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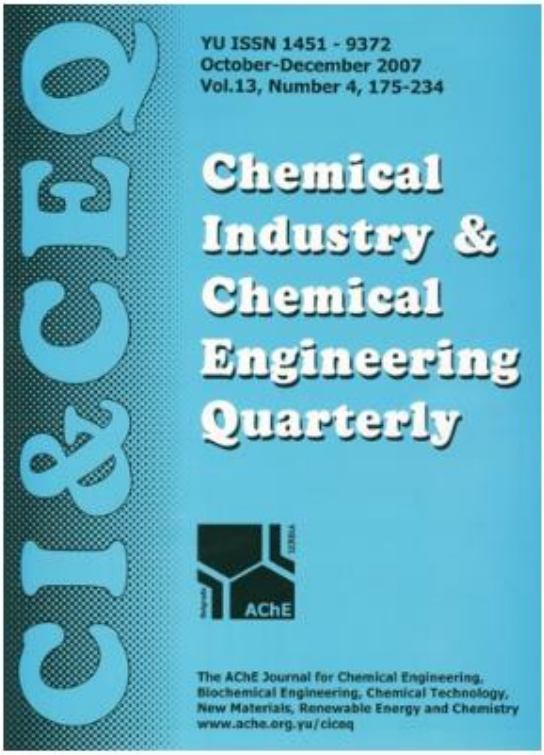

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SCIENTIFIC PAPER

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MANGO SEED KERNEL OIL EXTRACTION WITH ETHANOL: OPTIMIZATION OF OIL YIELD AND POLYPHENOL

Article Highlights

- MSKO was extracted with ethanol without heating and can implement the concept of green solvent
- The effects of extraction time, ethanol concentration and ethanol volume on the MSKO were studied
- The optimization model of the MSKO extraction has been verified on a laboratory scale

Abstract

Mango seed kernel oil (MSKO) contains a high concentration of polyphenol and has potential as raw material of oil-based food products and as a natural antioxidant of functional food. Ethanol was used for the extraction of MSKO by response surface methodology (RSM) as a tool to study the optimum extraction conditions of the oil yield and total polyphenol content (TPC). Three-factor-three-level central composite design (CCD) was employed to optimize extraction time (X_1), ethanol concentration (X_2), and ethanol volume (X_3) to obtain a high oil yield and TPC. The central points for treatment were 5 h for X_1, 86% for X_2, and 250 mL for X_3. The results showed that the optimum conditions of MSKO extraction were X_1 = 5.18 h, X_2 = 87.84% and X_3 = 233.43 mL, respectively. Under these conditions, the experimental oil yield and TPC of MSKO were 34.79% and 61.17 mgGAE/g, which was agreed closely to the verification value. The results indicated that MSKO extraction using ethanol could be an effective and advisable method for large-scale production of MSKO extraction.

Keywords: edible oil extraction, ethanol, mango seed kernel oil, response surface methodology, solvent extraction, total phenolic content.

Mango seeds (*Mangifera indica*) are by-products of industrial processing of mango. The kernel represents about 45-75% of the seed [1], and approximately 10 to 25% of the fruit depending on the varieties [2]. The mango seed kernel contains 15 wt.% of oils [3]. MSKO could be used as a source of functional food ingredients because it contains high quality oil, which contains antioxidants in high concentrations [4]. Mango seed kernel oil (MSKO) is a good source of phenolic compounds and has been used in the cosmetics industry as an ingredient in soaps, shampoos, and lotions production [5]. It can be used in the formulations of food products such as chocolate and biscuits as a natural nutritional additive due to its fatty acid composition and antioxidant activities [6]. MSKO has attracted considerable interest because it is a semi-solid oil [7]. The physical and chemical characteristics of the oil are similar to cocoa butter [8,9].

These attributes have prompted this research to evaluate the optimization of the MSKO extraction process using ethanol. The choice of ethanol as a solvent is deemed necessary to implement green extraction concepts and principles. The direct solvent extraction is most commonly used in the extraction of vegetable oil [10], and ethanol has gained attention as a solvent [11]. A study on vegetable oil extraction has revealed that the polar solvent is better than the non-polar solvent, it could be explained by the interaction between the unsaturated fatty acids with a polar solvent, compared with non-polar solvents [12].

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Extract of MSKO using n-hexane, a non-polar solvent, resulted in an oil yield of 7.03% [13].

