Food Research 7 (2) : 289 - 296 (April 2023)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Rice bran oil extraction by ethanol: optimization of γ-oryzanol and polyphenol

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Article history:
Received: 8 January 2022
Received in revised form: 25 February 2022
Accepted: 19 April 2022
Available Online: 22 April 2023

Keywords:
Rice bran oil,
Reflux method,
Ethanol,
Response surface methodology

DOI:
https://doi.org/10.26656/fr.2017.7(2).011

Abstract

Rice bran oil (RBO) contains γ-oryzanol and polyphenols which are very beneficial for health. The work investigated the effect of temperature (°C) (X_1), time (hr) (X_2), and volume of ethanol (mL) (X_3) as a solvent on the oil yield (%) (Y_1), γ-oryzanol (Y_2), and total polyphenol content (TPC) of RBO (Y_3) with apply reflux extraction method. The study was conducted using Response Surface Methodology (RSM) based on Central Composite Design (CCD) model with three levels and six centre points. The data were analyzed using Design-Expert 10 software to create and evaluate models and to plot the response curves of three-dimensional surfaces. The maximum of Y_1 = 16.4%, Y_2 = 195.49 mg.L^{-1}, and Y_3 = 1.02 mg gallic acid equivalent (GAE).g^{-1} were achieved under the optimum conditions process of X_1, X_2, and X_3 were 78.7°C, 1.6 hrs, and 496.5 mL ethanol/100 g rice bran, respectively. The average of Y_1, Y_2, and Y_3 on verified models were 16.1±0.66%, 189.12±1.18 mg.L^{-1}, and 0.91±0.54 mg GAE.g^{-1}, respectively. These indicated that this method is very promising to be applied in industry and as an effort to apply the concept of "green" solvents that is safe for humans and the environment.

1. Introduction

Rice bran, a low-value co-product obtained from rice processing, represents a potential source of healthy products due to its nutritional aspects, especially the oil it contains. RBO is rich in natural antioxidants or various bioactive compounds, including γ-oryzanol, phytosterols, sterol esters, triterpene alcohols, tocopherols, tocotrienols and other phenolic compounds (Patel and Naik, 2004). These minor compounds have been cited in the scientific literature as powerful antioxidant agents that are effective in preventing degenerative diseases (Lerma-García *et al.,* 2009). The antioxidants of RBO have the potential use as additives to improve the storage stability of foods (Iqbal *et al.,* 2005).

Several studies have reported the potential of RBO. Therefore, this study was directed at optimizing the extraction process. In recent years, the oil industry has shown increased interest in alternative solvents because of environmental and safety concerns. Ethanol has gained attention as a potential solvent for vegetable oils (Rodrigues *et al.,* 2010). As a result of study before, ethanol was feasible to use for RBO extraction by maceration method (Mas'ud *et al.,* 2019), furthermore, Mas'ud *et al.* (2020) reported that ethanol has proven as an effective and efficient solvent for extracting oil with high yield, and it feasible to be applied on the extraction of mango seed kernel oil (Mas'ud *et al*., 2021). Ethanol has been widely applied as a viable solvent due to its ease of recovery and low cost in an application and is classified as an environmentally friendly green solvent (Péres *et al.,* 2006). Green solvents have several benefits such as biodegradability, low toxicity, non-flammability, and renewability, making them potential candidates in separation/extraction science (Mas'ud *et al.,* 2017).

Do *et al*. (2014) reported that ethanol is a good solvent for polyphenols, and in this work, the feasibility of ethanol as a solvent for RBO on reflux method was investigated from the perspective of the extracted oil, γ-oryzanol, and polyphenols. Previous studies showed that temperature, time, and solvent were the main parameters affecting the extraction yield from different sources (Minjares-Fuentes *et al.,* 2014). These parameters also

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eISSN: 2550-2166
Published by Rynnye Lyan Resources

had a significant influence on the endogenous bioactive compounds, such as certain from the TPC (Rodrigues *et al.,* 2008; Chanioti *et al.,* 2016). Oliveira *et al.* (2012) have used ethanol in RBO extraction to study its effect on γ-oryzanol and tocols. It was observed that γ-oryzanol and tocols behave in different ways during the extraction process, and ethanol is feasible to obtain enriched oil when this renewable solvent is used.

