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Domestic Microwave-Maceration Extraction (DMME) of PhenolicCompounds from Peanut (*Arachishypogeae L.*) Shell using Full Factorial Design of Experiment

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Abstract

In this study, the applicability of domestic microwave-maceration extraction (DMME) for phenolic compounds extraction from peanut (Arachishypogeae L.) shell was determined using a statistical full factorial design of experiment (DOE) method. The influence of ethanol concentration (%), solvent to solid ratio (v/w), and irradiation time (s) on total phenolic content (TPC) of peanut shell extracts was investigated. The significance of each variable and their interaction effects were also evaluated in response to TPC. Results showed that within the range of variables studied TPC of peanut shell extracts increased with the increase in ethanol concentration and irradiation time. The TPC of extracts ranged from 0.6318 to 7.7901 g GAE/100 g extract. Extract with the highest TPC value was obtained at DMME condition as follows: ethanol concentration of 96%, irradiation time of 150 second, and solvent to solid ratio of 10:1.The DOE result showed that TPC of peanut shell extracts were significantly affected by irradiation time ($p<0.0001$), followed by ethanol concentration and solvent to solid ratio. The relationship between the controlled variables and response may be predicted by the equation obtained. Under the optimised conditions, the experimental TPC value was reasonably close to the DOE predicted value.

Keywords: Antioxidants, Design of experiment, Microwave-maceration extraction, Peanut shell, Phenolics.

Introduction

Paper Code:

Free radicals are highly reactive and unstable molecules that likely to react with food lipids,causing the lipid oxidation [1]. Lipid oxidation is one of the major concerns in food industry as they may contribute to the loss in fatty food quality leading to deterioration on taste, flavour, colour, nutritional value, and storage stability of food products [2]. In order to suppress the oxidative deterioration, synthetic antioxidants such as butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT) are usually added to fresh or processed foods [3] However, the use of synthetic antioxidants has caused anxiety due to their possible toxic and carcinogenic effects [4]. The search of natural and safe antioxidants, especially of plant origin, has therefore increased considerably in recent years.

Peanut shells are low value byproducts of peanut blanching operations [5]. Several authors [6,7,8,9,10,11] Yen *et al*., 1993) have reported that the shells, hulls, and roots of peanuts have high levels of polyphenols with demonstrated antioxidant properties. Lou *et al*. identified six A-type proanthocyanidins from the water-soluble fraction of peanut shell extracts [7].Yu *et al*. observed three classes of compounds in peanut shell extracts: phenolic acids, flavonoids and stilbene (resveratrol). Despite being an abundant source of these health-promoting compounds, peanut shells have not been considerably exploited as a valuable natural resource [12]. The development of more efficient methods for extracting antioxidant compounds from peanut shells is thus needed in order to increase its commercial appeal.

Some authors[9,12,13,14] have employed conventional solid–liquid extraction techniques, by using different organic solvents,in order to extract antioxidants from peanut shells. Study by Nepote*et al*. [15]reported different total phenolic content of peanut skins than that reported by Wang*et al*.[16],0.118 g/g compared to 90 mg/g extract. The difference might be due to the type of peanut as raw material, method of extraction, and solvent concentration.

The availability of phenolic compounds in peanut shell as antioxidant source is verified. However, the economic feasibility of an industrial process also requires further study in order to obtain high extraction efficiency. Many factors have been established to influence the extraction efficacy, for instance extraction methods, particle size, solvent type, solvent concentration, solvent to solid ratio, extraction temperature, extraction time and pH [17,18,19]. Over the past decade, microwave-assisted extraction method has attracted tremendous attenti,n mainly due to considerable savings in processing time and solvent - energy consumption [20,21].

In general, process optimization could be achieved by either empirical or statistical methods [2,22]. Empirical method adopts one factor at a time approach, in which one factor is varying at a time while all others are kept constant [2,17]. The main drawbacks of this method include the inability to determine the interaction between variables, time-consuming, costly and less effective [23]. The optimisation of DMME extraction of phenolic compounds from peanut shells has not yet been reported. Therefore in this study, the applicability of domestic microwave-maceration extractionfor phenolic compounds from peanut shell was investigated. Besides the optimum extraction condition, the effects of ethanol concentration, solvent to solid ratio, and irradiation time were also studied using a full factorial design of experiment (DOE).