Ethanol has advantages as a solvent of extraction, including low toxicity, adequate operational security, and is obtained from a bio-renewable source [14]. The most feasible alternative for a solvent for the extraction process is bio-solvent such as ethanol, which has been recognized as an environmentally safe solvent. Safety, health, and environmental concerns have increased the interest in alternatives solvents that are relatively safe. Ethanol has been widely applied as a viable solvent due to its ease of recovery and low cost in application and is classified as an environmentally friendly green solvent [15]. Green solvents have several benefits such as biodegradability, low toxicity, non-flammability, and renewability, making them potential candidates in separation/extraction science [16].

The solvent extraction may be affected by various factors such as time, the solvent's concentration, and the amount of solvent. RSM is a useful tool to obtain optimal process conditions, when many factors and interactions are involved during the process and affect the desired response [17]. RSM is a statistical technique used to design experiments to obtain relevant information in the shortest time at the lowest cost. The basic principle of RSM is to relate product properties to regression equations that describe interactions between input parameters and responses [18]. RSM has been utilized to optimize the extraction process variables, and it is more efficient and more comfortable to arrange and interpret experiments than others [19,20].

Based on the current MSKO study results, and that optimization of the extraction process is related to production costs, and the food industry's needs are associated with optimizing MSKO extraction, this research was conducted. It is a novel study on MSKO extraction using ethanol and studying the effects of extraction time, ethanol concentration, ethanol volume on MSKO yield and TPC, and applied RSM for optimizing, standardizing, and analyzing the resulting model.

MATERIALS AND METHODS

The samples used were Arumanis mango seeds, a local Indonesian mango from highlands >1.500 m.a.s.l., Folin-Ciocalteau reagent and all chemicals used were from Merck. Preparation of mango seed kernel (MSK): the seeds were washed, and the kernels enclosed in the hardcover were separated manually. The kernels were dried in the oven at 50 °C for 12 h to a constant weight to reduce their moisture content. Separation of the thin cover from the kernel was carried out using the tray to blow away the cover to achieve a very high kernel. Stainless steel grinder was used to grind the seeds to a powder, sealed in a plastic container, and stored in a freezer until extraction to prevent its oxidation.

The design of experiments was conducted using RSM with CCD to study the optimum condition of the oil yield and TPC as the responses. The variables investigated were: extraction time (h, X_1), ethanol concentration (%,X_2), and ethanol volume (mL, X_3) as the independent variables. The central points for X_1, X_2 and X_3 in the extraction of MSKO were 5 h for X_1, 86% forX_2 and 250 mL for X_3. The low and high limits for treatment were 4 and 6 h for X_1, 76 and 96% for X_2, 200 and 300 mL for X_3 (Table 1). Statistic software Design-Expert Version 10 was used for the regression analysis of the experimental data. The significance between treatments were determined by the F test obtained from analysis of variance (ANOVA). The adequacy of each model was determined by evaluating the lack of fit and the coefficient of determination (R^2). The regression coefficients were then used to generate response surfaces. The 3D response surface graphs and profile for predicted values for variables were plotted using the software. In order to verify the validity of the statistical models, additional verification experiments were subsequently performed. Verification of the optimum extraction conditions suggested by solving the mathematical model equations were done on a laboratory scale. For this verification, they were carried out using the optimum conditions suggested in triplicate experiments.

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

Independent variable	Star low (-1.68)	Low (-1)	Centre point (0)	High (+1)	Star high (+1.68)
Time (h)	3.32	4	5	6	6.68
Ethanol concentration (%)	72.59	76	86	96	89.41
Ethanol volume (mL)	165.91	200	250	300	334.09

For the study, 50 g of powdered MSK was weighed into the 1 L Erlenmeyer flask, followed by the addition of ethanol accompanied by stirring at 200 rpm at room temperature to prevent damage to its bioactive components. The residue was separated by centrifugation at a speed of 3500 rpm for 20 min. The liquid part accommodated in the flask evaporator, solvents were removed on a rotary evaporator Buchi R-215 incorporating a vacuum pump V-700. MSKO

obtained was stored in a dark glass bottle and stored in a freezer for analysis. The percentage of oil yield was calculated as follows:

$$\text{Oil yield}(\%) = 100\frac{\text{Weight of extracted MSKO (g)}}{\text{Weight of MSK (g)}} \quad (1)$$

TPC was determined by the Folin-Ciocalteu method, as described by Waterhouse (2002), using gallic acid as the standard. The results were expressed in mg gallic acid equivalent (GAE)/g of MSKO (dw).

