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Model development to enhance the solvent extraction of rice bran oil

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Abstract – Rice bran oil (RBO) extraction with ethanol using maceration method accompanied by stirring has been optimized using response surface methodology (RSM) based on central composite design (CCD). Experiments were conducted to investigate the influence of extraction time, ethanol concentration, and ethanol volume on the oil yield, γ-oryzanol, and vitamin E of RBO as the response. The experiment consisted of twenty units including six replicates of the center points. The data were analyzed using Design-Expert 10 software to develop and evaluate models and to plot the response curve as 3D surfaces. The result showed that the maximum of the oil yield, γ-oryzanol, and vitamin E of RBO was achieved under the optimum conditions of $x_1 = 5.30$ h, $x_2 = 89.21\%$ and $x_3 = 686.66$ mL (50 g rice bran), respectively. Maximum of the response under these conditions was 14.47%, 783.65 $mg.L^{-1}$, and 127.01 $mg.L^{-1}$, respectively. This study has resulted in the development of a model for RBO extraction using ethanol as solvent, it is feasible to be applied to the RBO industry with an efficient process, as well as an implementation of the "green" solvent concept.

Keywords: maceration / edible oil / ethanol / green solvent / response surface methodology

Résumé – **Développement d'un modèle pour améliorer l'extraction solvant de l'huile de son de riz.** L'extraction de l'huile de son de riz (rice bran oil, RBO) à l'éthanol par la méthode de la macération accompagnée d'une agitation a été optimisée à l'aide de la méthode RSM (*response surface methodology*) basée sur un plan d'expérience de type composite centré (*central composite design*, CCD). Des expériences ont été menées pour étudier l'influence du temps d'extraction, de la concentration en éthanol et du volume d'éthanol sur le rendement en huile, en γ-oryzanol et en vitamine E de l'huile de son de riz. L'expérience consistait en vingt essais comprenant six réplicats des points centraux. Les données ont été analysées en utilisant le logiciel Design-Expert 10 pour développer et évaluer les modèles et pour tracer la courbe de réponse sous forme de surfaces 3D. Le résultat a montré que le maximum de rendement en huile, γ-oryzanol et vitamine E de l'huile de son de riz a été atteint dans les conditions optimales de $x_1 = 5,30$ h d'extraction, $x_2 = 89,21\%$ d'éthanol et $x_3 = 686,66$ mL de solvant (50 g de son de riz). La réponse maximale dans ces conditions était de 14,47 % d'huile, 783,65 $mg.L^{-1}$ d'γ-oryzanol et 127,01 $mg.L^{-1}$ de vitamine E. Cette étude a abouti à la mise au point d'un modèle d'extraction d'huile de son de riz utilisant l'éthanol comme solvant ; le process pourrait être appliquée à l'industrie de l'huile de son de riz de manière efficace et en répondant au concept de solvant « vert ».

Mots clés : macération / huile alimentaire / éthanol / solvant vert / méthodologie de surface de réponse

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1 Introduction

RBO is edible oil extracted from rice bran which is a by-product of rice mills. RBO is superior among the other edible oils because it contains unique antioxidants and nutraceutical complexes present in its composition (Oliveira *et al.*, 2012). In the unsaponifiable fraction of RBO contains γ-oryzanol and tocols. These compounds have been reported in the scientific literature as powerful antioxidant agents that are effective for preventing degenerative diseases (Lerma-García *et al.*, 2009). Seema (2015) suggests that bran antioxidants are mainly γ-oryzanol and vitamin E, as well as unsaturated fats capable of lowering cholesterol. γ-oryzanol component of RBO was first presumed to be a single component, but later it was determined to be a fraction containing ferulate (4-hydroxy-3-methoxy cinnamic acid) esters of triterpene alcohols and plant sterols (Rogers *et al.*, 1993). Cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, and campesteryl ferulate are the three major components of γ-oryzanol (Xu *et al.*, 2001).

On the other hand, the most important contribution of vegetable oils is their tocopherol content, which is generally collectively referred to as "vitamin E. Vegetable oils contain high concentrations of vitamin E (Bauernfeind and Desai, 1977) and can provide the most daily requirement of vitamin E (Desai *et al.*, 1980). High vitamin E levels in rice bran oils are reported to have antihypocholesterolemic, anticancer, and neuroprotective properties. Tocols are capable of reducing lipid peroxidation and lipid risk factors, in this case increasing LDL cholesterol and platelet aggregation, exhibiting anti-inflammatory properties, demonstrating anti-carcinogenic and cardiovascular protection effects (Tiwari and Cummins, 2009).