Based on the advantages of ethanol as solvent, this study is directed to investigate the effect of temperature, time, and volume of ethanol on the oil yield, γ-oryzanol, and total polyphenol content (TPC) of RBO, and apply the reflux extraction method using RSM based CCD model to optimize the RBO extraction process, standardize, and analyses the models.

2. Materials and methods

2.1 Materials

Celebes rice, local rice of Indonesia, as samples were obtained from milling rice grain in a local grinding mill in Makassar, Indonesia from March to April 2021. Ethanol (wt.%) was purchased from a local chemical shop, g-oryzanol standard from Sigma-Aldrich Co. All chemicals were from Merck, Germany.

2.2 Preparation of rice bran and extraction oil

Freshly milled rice bran was directly collected from the milling system in polyethylene bags, the rice bran was screened through a 60-mesh sieve to have a uniform particle size and stabilized at autoclave (Hiclave HV-85 Hirayama) at 100°C for 15 mins for inactivating endogenous lipase. For extraction oil, each experimental unit weighed 100 g of rice bran in the reactor 1.0 L four neck flasks, RBO was extracted by reflux method using a heating mantle connected with the thermometer set, agitator on the top with the speed of 100 rpm, the residue was separated by centrifugation (refrigerated AX-521 centrifuge) at a speed of 3500 rpm for 20 mins. The liquid part is accommodated in the flask evaporator and ethanol as solvent is removed on a rotary evaporator Buchi R-215 incorporates vacuum Pomp V-700. RBO obtained was packaged in a dark glass bottle and stored in a freezer for analysis. The percentage of oil yield was calculated by equation (1) according to Sani (2014):

$$Oil\ yield\ (\%) = \frac{\text{RBO (g)}}{\text{Rice bran sample (g)}} \times 100 \qquad (1)$$

2.3 Analysis of γ-oryzanol

Preparation of γ-oryzanol standard: 0.05 g of the pure γ-oryzanol was dissolved in ethyl acetate in a 100 mL flask, and diluted to respectively 100, 200, 300, 400, and 500 ppm at a 50 mL flask, homogenized by vortex, inserted in the vial GC-MS (gas chromatography-mass spectrometry). Preparation of sample of RBO for γ-oryzanol analysis: 0.012 g RBO dissolved in 2 mL ethyl acetate, homogenized by vortex, and inserted into the vial bottle GC-MS. Quantification of γ-oryzanol of RBO: γ-oryzanol of RBO was performed on a GC-MS QP2010 by Shimadzu equipped with a split/splitless injector. Separations were achieved using a Rxi SH-5Sil MS capillary column (30 m, 0.25 mm ID, 0.25 mm film thickness). Helium was used as the carrier gas at flow rates of 14.0 mL/min and a split ratio of 1:10. The oven temperature was programmed at 110°C for a hold of 2 mins and increased to 200°C at a rate of 10°C/min and hold at the final temperature for 9 mins. LabSolutions software was used to control the operation of GC-MS. MS spectra were obtained at range width m/z 40-450, interface temperature 280°C, and ion source temperature 200°C. γ-oryzanol of RBO peaks was identified by comparing their retention time and equivalent chain length with respect to the standard.

2.4 Determination of total phenolic content

The analysis method of TPC according to Brand-Williams *et al.* (1995), 0.25 mL RBO was mixed with 2 mL of 10% Folin Ciocalteau reagent and 1.6 mL of 7.5% Na_2CO_3 and left at room temperature for 30 mins. The mixing solution was measured absorbance at 750 nm by ultraviolet-visible spectrophotometer (UV 1800, Shimadzu, Japan). Gallic acid was used as a standard and the TPC were expressed as mg gallic acid equivalents (GAE).g^{-1}.