RESULTS AND DISCUSSION

Statistical analysis and the model fitting

The effects of X_1, $X_2$and X_3 on the oil yield and TPC of MSKO were studied. The results showed that the oil yield of MSKO ranged from 29.94 to 34.62%, while the TPC ranged from 35.23 to 69.04 mg GAE/g (Table 2). The maximum oil yield and TPC of MSKO were observed under the experimental conditions of X_1 = 5.18 h, X_2 = 87.84% and X_3 = 233.43 mL. The maximum oil yield and TPC of MSKO under these conditions were 34.79% and 61.17 mgGAE/g, respectively.

According to the results of ANOVA for oil yield, the model is significant (Table 3). The quality of the models developed were evaluated based on the coefficient of determination (R^2). The R^2 value of oil yield was 0.77. A similar phenomenon is also seen in TPC, that the model is significant (Table 4). The R^2 value of TPC was 0.84. The significant difference terms of each coefficient were 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 [21]. The actual quadratic equations for predicting the oil yield and TPC responses are as follows:

$$\begin{aligned}\text{Oilyield }(\%) = &-74.167 + 13.025X_1 + 1.142X_2 + \\ &+0.225X_3 + 0.015X_1X_2 - 0.013X_1X_3 - \\ &-0.0004X_2X_3 - -1.114X_1^2 - 0.006X_2^2 - 0.0003X_3^2\end{aligned} \quad (2)$$

$$\begin{aligned}TPC(\text{mg GAE/g}) = &-983.122 + 45.057X_1 + \\ &+14.817X_2 + 2.315X_3 + 0.072X_1X_2 - \\ &-0.053X_1X_3 - 0.008X_2X_3 - \\ &-3.697X_1^2 - 0.076X_2^2 - 0.002X_3^2\end{aligned} \quad (3)$$

Statistical analysis results showed that the best model to describe the effect of X_1, X_2, X_3 for the oil yield is a quadratic model. The F value of 3.71 and the low probability value (p < 0.02) imply the model is significant, values of $Prob > F$ less than 0.05 indicate model terms are significant. The variables with the

Table 2. Central composite design, showing coded and actual values of independent variables, with observed oil yield and TPC of MSKO

Run	Coded values of independent variables			Actual values of independent variables			Response	
	Extraction time (h)	Ethanol concentration (%)	Ethanol volume (mL)	Extraction time (h)	Ethanol concentration (%)	Ethanol volume (mL)	Oil yield (%)	TPC (mg GAE/g)
1	-1	-1	-1	4	76	200	32.46	37.14
2	1	-1	-1	6	76	200	31.78	42.85
3	-1	1	-1	4	96	200	32.42	46.66
4	1	1	-1	6	96	200	33.47	54.76
5	-1	-1	1	4	76	300	32.26	43.33
6	1	-1	1	6	76	300	30.17	38.09
7	-1	1	1	4	96	300	32.54	37.14
8	1	1	1	6	96	300	29.94	35.23
9	-1.68	0	0	3.32	86	250	30.28	49.52
10	1.68	0	0	6.68	86	250	33.08	54.76
11	0	-1.68	0	5	72.59	250	32.65	40.47
12	0	1.68	0	5	89.41	250	33.41	41.42
13	0	0	-1.68	5	86	165.91	34.62	37.61
14	0	0	1.68	5	86	334.09	30.97	47.61
15	0	0	0	5	86	250	33.69	60.47
16	0	0	0	5	86	250	34.19	69.04
17	0	0	0	5	86	250	35.36	66.19
18	0	0	0	5	86	250	35.85	60.01
19	0	0	0	5	86	250	33.80	56.66
20	0	0	0	5	86	250	34.32	54.76

Table 3. Statistical analysis of the oil yield. Response 1: oil yield; ANOVA for response surface quadratic model; analysis of variance table (partial sum of squares)