Some scientific reports on the benefits of RBO for health have led this study to evaluate the optimization of the extraction process of RBO. The direct solvent extraction method, which does not require specific extraction instrumentation, has been most commonly used (Chen and Bergman, 2005), and ethanol has gained attention as a potential solvent for vegetable oils (Rodrigues and Oliveira, 2010). Study on sesame oil revealed that the polar solvent such as ethanol was a good solvent compared to non-polar solvents. According to Péres *et al.* (2006), these results could be explained by the interaction between the unsaturated fatty acids with a polar solvent, compared with non-polar solvents. In fact, the oils extracted with ethanol presented the typical composition of RBO (Firestone, 1999). Ethanol has attractive advantages to use as a solvent, including low toxicity, good operational security, as well as being obtained from a bio-renewable source (Bessa *et al.*, 2017). The choice of ethanol as a solvent is deemed necessary to implement the concepts and principles of green extraction.

According to Chemat *et al.* (2012), green extraction is a new concept to protect both the environment and consumers and in the enhanced competition of industries to be more ecologic, economic, and innovative. Within the green extraction approach, the concept of the green extract is an extract obtained in such a way have the lowest possible impact on the environment. Because of environmental concerns, a suitable solvent which gives less impact to the environment is more preferred nowadays. Further, according

to Tekin *et al.* (2018), ethanol has been widely applied as a viable solvent due to their ease of recovery and low cost in an application and classified as an environmentally friendly green solvent. Although alcohols such as ethanol, methanol and isopropyl alcohol have similar solvent properties, ethanol has become the foremost among others because of its non-toxic nature. Li *et al.* (2016) stated that the green solvents have several benefits such as biodegradability, low toxicity, non-flammability, and renewability making them potential candidates in separation science.

Many studies have proven the technical feasibility of employing ethanol in the process of extraction oils (Saxena *et al.*, 2011). On oil extraction from sunflower collets, ethanol gave a higher yield of extracted material, the content of oil phase was similar to that obtained when n-hexane is used. When ethanol was used, about 70% less crystallizable waxes and about 38% more tocopherols and phospholipids were extracted, this shows the feasibility of using ethanol as an alternative solvent to hexane in extracting oil (Baümler *et al.*, 2016), including RBO (Oliveira *et al.*, 2012; Rodrigues and Oliveira, 2010). Previously, Imsanguan *et al.* (2008) have reported that ethanol was a better solvent for γ-oryzanol fraction compared to hexane. It can be explained by the relatively high polarity of the γ-oryzanol molecule (consisting of triterpene alcohols and phytosterols esterified with ferulic acid), where the polarity of the solvent may significantly affect the extractability of γ-oryzanol (Xu and Godber, 2001).

Based on the results of the existing study on the RBO, and that the optimization of the extraction process is very much related to production costs, as well as considering the needs of the RBO extraction industry for the pharmaceutical and food industries related to the optimization of the extraction process, then this research was conducted. To the best of the author's knowledge, this is the first study, RBO extraction uses ethanol and monitors the extraction time, ethanol concentration, and volume of ethanol on oil yield, γ-oryzanol, and vitamin E of RBO in the maceration method accompanied by stirring, and applies RSM to optimize the RBO extraction process, standardize, and analyze the resulting model.

2 Materials and methods

Ciliwung rice, local rice of Indonesia, as samples were obtained from milling rice grain in a local grinding mill Makassar Indonesia during March to April in 2017. Ethanol (wt.%) purchased from a local chemical shop, γ-oryzanol standard from Sigma-Aldrich Co and α-tocopherol standard from Sigma, St. Louis, USA. Ethyl acetate, methanol, and chloroform from Merck, Germany.

2.1 Preparation of rice bran and extraction oil

Freshly milled bran samples were 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 min for inactivating endogenous lipase. For extraction oil, each experimental unit weighed 50 g of rice bran in the Erlenmeyer 1000 mL, RBO was extracted using maceration method with stirring under the 30 °C of room

Table 1. Five levels of independent variables of central composite design.