Materials and Methods

Materials

Peanut shells were purchased from local farmers in Jember, East Java, Indonesia. The shells were washed with tap water and sun dried for about 5 days. The dried shells were ground into fine powder in a grinder (Miyako Type BL-152 PF-AP) and passed through a 60-mesh sieve. The peanut shell powder was stored in a freezer at -4°C in sealed plastic bags prior to further use. Folin-Ciocalteu reagent (Merck, Germany), gallic acid standard (Sigma–Aldrich, USA), and other chemicals were obtained from local distributors. All solvents and chemicals used were of analytical grade.

DMME Extraction Procedure

Polyphenolic compounds were extracted from peanut shells in a domestic microwave (Inextron WD9000SL23-2 2.450MHz). The system supplies 900W of microwave energy at 20% power. The extraction variables evaluated were ethanol concentrations (0, 48, 96%), solvent to solid ratio (5, 10, 15 v/w), and microwave irradiation time (30, 90, 150 s). Constant variables employed were particle size (-20/+80 mesh), volume of solvent (100 mL), and microwave power (180 W). All the extractions were replicated once. Following extraction, the total phenolic content(TPC) of the crude extracts were then determined.

Determination of Total Phenolic Content

The concentration of total phenolic compounds was determined spectrophotometrically using the Folin–Ciocalteu total phenol procedure described by Spanos and Wrolstad[24], with minor modifications. Gallic acid standard solutions were prepared at 0.001, 0.002, 0.003, 0.004, 0.005, and 0.006 mg/mL. One milliliter of extracts and 1 mL of gallic acid standard (Sigma–Aldrich, USA) were transferred to 15 mL test tubes. Five milliliter of 0.2 N Folin–Ciocalteu reagent (Merck, Germany) were added to each test tube and mixed. After 1 min, 4.0 mL of 7.5% (w/v) Na_2CO_3 in water were added and mixed. The absorbance of samples was measured spectrophotometrically at 765 nm using a Shimadzu UV-VIS 1700 spectrophotometer, after being left for 1 h at room temperature. A calibration curve of gallic acid was plotted by plotting absorbance vs concentrations of gallic acid (mg/L). Total phenolic compounds concentration in the extracts was determined by comparing the absorbance of the extract samples to that of gallic acid standard solutions. All samples were analysed in duplicate. TPC value was expressed as g gallic acid equivalents (GAE) per 100 g extracts.

Statistical Analysis

A full factorial design of experiment (DOE) method was employed to determine the optimum conditions for DMME peanut shell extraction. The significant levels of each variable and the effects of interactions between variables were studied in

response to TPC. DOE was performed using the Minitab software (Minitab Version 15.1.1.0.). A central composite design was used to investigate the effects of three controlled variables (ethanol concentration (X_i), solvent to solid ratio (X_j), and irradiation time (X_k)). This design uses the method of least squares regression to fit the data to a linear model. The linear model for each response was as follows:

$$Y = b_0 + \Sigma\, b_i.x_i + \Sigma\, \Sigma b_{ij}.x_i.x_j + \Sigma\, \Sigma\Sigma b_{ijk}.x_i.x_j.x_k \quad (1)$$

where Y is the predicted response, b_0 a constant, b_i the linear coefficient, b_{ij} the interaction coefficient of variables i and j, b_{ijk} the interaction coefficient of variables i, j, and k, and X_i, X_j and X_k are controlled variables. The software uses this linear model to build response surfaces. The adequacy of the model was determined by evaluating the lack of fit, coefficient of determination (R^2) that was generated by the software. Statistical significance of the model and model parameters was determined at the probability level (p = 0.0001). The codes used in the response surface analysis and the corresponding parameter values are given in Table 1. The complete design consisted of 18 experimental points, including two replications of the centre point.