Source	Sum of squares	*df*	Mean square	*F* value	*p*-value *Prob* > *F*	Significance
Model	40.39	9	4.49	3.71	0.02	Significant
X_1	0.01	1	0.01	0.01	0.93	
X_2	0.65	1	0.65	0.54	0.48	
X_3	9.45	1	9.45	7.79	0.02	
X_1X_2	0.19	1	0.19	0.15	0.7	
X_1X_3	3.2	1	3.2	2.64	0.14	
X_2X_3	0.32	1	0.32	0.26	0.62	
X_1^2	17.9	1	17.9	14.78	0.003	
X_2^2	5.85	1	5.85	4.83	0.05	
X_3^2	7.49	1	7.48	6.17	0.03	
Residual	12.11	10	1.214			
Lack of fit	8.28	5	1.66	2.16	0.21	Not significant
Pure error	3.83	5	0.77			
Cor Total	52.5	19				

Table 4. Statistical analysis of the TPC . Response 2: TPC; ANOVA for response surface quadratic model; analysis of variance table (partial sum of squares)

Source	Sum of squares	*df*	Mean square	*F* value	*p*-value *Prob* > *F*	Significance
Model	1721.21	9	191.25	6.12	0.004	Significant
X_1	17.53	1	17.53	0.56	0.47	
X_2	14.31	1	14.31	0.49	0.51	
X_3	8.54	1	8.54	0.27	0.61	
X_1X_2	4.09	1	4.09	0.13	0.72	
X_1X_3	54.91	1	54.92	1.76	0.21	
X_2X_3	116.13	1	116.13	3.72	0.08	
X_1^2	197	1	197	6.31	0.03	
X_2^2	844.56	1	844.56	27.05	0	
X_3^2	719.69	1	719.67	23.05	0	
Residual	312.24	10	31.22			
Lack of fit	161.82	5	32.36	1.08	0.47	Not significant
Pure error	150.42	5	30.08			
Cor Total	2033.45	19				

most considerable significant effect on oil yield at 5% confidence level were the linear terms of ethanol volume (X_3), the quadratic term of time (X_1^2), the quadratic term of ethanol concentration (X_2^2), and the quadratic term of ethanol volume (X_3^2). The lack of fit *F* value of 2.16 implies the lack of fit is not significant, which shows that the model is suitable to describe the extraction process. The model can be considered appropriate if the lack of fit value model is not significantly different at the level of specific α [22].

These results are in agreement with other studies. Time and amount of solvent were the parameters affecting the extraction yield from different sources [23-25]. The more time the seeds are in contact with the solvent, the higher the extraction yield percentage [26]. The amount of salmon liver oil yield increased by increasing extraction time [27]. The solid-liquid ratio had the most significant influence on the oil yield [17,28].

As for the results of the statistical analysis of the TPC (Table 4), statistical analysis results showed that the best model to describe the effect of X_1, X_2, X_3 for the TPC is a quadratic model. The R^2 value of TPC was 0.85. The *F* value of 6.12 and the low probability value ($p < 0.02$) imply the model is significant, values of *Prob* > *F* less than 0.05 indicate model terms are significant. The variables with the most considerable significant effect on TPC at 5% confidence level were the quadratic term of time (X_1^2), the quadratic term of ethanol concentration (X_2^2), and the quadratic term of ethanol volume (X_3^2). The lack of fit *F* value of 1.07 was non-significant ($p > 0.05$), which shows that the model is suitable to describe the extraction process.

Several studies have reported that the extraction time significantly influenced the endogenous bioactive compounds, such as individual ones from the TPC [29-33]; hexane extract of MSKO contains phenolic content of 98.7 mg/g [34]. The solubility of phenolic compounds increases with increasing extraction time [33]. The solid-liquid ratio is the most significant parameter affecting an increase of TPC [31,35]. The effect of the most significant variable influencing the acquisition of TPC extract was the solid-liquid ratio [29].

Regarding the polyphenol content, the same study was carried out on mango seeds taken from the lowlands (3-50 m.a.s.l.), showing high polyphenol content [36]. It is thought to be due to the influence of the photosynthesis process. Polyphenol in plants need sugar during their synthesis, and they are produced during photosynthesis. The elevation of the location influences the process of photosynthesis; the greater the elevation, the smaller the intensity of sunlight so that the sugar produced by plants will be smaller, and cause the production of polyphenol to be lesser.