Independent variables	Star low (−1.68)	Low (−1)	Centre (0)	High (+1)	Star high (+1.68)
Extraction time (h)	3.32	4	5	6	6.68
Ethanol concentration (wt.%)	69.18	76	86	96	100
Ethanol volume (mL)	431.82	500	600	700	768.18

temperature, and pulp was separated by centrifugation (refrigerated AX-521 centrifuge) at a speed of 3500 rpm for 20 min. The liquid part was accommodated in the evaporator flask. Then, the solvent was removed in a Buchi R-215 rotary evaporator equipped V-700 vacuum Pomp speed of 60 rpm, the heating temperature was of 35 °C, and the evaporation temperature was of 21 °C. RBO was packaged in a dark glass bottle and stored in a freezer before analysis. The percentage of oil yield was calculated as follows (Sani, 2014):

$$\text{Oil yield (\%)} = \frac{\text{Weight of oil extracted (g)}}{\text{Weight of sample (g)}} \times 100. \quad (1)$$

2.2 Analysis of γ-oryzanol and vitamin E

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 vitamin E standard: 0.05 g of the pure vitamin E was dissolved in methanol-chloroform (1:1) in a 100 mL flasks, and diluted to respectively 100, 200, 300, 400, and 500 mg.L^{-1} at a 50 mL flask, homogenized by vortex, inserted in the vial GC-MS. 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. Preparation of sample of RBO for vitamin E analysis: 0.012 g RBO dissolved in 2 mL methanol-chloroform (1:1), homogenized by vortex and inserted into the vial bottle GC-MS.

Quantification of γ-oryzanol and vitamin E of RBO: γ-oryzanol and vitamin E of RBO were performed on a GC-MS QP2010 by Shimadzu equipped with a split/splitless injector. Separation were achieved using a Rxi SH-5Sil MS capillary column (30 m, 0.25 mm ID, 0.25 μm 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 min and increased to 200 °C at a rate of 10 °C/min and hold at the final temperature for 9 min. LabSolution 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 and vitamin E of RBO peaks were identified by comparing their retention time and equivalent chain length with respect to the standards.

2.3 Experimental design for optimization and statistical analysis

RSM was employed to optimize the parameters of time, ethanol concentration, and ethanol volume for oil yield, γ-oryzanol, and vitamin E maximum of RBO on extraction process by maceration method accompanied by stirring. RSM provides the optimal conditions of a process, using multivariate statistical techniques to obtain responses from the observed process variables. RSM consists of mathematical and statistical techniques based on polynomial equations that best suit experimental data to describe the behavior of data sets with the aim of obtaining statistical predictive values (Rafi *et al.*, 2015). It is a rapid analytical approach and more economical (Banga and Tripathi, 2009). Nowadays, the procedures based on the statistical evaluation using RSM are widely used to determine the interaction between the factors that influence reactions for optimization.

The CCD is one of the most effective RSM designs, which are widely used in the study and optimization of the linear, quadratic, and interaction effects of variables on observed responses (Soundararajan *et al.*, 2016). The CCD is still the symmetrical second order experimental design most utilized for the development of analytical procedures (Bezerra *et al.*, 2008). Three independent variables examined in this study were time (h) (x_1), ethanol concentration (%) (x_2), and ethanol volume (mL) (x_3) while the oil yield, γ-oryzanol, and vitamin E concentration were considered as response variables. The value of independent variables was determined based on the results of the preliminary study. The middle values for the independent variables were 5 h, 86%, and 600 mL, respectively. The lower and upper limits for each treatment were 4 and 6 h for time, 76 and 96% for ethanol concentrations, 500 and 700 mL for ethanol volume (Tab. 1). The CCD consists of 14 experimental points and 6 replications of the center point. Replication of the center point was aims 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; Keshtegar *et al.*, 2018).

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

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 (Awad *et al.*, 2017).

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 (Chan *et al.*, 2017; 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. R^2 values are close to 1, indicating a reasonable adjustment of the model to experimental

Table 2. Central composite design, showing coded values of independent variables, with observed of yield, γ-oryzanol, and vitamin E of RBO.