2.5 Experimental design and statistical analysis

The study was conducted using RSM to optimize the temperature (°C) (X_1), time (h) (X_2), and volume of ethanol (mL) (X_3) to maximize the oil yield (%) (Y_1), γ-oryzanol (mg. L^{-1}) (Y_2), and TPC (mg GAE.g^{-1}) (Y_3) of RBO as responses. The middle values were determined based on the results of the preliminary study, i.e., 80°C, 1.5 hrs, and 450 mL ethanol, respectively. The lower and upper limits for each treatment were 70°C and 90°C for X_1, 1 hr and 2 hrs for X_2, 350 mL and 550 mL ethanol for X_3 (Table 1). The CCD consists of 14 experimental units and 6 replications of the centre point. Replication of the centre point was aimed to evaluate the pure error variance as the experimental error and to control the adequacy of the model. To estimate the coefficients of the response function and predict the system's responses, analysis of the experimental results of CCD was realized using empirical second-order polynomial equations as follows Amiri *et al*. (2018).

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

https://doi.org/10.26656/fr.2017.7(2).011

Where Y is a response, β_0 denotes the constant-coefficient; X_i and X_j the independent factors, β_i, β_{ii}, and β_{ij} the regression coefficients for the linear, quadratic, and interaction effects, respectively; k the number of variables; and e stands for the statistical error occurring to response Y.

Table 1. Five levels of independent variables of the CCD

Independent variables	Star low	Low	Centre	High	Star high
	-1.68	-1	0	1	1.68
X_1 (°C)	63	70	80	90	97
X_2 (h)	0.7	1	1.5	2	2.3
X_3 (mL)	282	350	450	550	619

Statistic software Design-Expert 10 was used to design, analyze, and optimize experimental models. Analysis of variance (ANOVA) was used to validate the statistical significance of the parameters that influence the responses, and the quality of the predicted model (Mohammed *et al.,* 2018). The coefficient of determination (R^2) shows the total predictive performance of the model, it represented the validity and fit quality of the model's quadratic polynomials. A *p*-value of ≤0.05 at a confidence level of 95% and an *F*-value of the Lack-of-Fit test were used for statistical analysis to evaluate the significance of model statistics (Tan *et al.,* 2017).

3. Results and discussion

3.1 Model fitting

Based on the correlation coefficient value, the quality of the model's responses can be evaluated. According to the ANOVA of Y_1, Y_2, and Y_3, the R^2 values of Y_1, Y_2, and Y_3 were 0.94, 0.93, and 0.94, respectively (Table 2). The value of R^2 close to 1 indicates that the models adequately represented the real relationship between the parameters chosen and indicated a reasonable adjustment of the model to experimental data. R^2 is more than 80% implying that the regression model shows good fit (Singh *et al*., 2018), the model will be significant at a 95% confidence interval if the *F* test has a *p*-value of less than 0.05. In the case of lack of fit ($p > F$), the *p*-value is greater than 0.05 showing the failure of the model in finding data points in the experimental domain. The significance of different terms of each coefficient was determined using the *F*-value and *p*-value. According to Yolmeh *et al*. (2014), a large *F*-value and a small *p*-value would imply a more significant effect on the corresponding response variable. According to Li *et al*. (2014), the model is important and can be used to navigate the design domain.

3.2 Interpretation result of analysis of variance

3.2.1 Oil yield

The result of ANOVA for the response surface quadratic model of Y_1 showed that the *F*-value model of 17.04 implies the model is significant (Table 3). There is only a 0.01% chance that an *F*-value this large could occur due to noise. Values of Prob > F of < 0.0001 indicate that the model terms are significant, in this case, the linear term of temperature (X_1), the linear term of time (X_2), the linear term of ethanol volume (X_3), the quadratic terms of temperature (X_1^2), and the quadratic terms of time (X_2^2) are significant model terms. On the other hand, values Prob > *F* greater than 0.1000 indicate the variable terms are not significant, in this case, the quadratic terms of ethanol volume (X_3^2), the interaction between temperature and time (X_1X_2), the interaction between temperature and volume of ethanol (X_1X_3), and the interaction between time and volume of ethanol (X_2X_3) did not give any significant contribution on Y_1.