Response surface plots

The relationship between variables and responses were illustrated in the 3D representation of the response surfaces and 2D contour plots generated by the model for oil yield (Figures 1-3) and TPC (Figures 4-6). These plots depict two variables within the experimental range, and keeping the third variable

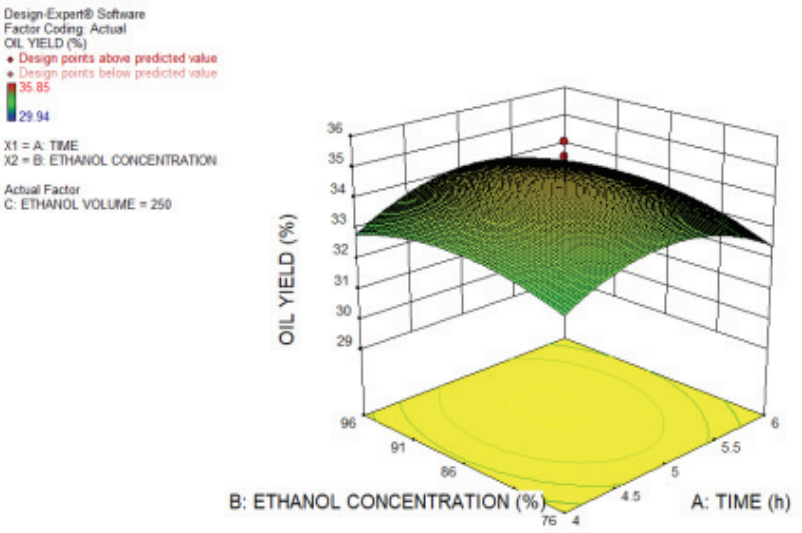

Figure 1. Response surface plots of the effect of X_1 and X_2 for oil yield.

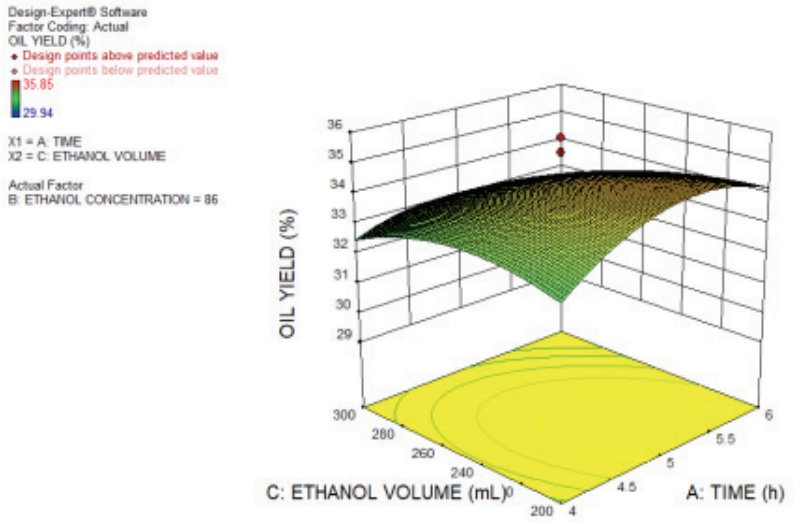

Figure 2. Response surface plots of the effect of X_1 and X_3 for oil yield.

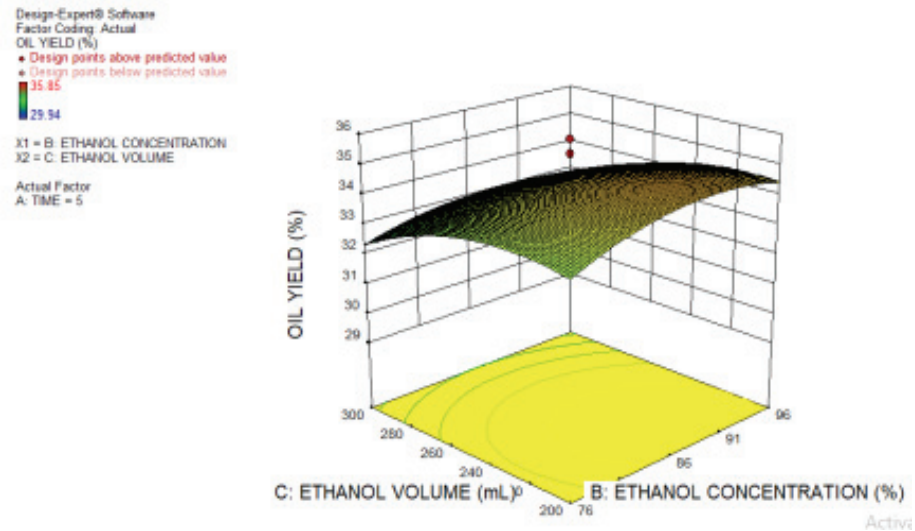

Figure 3. Response surface plots of the effect of X_2 and X_3 for oil yield.