Exp.	Parameters			Results		
	Extraction time (h)	Ethanol concentration (%)	Ethanol volume (mL)	Oil yield (%)	γ-oryzanol concentration (mg.L^{-1})	Vitamin E concentration (mg.L^{-1})
1	−1	−1	−1	10.02	607.93	120.51
2	1	−1	−1	14.01	728.98	121.33
3	−1	1	−1	11.89	733.80	123.47
4	1	1	−1	10.73	747.09	123.02
5	−1	−1	1	12.12	698.88	121.74
6	1	−1	1	14.07	762.98	123.99
7	−1	1	1	16.05	755.86	122.45
8	1	1	1	13.99	783.93	124.76
9	−1.68	0	0	11.46	645.42	115.63
10	1.68	0	0	10.95	882.11	124.38
11	0	−1.68	0	11.53	654.11	117.66
12	0	1.68	0	14.33	450.27	123.60
13	0	0	−1.68	13.01	568.47	122.40
14	0	0	1.68	15.29	584.87	125.44
15	0	0	0	13.49	738.25	126.20
16	0	0	0	11.98	823.64	128.43
17	0	0	0	12.78	804.40	126.45
18	0	0	0	13.73	813.03	127.03
19	0	0	0	13.62	900.01	129.83
20	0	0	0	13.38	782.93	126.45

data. A p-value of ≤ 0.05 at a confidence level of 95% and a F-value of the lack-of-fit test were used for statistical analysis to evaluate the significance of model statistics, at a confidence level of 95% and a 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

Extraction of edible oil is generally a multi-parameter process so that the optimization of the process conditions represents a critical step in the development of the model. In the present study, RSM has been used as a tool to develop the model of RBO extraction apply maceration method with stirring to see the effect of x_1, x_2, and x_3 on the oil yield (%), γ-oryzanol (mg.L^{-1}), and vitamin E (mg.L^{-1}) of RBO. The effects of x_1, x_2, and x_3 on the yield of oil (%), γ-oryzanol (mg.L^{-1}), and vitamin E (mg.L^{-1}) of RBO have been studied during experimentation. The data results of 20 runs using a CCD showed that oil yield, γ-oryzanol, and vitamin E concentration of RBO ranged from 10.02 to 15.29%, 450.27 to 900.013 mg.L^{-1}, and 120.51 to 129.83 mg.L^{-1}, respectively (Tab. 2). The maximum of the oil yield, γ-oryzanol, and vitamin E concentration of RBO has been achieved at the optimum conditions of $x_1 = 5.30$ h, $x_2 = 89.21\%$ and $x_3 = 686.66$ mL (50 g rice bran), respectively. Maximum oil yield, γ-oryzanol, and vitamin E concentration of RBO under these conditions were 20.47%, 783.65 mg.L^{-1}, and 127.01 mg.L^{-1}, respectively. The experimental data were fitted with the second-order equation model suggested by Design Expert 10 software.

3.1 Model fitting

The quality of the model developed was evaluated based on the correlation coefficient value. According to the result of ANOVA for oil yield, γ-oryzanol, and vitamin E of RBO, the R^2 values of oil yield, γ-oryzanol, and vitamin E were 0.89, 0.83, and 0.84, respectively, indicating that the models adequately represented the real relationship between the parameters chosen. According to Singh *et al.* (2018), when R^2 is more than 80%, the regression model shows good fit. The significance of different terms of each coefficient was determined using the F-value and p-value. According to Yolmeh *et al.* (2014), 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. 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 which shows the failure of the model in finding data points in the experimental domain. The reduced quadratic model equation developed from the experimental data to predict the oil yield, γ-oryzanol, and vitamin E of RBO in terms of coded factors are given in equations (3–5), respectively.

$$\text{Oil yield}(\%) = 13.40 + 0.14x_1 + 0.52x_2 + 0.98x_3$$
$$- 1.14x_1x_2 + 0.66x_2x_3 - 0.69x_1^2, \quad (3)$$

$$\gamma-\text{oryzanol}\,(\text{mg.L}^{-1}) = 811.98 + 45.73x_1 - 8.85x_2 \\ + 15.48x_3 - 69.22x_2^2 - 60.57x_3^2, \quad (4)$$

$$\text{vitamin E}\,(\text{mg.L}^{-1}) = 126.60 + 1.44x_1 + 1.18x_2 \\ - 2.2x_1^2 - 1.99x_2^2, \quad (5)$$

where: x_1 = time; x_2 = ethanol concentration; x_3 = ethanol volume.