The Lack of Fit *F*-value of 1.26 implies the Lack of Fit is not significant relative to the pure error. Non-significant lack of fit is good, which indicates that the model is suitable to describe the effect of variables for Y_1 and that the model is adequate for predicting the response. According to Bas and Boyaci (2007), the model will be considered appropriate if the lack of fit value model is not significantly different at the level of specific α. A model will be well fitted to the experimental data if it presents a significant regression and a non-significant lack of fit (Bezerra *et al.*, 2008).

Y_1 was calculated at about 16.38%. The value is comparable to those findings by Anwar *et al.* (2005) of about 15-20% and Pourali *et al.* (2009) of about 10-26% but slightly higher than reported by Mas'ud *et al*. (2019) of about 14.47%. Mas'ud *et al*. (2017) reported the effects of extraction time, temperature, and volume of solvent on mango seed kernel oil above room temperature had a significant effect on oil yield.

3.2.2 γ-Oryzanol of rice bran oil

According to the ANOVA of Y_2, the *F*-value model

Table 2. Y_1, Y_2, and Y_3 of RBO in terms of actual factors

	R^2	a	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	X_1^2	X_2^2	X_3^2
Y_1 (%)	0.94	-53.87	1.22	9.48	0.05	-0.01	-2.56	-3.68	-6.62	-1.98	-2.03
Y_2 (mg.L^{-1})	0.93	-1005.08	17.5	227.72	1.18	0.10	-1.35E-003	-0.17	-0.1	-47.43	-8.20E-004
Y_3 (mg GAE.mg^{-1})	0.94	-6.03	0.07	2.40	0.01	-0.01	2.45E-005	-6.78E-004	-2.35E-004	0.29	-9.21

https://doi.org/10.26656/fr.2017.7(2).011

Table 3. ANOVA for the response variables

Source	df	Oil Yield		γ-oryzanol		TPC	
		SS	*p-value*	SS	*p-value*	SS	*p-value*
Model[a]	9	18.58	6.11E-05	7070.22	0.0001	0.34	5.20E-05
X_1	1	0.64	0.0445	826.35	0.0029	0.04	0.0013
X_2	1	2.87	0.0006	979.54	0.0016	0.02	0.0222
X_3	1	5.11	6.93E-05	894.09	0.0022	0.05	0.0009
X_1X_2	1	0.03	0.6294	2.17	0.8451	0.04	0.0012
X_1X_3	1	0.53	0.0639	14.61	0.6145	0.004	0.1655
X_2X_3	1	0.27	0.1662	582.94	0.0082	0.01	0.0648
X_1^2	1	6.32	2.85E-05	1491.02	0.0004	0.01	0.082
X_2^2	1	3.52	0.0003	2026.42	0.0001	0.07	0.0001
X_3^2	1	0.59	0.0516	969.98	0.0017	0.12	1.90E-05
Residual	10	1.21		540.58		0.02	
Lack of Fit[b]	5	0.68	0.4027	327.086	0.3255	0.01	0.8565
Pure Error	5	0.54		213.49		0.02	
Cor Total	19	19.79		7610.79		0.361207	

[a] Significant, [b] Non-significant, X_1: Temperature (°C), X_2: Extraction time(h), X_3: Volume of ethanol (mL)

of 14.53 implies the model is significant. Values of Prob > F of 0.0001 (less than 0.0500) indicate model terms are significant. Variables that have a significant effect on Y_2 were X_1, X_2, X_3, X_2X_3, X_1^2, X_2^2, and X_3^2. Values greater than 0.1000 indicate the model terms are not significant, and that X_1X_2 and X_1X_3 did not give any significant contribution to γ-oryzanol concentration. The Lack of Fit *F*-value of 1.53 implies the Lack of Fit is not significant relative to the pure error. This indicates that the model is suitable to describe the effect of a parameter observed on γ-oryzanol and that the model is adequate for predicting the response.