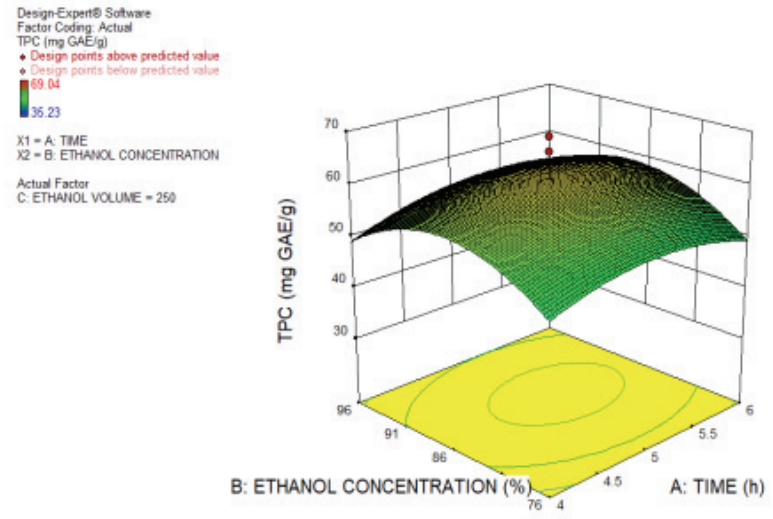

Figure 4. Response surface plots of the effect of X_1 and X_2 for TPC.

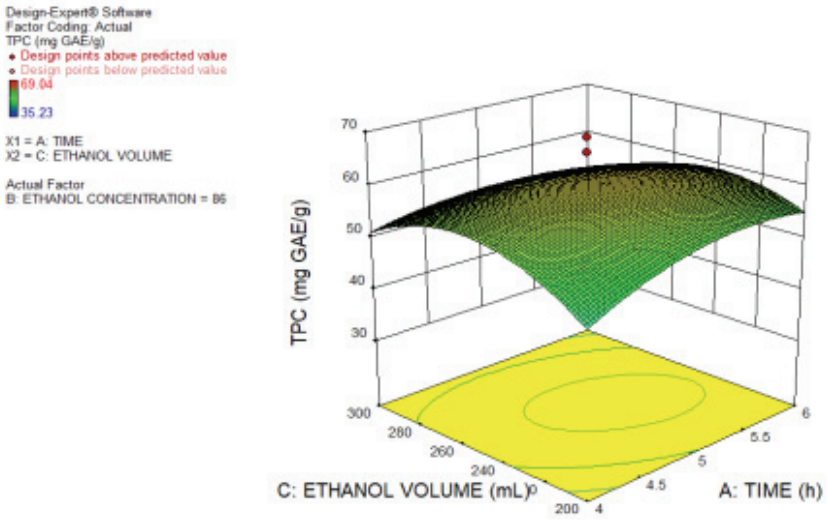

Figure 5. Response surface plots of the effect of X_1 and X_3 for TPC.

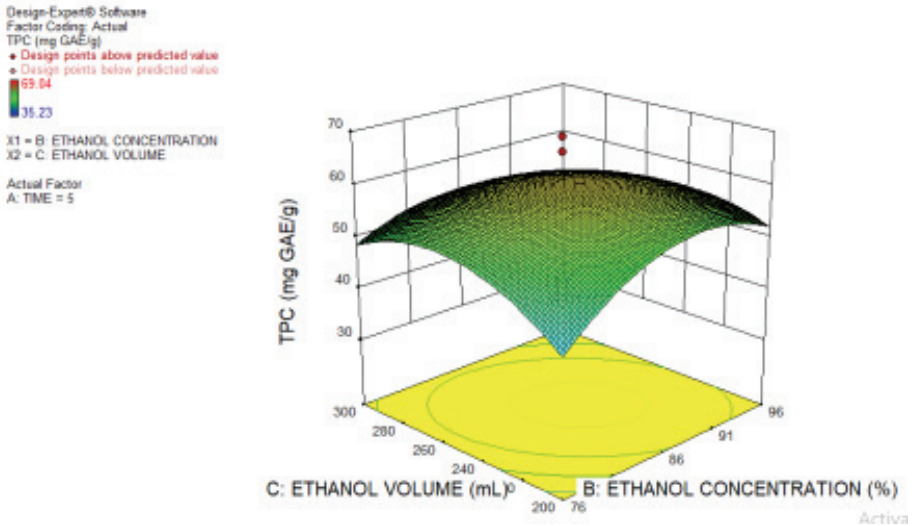

Figure 6. Response surface plots of the effect of X_2 and X_3 for TPC.