3.2 interpretation result of ANOVA

3.2.1 Extraction yield of RBO

The result of ANOVA for response surface reduced quadratic model of the oil yield showed that the model F-value of 9.99 implies the model is significant. Values of Prob > F of 0.0003 (less than 0.05) indicate model terms are significant. Values of Prob > F less than 0.05 indicate variable terms are significant, in this case, the linear term of ethanol concentration (x_2), the linear term of ethanol volume (x_3), the quadratic terms of extraction time (x_1^2), the interaction between extraction time and ethanol concentration (x_1x_2), and the interaction between ethanol concentration and ethanol volume (x_2x_3) are significant variables terms. On the contrary, values Prob > F greater than 0.1000 indicate the variable terms are not significant, in this case, the linear term of extraction time (x_1), the quadratic terms of ethanol concentration (x_2^2), the quadratic terms of ethanol volume (x_3^2), and interaction between extraction time and ethanol volume (x_1x_3) did not give any significant contribution on oil yield.

The lack-of-fit F-value of 1.69 implies the lack-of-fit is not significant related 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 the oil yield and that the developed model was 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. Further, according to Bezerra *et al.* (2008), a model will be well fitted to the experimental data if it presents a significant regression and a non-significant lack-of-fit. These values would give a relatively good fit to the mathematic model in terms of coded factors are given in equation (3).

In this study obtained a yield of RBO was 14.47% in agreement with the previous study. Anwar *et al.* (2005) has reported that the rice bran contained 15–20% oil, and 10–26% according to Pourali *et al.* (2009). The simplest method of solvent extraction is the single contact batch operation, where the solid to be leached and the solvent is mixed and the extract solution and raffinate solid phases are separated (Bessa *et al.*, 2017). Considering the time effect, it greatly affects the yield especially on the value of the mass transfer. The longer the contact time between the solvent with the solute during the extraction process, the more the number of elements extracted chemical content. The more time is given for contact of the sample with the solvent, the higher the extraction yield percentage (Elkhaleefa and Shigidi, 2015). The effect of solvent concentration on the yield was also explained by Chen *et al.* (2016), that the extraction yield was

increased with the increase of ethanol concentration in the extraction solvent.

The effect of volume of solvent is consistent with mass transfer principles. The driving force during mass transfer is the concentration gradient between the solid and the bulk of the liquid, which is greater when a higher solvent to solid ratio is used. Distribution of solvent to solids will greatly affect oil yield, the ratio solids with a solvent will affect the oil yield. The amount of solvent affects the wide contact between solids and solvents. The more solvent, the greater the contact area, so the distribution of the solvent will be even greater. Equitable distribution of solvent to solids increases the oil yields, the amount of solvent will reduce the saturation level of the solvent so that the desired component will be extracted perfectly. In general, the yield increased with increasing volume of the solvent used. The more the volume of solvent used, the greater the ability of a solvent to extract oil contained in the material. The increasing volume of solvent also leads to increasing contact time, which occurs between materials with a solvent.

In the extraction process, an optimum point will be reached. Mas'ud *et al.* (2017) conducted a study of the effects of extraction time, temperature, and volume of solvent in mango seed kernel oil extraction above room temperature, they reported that the variables had a significant effect on oil yield. Effect of the combination of reactions between variable time and temperature at high-level experimental process will obviously result in a decrease in oil yield. Similar phenomena have also been reported by Oniya *et al.* (2017) and Pichai and Krit (2015). A decrease in oil yield at high temperatures and long periods of time in oil extraction is thought to be a result of degradations of some oil components such as phenolic compounds degradations as reported by Chew *et al.* (2011). They conducted a study of the effects of ethanol concentration, extraction time, and extraction temperature on the recovery of phenolic compounds and antioxidant capacity, they were reported that the extraction time of 120 min was achieved the maximum concentration of phenolic compounds, and after this point, total phenolic content and thick tannin content were decreased. According to Chirinos *et al.* (2007), oxidation of phenolic compounds due to excessive oxygen exposure can occur if the extraction time is longer.