γ-oryzanol was extracted at about 783.65 mg. L^{-1}. The value is comparable to those findings by other studies. Arab *et al.* (2011) reported approximately 0.9-2.9% (9.000 - 29.000 mg.L^{-1}), 1.5 - 2.9% (15.000 – 29.000 mg.L^{-1}) reported by Krishna *et al.* (2001), even up to 3.000 mg.kg^{-1} reported by Shin *et al.* (1997), and 119.750–281.950 mg.kg^{-1} reported by Sukanya *et al.* (2017). According to Iqbal *et al.* (2005), the exact composition of γ-oryzanol depends on the rice cultivars. Furthermore, according to Butsat and Siriamornpun (2010), the content of γ-oryzanol in rice is affected by the variety and growing conditions, as the antioxidant component will respond differently to environmental changes.

3.2.3 Total phenolic content for rice bran oil

Based on the ANOVA of Y_3 showed that the *F*-value model of 17.66, value implies the model is significant. Values of Prob > *F* of 0.0001 (less than 0.0500) indicate model terms are significant. In this case, X_1, X_2, X_3, X_1X_2, X_2^2, and X_3^2. Values greater than 0.1000 indicate the model terms are not significant, therefore X_1X_2 and X_1X_2 did not give any significant contribution to Y_3. The Lack of Fit F-value of 1.53 implies the Lack of Fit is not significant relative to the pure error, this indicates that the model is suitable to describe the effect of a variable observed for Y_3 and that the model is adequate for predicting Y_3. This study obtained a TPC of RBO of 1.02 mg GAE.g^{-1}, this value is in accordance with Bopitiya and Madhujith (2014), they are reported that RBO contains TPC of about 0.8931- 1.2239 mg GAE/mg of bran.

3.3 Interpretation of response surface and contour plots

Based on the ANOVA and the fitted models, the responses surfaces were generated by the model for extraction of Y_1, Y_2, and Y_3 as responses. To gain a better understanding of this study, related to the effect of observed variables on the responses, then the predicted models are presented as the 3D plot (Figures 1-3). Response surface plot is a representation of the surface plot in 3-D space as the plot determining optimum operating conditions reaching maximum from the best-fitted model. These plots are obtained by depicting two variables within the experimental range and keeping the third variable at a constant level. According to Bezerra *et al*. (2008), a two-dimensional representation of a three-dimensional plot can be explained. Thus, if there are three or more variables, the plot visualization is possible only if one or more variables are set at a constant value.

Figure 1 shows a 3D plot corresponding to the effect of X_1 and X_2 on Y_1 at the fixed of X_3. Y_1 had a maximum point, and the effect of X_2 is stronger than the effect of X_1 on increasing Y_1. In the 3D plot curve, the curve at X_2 is more convex than the curve at X_1, meaning that a small change in X_2 has greatly affected the acquisition of

https://doi.org/10.26656/fr.2017.7(2).011

Y_1 compared to the change in X_1. It is evident in the coefficient variable of X_1 and X_2, where the coefficient variable of $X_2 > X_1$, and it is evident in the coefficient estimate of the ANOVA, where the coefficient variable in terms of actual factor of X_2 (9.48) is higher than X_1 (1.21). On the effects of X_1 and X_3 on increasing Y_1 at a fixed of X_2 (1.6 h), the coefficient variable in terms of the actual factor of X_1 (1.21) is higher than X_3 (0.61), whereas the effects of X_2 and X_3 on increasing Y_1 at a fixed of X_1, the coefficient variable in term of the actual factor of X_2 (9.48) is higher than X_3 (0.61).

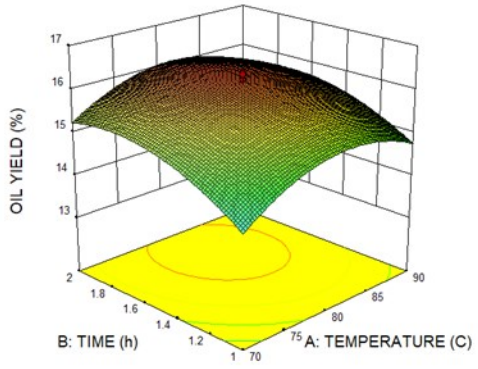

Figure 1. Response surface 3D plot the effect of X_1 and X_2 on Y_1 at the fixed of X_3