Table 1: The controlled variables for DOE and their levels

Parameters	Low Level (-1)	Centre Point (0)	High Level (+1)
Ethanol concentration (%w) (X_i)	0	48	96
Solvent to solid ratio (v/w) (X_j)	5:1	10:1	15:1
Irradiation time (s) (X_k)	30	90	150

Results and Discussion

The Effect of Ethanol Concentration on TPC

The effect of ethanol concentration on TPC of peanut shells extracts by DMME extraction method is shown in Figure 1. Within the range studied, TPC of peanut shells increased with the increase in ethanol concentration. The optimized ethanol concentration for phenolic compounds extraction from peanut shells was 96% which resulting extract with TPC value of 7.7901 g GAE/100 g extract.

Similar trend was reported by Nepote*et al*.[15]. This may be due to the higher dissipation factor (tan δ) possesed by ethanol with higher concentration [25].As reported, in microwave-assisted extraction, theextraction effiencyis mainly influenced by the ability of solvent to absorb microwave energy and to pass the energy on as heat to surrounding molecules (dissipation factor)[25,26].This thus effects the TPC value of extracts. In addition, peanut shells contain phenolic compounds such asp-hydroxybenzoic acid, chlorogenic acid, p-coumaric acid, ferulic acid, resveratrol, epicatechin and quercetin[27].Some of the compounds are more soluble in ethanol than in water.

The Effect of Solvent to Solid Ratio on TPC

The effect of solvent to solid ratio on extraction of phenolic compounds from peanut shells was shown in Figure 1. Extracts obtained at condition solvent to solid ratio of 5:1 had lower TPC value than that of 10:1 ratio. With the same amount of solvent, the solid amount used in ratio of 5:1was greater than any other ratios. The large amount of solid has caused the microwave energy scattered. The energy absorbed by the solid per mass unit thus decreased and caused the imperfection of cell rupture [28]. Since the cell wall was not perfectly broken, the phenolic compounds could not freely escaped from the cells, thus, the diffusion rate of phenolic compounds from peanut shell into solvent became more slowly.Referring to Figure 1,the optimum solvent to solid ratio which gave the highest TPC value was 10:1 (v/w).

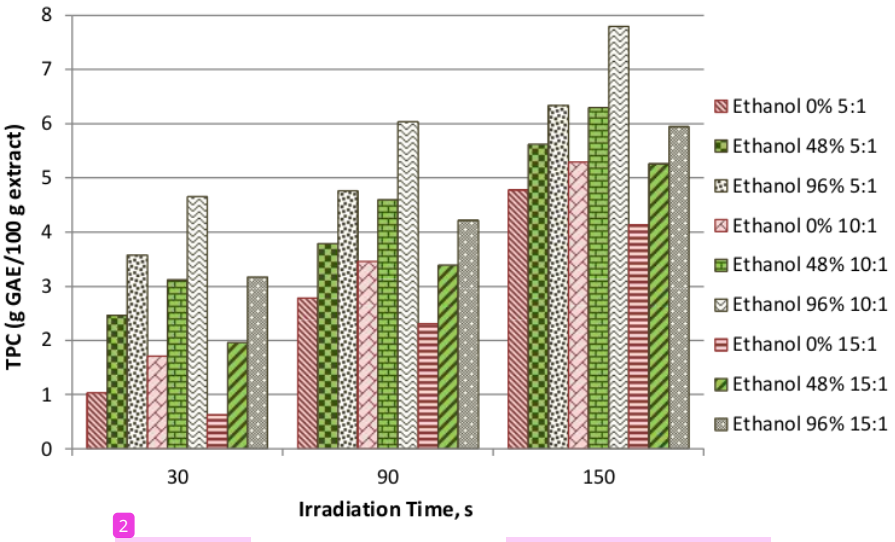

Figure 1. The effect of ethanol concentrations, solvent to solid ratio, and irradiation time on TPC extracts (g GAE/100 g extract)

The Effect of Irradiation Time on TPC

Figure 1 also showed that at the same ethanol concentrations and solvent to solid ratio, TPC of peanut shells extracts increased with the increase in microwave irradiation time. Longerirradiation time may increasethe mass transfer of phenolic compounds from peanut shells to solvent. In addition, the longer irradiation time allows the solvent absorb more microwave energy. With the increased amount of microwave energy absorbed, the peanut shell cell wall will be fragile, so that the phenolic compounds could be easily extracted out of the cells and more rapidly diffused into the solvent [25].In this study, the optimum irradiation time was 150 s.