3.2.2 γ-Oryzanol concentration of RBO

The Model F-value of 4.62 implies the model is significant. Values of Prob > F of 0.0106 (less than 0.05) indicate model terms are significant. Variables that have a significant effect on γ-oryzanol concentration were the quadratic terms of ethanol concentration (x_2^2) and the quadratic terms of ethanol volume (x_3^2). Values greater than 0.1000 indicate the model terms are not significant so that the linear term of extraction time (x_1), ethanol concentration (x_2), and ethanol volume (x_3), the quadratic term of extraction time (x_1^2), the interaction between time and ethanol concentration (x_1x_2), the interaction between time and ethanol volume (x_1x_3), and the interaction between ethanol concentration and ethanol volume (x_2x_3) did not give any significant contribution on γ-oryzanol concentration. The reduced quadratic model equation in terms of coded factors was developed from the experimental data to predict the γ-oryzanol of RBO produced from ANOVA as given in equation (4). The coefficient values of variables x_1, x_2, and x_3
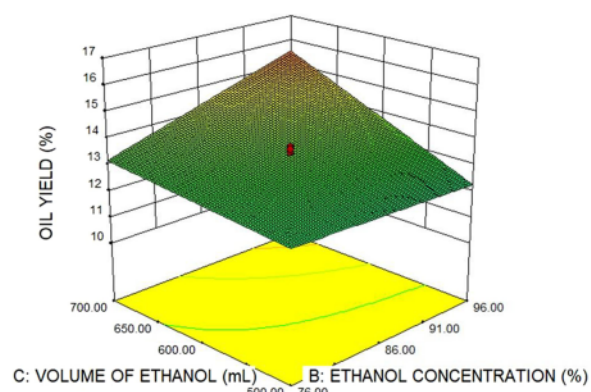
Fig. 1. Response surface plot of oil yield RBO at a fixed extraction time of 5 h.

still appear in the model because the Design Expert 10 software work system used was hierarchical, so that if the quadratic effect is significant then the linear effect will also appear in the model even though the linear effect is not significant based on the ANOVA results. The lack-of-fit F-value of 2.90 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 developed model is adequate for predicting the response.

In this study obtained a γ-oryzanol of 783.65 mg.L^{-1}. According to Arab *et al.* (2011), RBO contains about 0.9–2.9% of γ-oryzanol, 1.5 to 2.9% according to Krishna *et al.* (2001), 119.75–281.95 mg.g^{-1} oil according to Sukanya *et al.* (2017), 3.33 g.100g^{-1} according to Al-Okbi *et al.* (2014), even up to 3000 mg.kg^{-1} according to Shin *et al.* (1997). γ-oryzanol extracted with 3:2 chloroform: methanol mixture yielding 23.7–43.0 mg.g^{-1} in the crude RBO (Azrina *et al.,* 2008). According to Iqbal *et al.* (2005), the exact composition of γ-oryzanol depends on the rice cultivars. Furthermore, according to Butsat and Siriamornpun, (2010), that the content of γ-oryzanol in rice affected by the variety and growing conditions, as the antioxidant component will respond differently to environmental changes.

The effect of different solvent concentrations can produce different yields have been also explained by Cacace and Mazza (2003), that the change in concentration in the solvent will modify the physical properties of the solvent such as density, dynamic viscosity, and dielectric constant. The solubility of the compound will also be modified by changes in solvent concentration, and this can affect yield. According to Japon-Lujan *et al.* (2006), 80% aqueous ethanol (v/v) was the optimum solvent for extraction of the targeted phenolics from olive leaf and it can be used as a replacement of toxic solvents (methanol, diethyl ether, chloroform) to obtain bioactive phenols for human use. Further, according to Malik and Bradford (2008), extraction with 80% methanol (v/v) was reported as the most effective method for olive leaves polyphenols. Related to the amount of solvent, the ratio of solvents to solids used by researchers for extraction varies greatly from 4 to 100, but the ratio between 10 and 50 is mostly reported in the literature (Kiritsakis *et al.*, 2010).