Figure 2 shows a 3D plot corresponding to the effect of X_1 and X_2 on Y_2 at the fixed of X_3. It can be seen that Y_2 had a maximum point, and the effect of X_2 is stronger than the effect of X_1 on increasing Y_2. In the 3D plot curve, can be seen that the curve at X_2 is more convex than the curve at X_1, meaning that a small change in X_2 has greatly affected the acquisition of Y_2 compared to X_1. It's evident in the coefficient variable of X_1 and X_2, where the coefficient variable of $X_2 > X_1$, and it is evident in the coefficient estimate of ANOVA, where the coefficient variable in terms of actual factor of X_2 (8.47) is higher than X_1 (7.78). On the effects of X_1 and X_3 on increasing Y_2 at a fixed of X_2, the effect of X_2 is stronger than the effect of X_1 on increasing Y_2, the coefficient variable in terms of actual factor of X_1 (7.78) is lower than X_3 (8.09), whereas the effects of X_2 and X_3 on increasing Y_2 at a fixed of X_1, the effect of X_2 is stronger than the effect of X_3 on increasing Y_2, the coefficient variable in term of the actual factor of X_2 (8.47) is higher than X_3 (8.09).

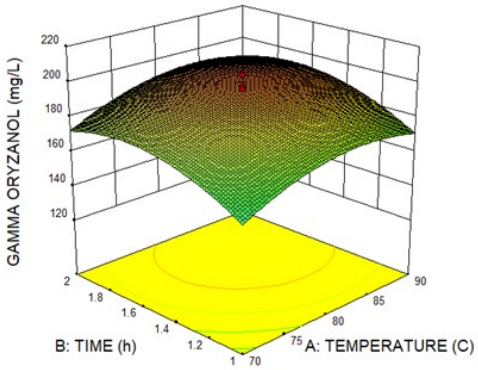

Figure 2. Response surface 3D plot the effect of X_1 and X_2 on Y_2 at the fixed of X_3

Figure 3 shows a 3D plot corresponding to the effect of X_1 and X_2 on Y_3 at the fixed of X_3. It can be seen that Y_3 had a maximum point, and the effect of X_2 is stronger than the effect of X_1 on increasing Y_3. A 3D plot curve can be seen that the curve X_2 is more convex than the curve X_1, meaning that a small change in X_2 has greatly affected the acquisition of Y_3 compared to X_1, it is evident in the coefficient variable of X_1 and X_2, where the coefficient variable of $X_2 > X_1$, and it is evident in the coefficient estimate of ANOVA, where the coefficient variable in term of the actual factor of X_2 (0.03) is higher than X_1 (0.06). On the effects of X_1 and X_3 on increasing Y_3 at a fixed of X_2, can be seen that Y_3 had a maximum point, and the effect of X_1 is lower than the effect of X_3 on increasing Y_3. At a 3D plot curve, can be seen that curve X_3 is more convex than the curve X_1, meaning that a small change in X_3 has greatly affected the acquisition of Y_3 compared to X_1, its evident in the coefficient variable of $X_1 < X_3$, and it is evident in the coefficient estimate of ANOVA, where the coefficient variable in term of the actual factor of X_1 (0.05) is lower than X_3 (0.06), whereas of the effects of X_2 and X_3 on increasing Y_3 a fixed of X_1, the coefficient variable in term of the actual factor of X_2 (0.03) is lower than X_3 (0.06).

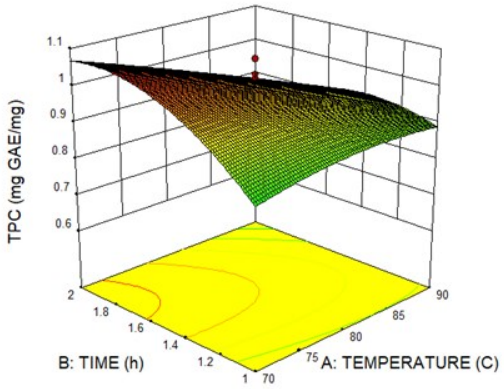

Figure 3. Response surface 3D plot the effect of X_1 and X_2 on Y_3 at the fixed of X_3