Model Fitting

Theexperimental results showed that TPC of peanut shells ranged from 0.6318 to

6.3898 g GAE/100g extract. The design of experiment for the linear model in response to TPCrevealed that the model was significant ($p< 0.0001$) with an F-value of 0.01 (Table 2). The R^2 value for the model was 99.98% with no significance in the lack of fit ($p> 0.0001$). These factors indicated that the model could be used to predict the responses. The software has generated the following regression equation (Eq. 2) which demonstrates the empirical relationship between ethanol concentration (X_i), solvent to solid ratio (X_j), irradiation time (X_k), and TPC (Y). By applying regression analysis, relationship between the tested controlled variables and the response was explained in equation (2).

$$TPC = 3.6950 + 1.0532A - 0.2430B + 1.6069C + 0.0277AB - 0.1945AC - 0.0318BC + 0.0428ABC \quad (2)$$

Table 2.DOE for the effect of controlled variables on TPC using a linear response surface model

Model	Effect	Coefficient	SE Coefficient	T	P
Constant		3.6950	0.1321	250.24	0.000
A	2.1064	1.0532	0.1321	70.36	0.000
B	-0.4859	-0.2430	0.1321	-14.87	0.000
C	3.2138	1.6069	0.1321	97.50	0.000
AB	0.0555	0.0277	0.1321	5.87	0.000
AC	-0.3889	-0.1945	0.1321	-1.91	0.088
BC	-0.0635	-0.0318	0.1321	-3.70	0.005
ABC	0.0857	0.0428	0.1321	3.35	0.009
	S = 0.389852	R^2 = 99.98%	R^2-Sq(adj) = 99.96%		

To fit the response function and experimental data, the linearity effect of the independent variables, their interactions and regression coefficients on the response variables were evaluated by design of experiment (DOE) (Table 2). The DOE of the regression model showed that the model was highly significant due to a very low probability value ($p < 0.0001$). The fitness and adequacy of the model was justified by the coefficient of determination (R^2) and the significance of lack-of-fit. R^2, which was defined as the ratio of the explained variation to the total variation, was a measure of the degree of fit [29]. The closer the R^2value to unity, the better the empirical model fits the actual data [30]. By referring to Table 2, R^2value for the regression model of TPC was 99.98%, which was closed to 100%. In this study, the adjusted $R^2$was very close to the R^2value. A small SE coefficient (0.1321) revealed that the experimental results were precise and reliable.

Significant Levels of Variables and Interaction of Variables in Response to TPC

Significant levels of variables or interaction of variables in response to TPC is also indicated by the Pareto chart seen in Figure 2. Red line indicates significance cut-off line. Variables or interaction variables that have a large effect will pass standard significance cut-off line (length of bar graphs pass significance cut-off line) thus these

variables or interactions of these variables are significant to the response TPC, while those which not passing significance cut-off line are not significant. The farther the distance of bar graph from the significance cut-off line, the variables or interaction variablesaremore significant.