constant at zero levels. The results showed that an increase of X_1 from 4 h to 5.18 h and X_2 from 76% to 87.84% at fixed X_3 of 250 mL promoted an increase in the oil yield and TPC. Further, the increase of X_1 from 5.18 h to 6 h and an increase of X_2 from 87.84% up to 96% at fixed X_3 of 250 mL promoted a decrease in the oil yield (Figure 1) and TPC (Figure 4). An increase of X_1 from 4 h to 5.18 h and X_3 from 200 mL up to 233.43 mL at fixed X_2 of 86% promoted an increase in the oil yield and TPC. Further, the increase of X_1 from 5.18 h to 6 h and X_3 from 233.43 mL up to 300 mL promoted a decrease in the oil yield (Figure 2) and TPC (Figure 5). The same phenomenon at an increase of X_2 from 76% to 87.84% and addition of X_3 from 200 mL up to 233.43 mL at fixed X_1 of 5.18 h promoted an increase in the oil yield and TPC. Further, the increase of X_2 from 87.84 to 96% and addition of X_3 from 233.43 up to 300 mL promoted a decrease in the oil yield (Figure 3) and TPC (Figure 6).

Verification of the optimum extraction condition

The optimal condition suggested by the software was then verified to test the accuracy of the model. Triplicate experiments were carried out using the optimized parameters. The results showed that an average oil yield of 35.08% and TPC of 62.12 mg GAE/g could be achieved. MSKO produced in the verification process shows that the average yield and TPC are slightly higher than the predicted values. This observation shows that the models developed are quite valid and adequate in their predictions, and they can be used to optimize the MSKO extraction process.

CONCLUSIONS

Ethanol has proven to be an effective and efficient solvent for extracting MSKO with high yield and polyphenol content without involving heating and has given satisfying and auspicious results to be applied in the industry as an effort to apply the concept of green solvents, which are relatively safe for consumers and the environment. The optimization of the MSKO extraction process has been achieved and verified on a laboratory scale with adequate results. The predicted values produced by the model that approached the actual values have proven that the model developed is valid and feasible to be applied in the MSKO extraction process.

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NAUČNI RAD

EKSTRAKCIJA ULJA MANGOVOG SEMENA ETANOLOM: OPTIMIZACIJA PRINOSA ULJA I POLIFENOLA

Ulje semena manga sadrži visoku koncentraciju polifenola i potencijalna je sirovina za prehrambene proizvode i kao prirodni antioksidans funkcionalne hrane. Etanol je korišćen za ekstrakciju ulja manga, pri čemu je korišćena metodologija površine odziva kao alat za nalaženje optimalnih uslova ekstrakcije koji obezbeđuju makslimalni prinos ulja i sadržaj ukupnih polifenola. Primenjen je centralni kompozitni dizajn sa tri faktora na tri nivoa radi optimizacije vremena ekstrakcije (X_1), koncentracije etanola (X_2) i zapremine etanola (X_3) i postizanja visokog prinos ulja i ukupnih polifenola. Centralne tačke dizajna bile su 5 h za X_1, 86% za X_2 i 250 ml za X_3. Rezultati su pokazali da su optimalni uslovi ekstrakcije mangovog ulja X_1 = 5,18 h, X_2 = 87,84% i X_3 = 233,43 ml. Pod ovim uslovima, prinos ulja je bio 34,79%, a sadržaj ukupnih polifenol 61,17 mg GAE/g. Rezultati su pokazali da bi ekstrakcija mangovog ulja etanolom mogla biti efikasna i korisna metoda za njegovu industrijsku proizvodnju.

Ključne reči: ekstrakcija jestivog ulja, etanol, ulje semena manga, metodologija odzivne površine, ekstrakcija rastvaračem, ukupan sadržaj fenola.