3.4 Interpretation of the optimum conditions and verification

According to ANOVA, the optimum of X_1, X_2, and X_3 to obtain the maximum of Y_1, Y_2, and Y_3 can be achieved at X_1 of 78.7°C, X_2 of 1.6 hrs, and X_3 of 496.5 mL, under the conditions of the extraction process, the predicted values of Y_1, Y_2, and Y_3 were 16.4%, 195.49 $mg.L^{-1}$, and 1.019 mg $GAE.g^{-1}$, respectively. It can be explained that an increase X_1 of 70°C to 78.7°C, X_2 of 1 h to 1.6 hrs, and X_3 of 350 mL to 496.5 mL at fixed X_1 of 80°C, X_2 of 1.5 hrs, and X_3 of 450 mL cause an increase Y_1, Y_2, and Y_3. Further, the addition of X_1 of 78.7°C up to 90°C, X_2 of 1.6 hrs up to 2 h, and X_3 of 496.5 mL up to

https://doi.org/10.26656/fr.2017.7(2).011

550 mL did not cause an increase in Y_1, Y_2, and Y_3.

Loypimai *et al.* (2020) have noted an increase in extraction temperature caused an increase in the extraction of γ-oryzanol. Previously, Srisaipet and Nuddagu (2014) reported that the amount of γ-oryzanol did not vary significantly at a temperature lower than 120°C, a decrease in γ-oryzanol concentration above 120°C due to the degradation of γ-oryzanol in the oil. According to Nystrom *et al*. (2007), the good heat stability of RBO has been attributed to its high content of steryl ferulates and tocopherols present in the oil. Related to TPC, Ghafoor *et al.* (2019) reported that the TPC of fruits showed a significant decrease during the heating process at 60, 80, 90, 110, and 130°C. Vergara-Salinas *et al.* (2012) reported that the TPC was detected at 100°C and 5 mins, higher temperatures and longer exposure times reduced extract polyphenol diversity.

For verification models, the laboratory scale has been carried out by conducting triplicate experiments using the recommended/predicted values of X_1, X_2, and X_3 from the Design Expert 10 software. The result showed that the average of Y_1, Y_2, and Y_3 were 16.1±0.66%, 189.12±1.18 mg.L^{-1}, and 0.91±0.54 mg GAE.g^{-1}, respectively. The RBO produced in the verification process showed that the average value of Y_1, Y_2, and Y_3 was close to the predicted values of the response, although slightly lower than the predicted values of the response. These results indicate that the reflux method of RBO extraction with ethanol as the "green solvent" is feasible for industrial application.

4. Conclusion

The effect of temperature, time, and volume of ethanol as a solvent on the oil yield, γ-oryzanol, and TPC of RBO with apply reflux extraction method have been studied and proven to be able to produce RBO with high oil yield, γ-oryzanol, and TPC. The maximum oil yield = 16.4%, γ-oryzanol = 195.49 mg.L^{-1}, and TPC = 1.02 mg GAE.g^{-1} were achieved under the optimum conditions process of temperature, time, and volume of ethanol were 78.7°C, 1.6 hrs, and 496.5 mL ethanol/100 g rice bran, respectively. The average oil yield, γ-oryzanol, and TPC on verified models were 16.1±0.66%, 189.12±1.18 mg. L^{-1}, and 0.91±0.54 mg GAE.g^{-1}, respectively. This result indicates that ethanol is very promising for RBO extraction, as an effort to apply the concept of "green" solvents that are safe for humans and the environment. The optimization of the RBO extraction process has resulted in an extraction model and has been verified on a laboratory scale with satisfactory results. The predicted value of oil yield, oryzanol, and TPC close to the value of the verification results have proven that this RBO extraction model is quite valid and feasible to be applied to the RBO extraction process in the industry.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We would like to thank the Directorate General of Higher Education, Ministry of Education Indonesia, for their research grand No. B/549/PL10/PT.01.05/2021, and the Chemical Engineering Department, Politeknik Negeri Ujung Pandang, Makassar, Indonesia.

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