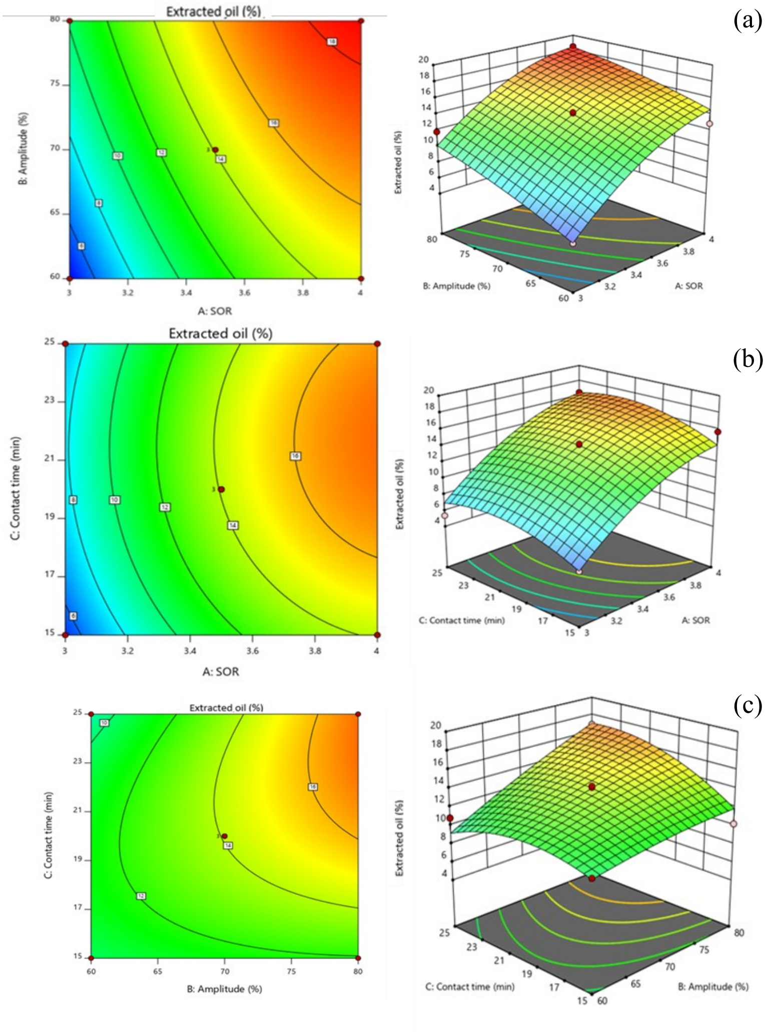

Figure 2. The response surface plot for the effects of extraction conditions for rice bran oil, (A) SOR and amplitude, (B) SOR and contact time, (C) amplitude and contact time.

the value 0 showed a small variance obtained during the optimization process. Therefore, the quadratic model used in the formula optimization process in this study has a high fit based on the relationship between residuals versus predicted and residuals versus the run. The data were distributed around a straight line (Esmaili *et al.,* 2011; Ahmad *et al*., 2015). These results showed the actual value of extracted oil has data like the predicted value of extracted oil in all the experiments. The closeness of the actual and the predicted values showed a normal fit of the model and the residual probability (Wang *et al*., 2020). Therefore, the quadratic model used in the formula optimization process has a high fit based on the predicted versus actual relationship.

Figure 2A shows that the optimum oil content response is when the high SOR ratio interacts with high ultrasonication amplitude at high levels. This is related to the increase in amplitude which will increase the cavitation energy, diffusion of the solvent into the material, and the rate of mass transfer (Luo *et al*., 2018). This affects the increase in the extracted oil. Also, the higher the SOR ratio, the greater the extracted oil. Figure 2B shows that the extracted oil response is optimum when a high amplitude interacts with a high ultrasonication contact time. Therefore, the higher the amplitude and contact time, the more the temperature is achieved. The local ultrasonic generation of micro cavitation around the material to be extracted also causes heating and releases the extracted compound. The longer the ultrasonic wave excitation, the higher the temperature in the extraction process. At amplitudes above 70%, the rate of temperature increase is greater (Garba *et al*., 2017; Luo *et al.,* 2018). The associated microfractures and cell wall disruption provide more evidence for the mechanical effects of ultrasound, thereby facilitating the release of its contents (Ahmad *et al*., 2015). The UAE has many advantages, including high extraction yields and reproducibility, low solvent usage, and short extraction times (Luo *et al*., 2018). Figure 2C shows that the response of rice bran oil content is optimum if a low ultrasonication amplitude interacts with a high contact time when the SOR is at a high level. Therefore, it is more efficient in the process of trapping rice bran oil in high concentrations.

3.2 Experimental validation of the optimum conditions

Table 4 shows the predicted and experimental values of the response variables under optimal conditions.

https://doi.org/10.26656/fr.2017.7(5).968

In this study, BBD predicted that the extracted oil has the optimal value when the SOR, amplitude, and contact time of ultrasonication are 3.947, 79.483%, and 23.940 mins, respectively. The optimal point was predicted to produce an extracted oil of 19.326%. The prediction results were further evaluated by experimental extraction at the optimal level. The experimental results showed an extracted oil of 18.52±0.04%, which is a difference of about 0.81% from the predicted values.

Table 4. The predicted and experimental values of the response variables under optimal conditions.

Parameter	Optimum values	
	Predicted Values*	Experimental Values*
SOR	3.947	4
Amplitude	79.483	80
Contact time	23.940	20
Extracted oil (%)	19.326	18.52±0.04

*Predicted using ridge analysis of response surface quadratic model, mean±standard deviation of triplicate determinations from different experiments.

4. Conclusion

The RSM was successfully used to optimize the extraction of rice bran oil with the SAAEP. This RSM with BBD is an effective method to determine the optimum conditions for extracting bran oil based on the response of the content. The optimum condition of SAAEP showed SOR, amplitude, and contact time of ultrasonication were 3.947, 79.483%, and 23.940 mins, respectively. The optimal point was predicted to produce an extracted oil of 19.326%. Furthermore, the prediction results were further evaluated by experimental extraction at the optimal level. The results showed an extracted oil of 18.52±0.04%, which is a difference of about 0.81% from the predicted values.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This research was funded by the Ministry of Research, Technology, and Higher Education, Republic of Indonesia grant number 8/E1/KPT/2021.

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