3.2.3 Vitamin E concentration of RBO

The result of ANOVA for response surface reduced quadratic model of vitamin E showed that the model F-value of 10.86 implies the model is significant. Values of Prob $> F$ of 0.0002 (less than 0.05) indicate model terms are significant. In this case, the linear terms of extraction time (x_1), the linear terms of ethanol concentration (x_2), the quadratic term of extraction time ($x_1{}^2$), and the quadratic term of ethanol concentration ($x_2{}^2$). Values greater than 0.1000 indicate the model terms are not significant, so that the linear term of ethanol volume (x^3), the quadratic term of ethanol volume ($x_3{}^2$), the interaction between extraction time and ethanol concentration (x_1x_2), the interaction between extraction time and ethanol volume (x_1x_3), and the interaction between ethanol concentration and ethanol volume (x_2x_3) did not give any significant contribution on vitamin E concentration. The lack-of-fit F-value of 2.26 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 variable observed for the vitamin E and that the developed model is adequate for predicting the response.

In this study obtained a vitamin E of 127.01 mg.L^{-1}. According to Xu *et al.* (2007), RBO contains 0.37–1.84 mg. g^{-1} oil, 170–218 μg.g^{-1} according to Schramm *et al.* (2007) and 665 μg.g^{-1} according to Al-Okbi *et al.* (2014). Short-chain alcohols, especially ethanol and isopropanol, have been proposed as alternative extraction solvents due to their greater safety. Alcohols tend to extract more non-glyceride materials than n-hexane, due to their greater polarity. Typically, alcohol-extracted oils contain more phosphatides and unsaponifiable compounds (Lusas *et al.,* 1991). Further, Hu *et al.* (1996) reported that the average amount of vitamin E of RBO with isopropanol extraction was greater than that with hexane extraction and related to the amount of solvent, an increase in isopropanol-to-bran ratio (w/w) from 2:1 to 3:1 extracted 9.4% more crude RBO that contained 10% more vitamin E which explains that the amount of solvent affects the extraction of vitamin E. They also reported that the extraction time did not have a significant effect on the amount of RBO or vitamin E extracted.

3.3 Interpretation of response surface and contour plots

Based on the fitted model, the response surface and contour plots were generated by the model for extraction of oil yield, γ-oryzanol, and vitamin E of RBO as a response. In order to gain a better understanding of this study, related to the effect of observed variables on the response, then the predicted models are presented as the 3D plot (Figs. 1–3, respectively). 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 depicting two variables within 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. If there are three or more variables, the plot visualization is possible only if one or more variables are set to a constant value.
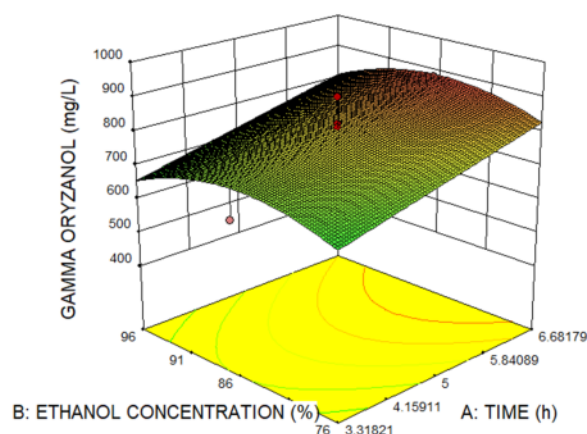
Fig. 2. Response surface plot of γ-oryzanol RBO at a fixed ethanol volume of 600 mL.

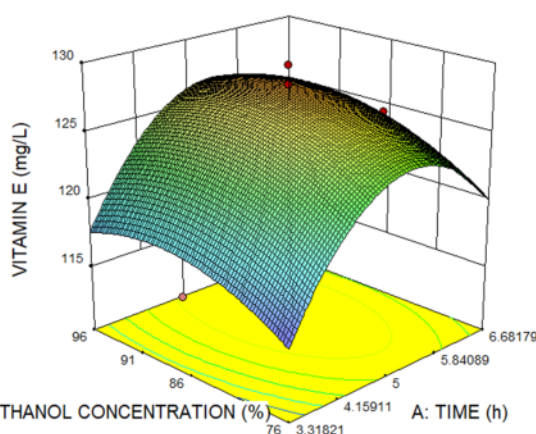**Fig. 3.** Response surface plot of vitamin E RBO at a fixed ethanol volume of 600 mL.

Figure 1 shows a 3D plot corresponding to the effect of x_2 and x_3 on oil yield at the fixed of x_1 (5 h). The effect of x_3 is stronger than the effect of x_2 on increasing the oil yield. It is evident in the coefficient variable of x_2 and x_3, where coefficient variable of $x_3 > x_2$, and it is evident in the coefficient estimate of the ANOVA, where the coefficient variable in term of actual factor of x_3 (0.98) is higher than x_2 (0.52), while the effect of x_1 is not significant in increasing the yield of the RBO, the estimated coefficient of x_1 is 0.14.

The plot that discloses the effects of x_1 and x_2 on increasing γ-oryzanol of RBO at a fixed of x_3 (600 mL) is shown in Figure 2. It can be seen that the γ-oryzanol had a maximum point. The combination of variables observed is an effect on increasing the γ-oryzanol of RBO. At x_3 constant (600 mL), the influence of x_2 is stronger than the effect of x_1 on increased of the γ-oryzanol. It is evident in the 3D plot 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 γ-oryzanol.

The 3D plot corresponding to explain the effect of x_1 and x_2 on the vitamin E at a fixed of x_3 (600 mL) is shown in Figure 3. It is showed that the vitamin E had a maximum point. x_1 and x_2 were effects on increasing the vitamin E. At x_3 constant (600 mL), the effect of x_1 is very strong compared to the effect of x_2 on the acquisition of vitamin E. It can also be proved from the coefficients of x_1 and x_2 in equation (5), where the value of coefficient $x_1 > x_2$.

3.4 Interpretation of the optimum conditions

Based on the optimization solution generated from the Design Expert 10 software that the optimum of x_1, x_2, and x_3 to obtain the maximum of oil yield, γ-oryzanol, and vitamin E of RBO can be achieved at x_1 of 5.30 h, x_2 of 89.21%, and x_3 of 686.66 mL. It can be explained that an increase x_1 of 4 h to 5.30 h and x_2 of 76% to 89.21% (at fixed x_3 of 600 mL) cause an increase in the oil yield, γ-oryzanol, and vitamin E of RBO.

The addition of x_1 of 5.30 h up to 6 h and addition x_2 of 89.21% up to 98% did not cause an increase in oil yield, γ-oryzanol, and vitamin E of RBO.

The same explanation at fixed x_1 of 5 h, an increase of x_2 of 76% to 89.21%, and x_3 of 500 mL to 686.66 mL cause an increase in the oil yield, γ-oryzanol, and vitamin E of RBO. Further, increasing x_2 of 89.21% up to 96%, and addition x_3 of 686.66 mL up to 700 mL did not cause an increase in the oil yield, γ-oryzanol, and vitamin E of RBO. The same phenomenon at fixed x_2 of 86%, an addition of x_1 of 4 h to 5.30 h, and x_3 of 500 mL up to 686.66 mL cause an increase in the oil yield, γ-oryzanol, and vitamin E of RBO. The addition of x_1 of 5.30 h up to 6 h and addition x_2 of 89.21% up to 98% did not cause an increase in the oil yield, γ-oryzanol, and vitamin E of RBO.

3.5 Verification model

An optimization model of RBO extraction by maceration method with stirring has been developed. The laboratory scale for verification of the model has been carried out by conducting triplicate experiments using the recommended value of variables from the software. The result showed that the average oil yield, γ-oryzanol, and vitamin E were $13.91\% \pm 0.44$, 775.36 ± 31.38, and 126.71 ± 0.61 mg.L^{-1}, respectively. The RBO produced in the verification process showed that the average value of the oil yield, γ-oryzanol, and vitamin E was close to the predicted values (14.47%, 783.65 mg.L^{-1}, and 127.01 mg.L^{-1}, respectively). This indicates that the model developed is quite valid in their predictions.

4 Conclusions

RBO extraction use ethanol by maceration method accompanied by stirring is very promising to be applied in the industry, as an effort to implement the concept of green solvents that are safe for consumers and the environment. The

effect of extraction time, ethanol concentration, and ethanol volume on the oil yield, γ-oryzanol, and vitamin E of RBO has been evaluated. Ethanol can be used to extract RBO without involving heating and proven to be able to provide satisfactory results. The optimization of the extraction process has been developed and verified at the laboratory scale with sufficient results. Predictive values produced by a model that approaches the actual value have proven that the model developed is quite valid and feasible to be applied in the RBO extraction process.

Conflicts of interest. The authors declare that they have no conflicts of interest in relation to this article.

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