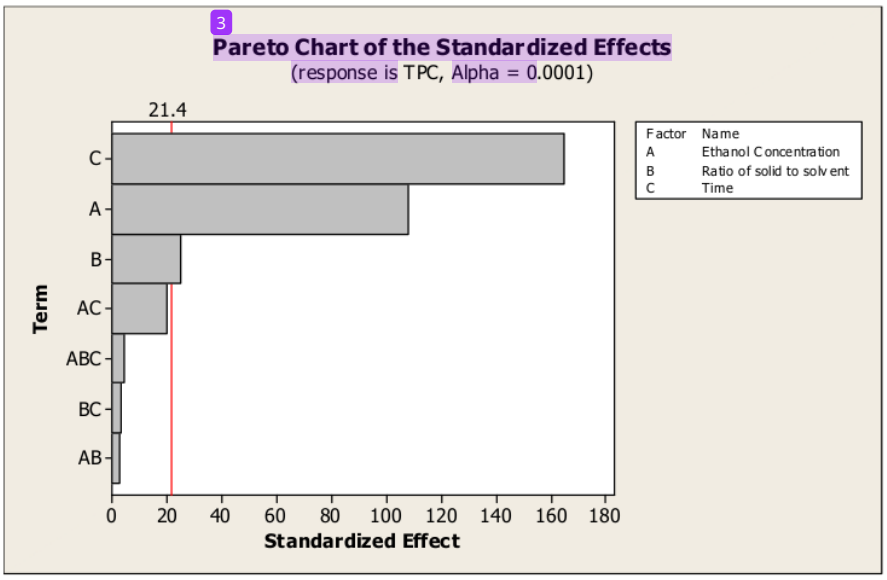

Figure 2. Pareto chart of the standardized effects

Analysis of Main Effect Plot

Main effectplot illustrates the increase or decrease in the value of the response (TPC) for each variable. Main effectplot can be seen in Figure 3.Based on Figure 3, the black line showed the increase or decrease in response (TPC) for each variable that is of a lower level (-1) to upper level (+1), while the dot-shaped red box indicates the mean (0) of a variable. Black line has a different slope for each variable. The greater the slope, the more significant a variable is. Figure 3 showed that variable with the greatest slope is the irradiation time, followed by ethanol concentration and ratio of solvent to solid, respectively.

As shownin Figure 3, TPC will be greater with the increasing concentrations of ethanol, 0% (level -1) to 96% (level 1). TPC will also increase with the increase in irradiation time of 30 seconds (level -1) to 150 seconds (level 1). On the other hand, the increasedin solvent to solid ratio from 5:1 (level -1) until 15:1 (level 1) has reduced TPC value of extracts. However, solvent to solid ratio of 10:1 gave the greates value of TPC amongst all ratios.

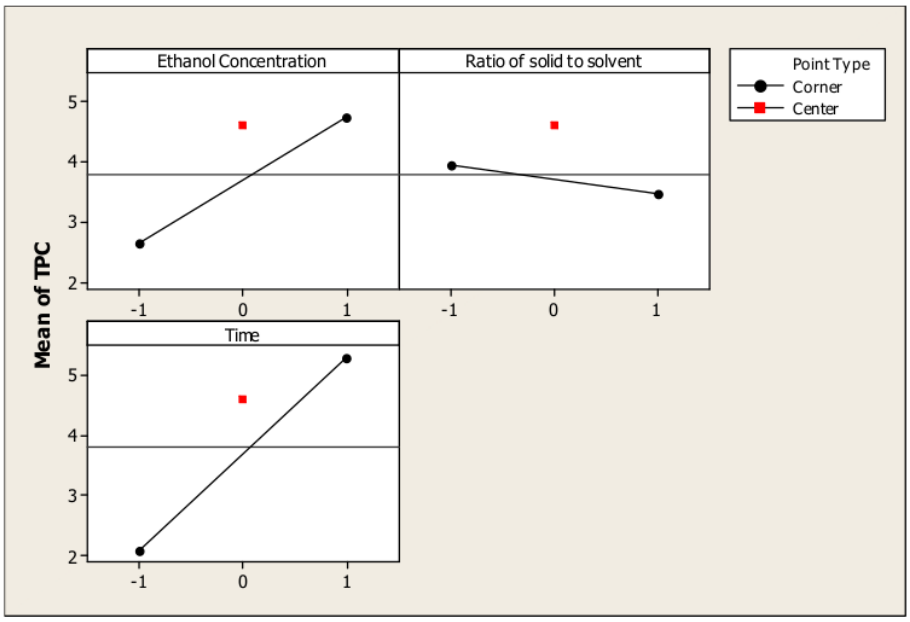

Figure 3. Main effect plot on TPC response

Interactions Plot Analysis

Interactions plot describes the interaction between two variables where each variable has an influence on other variables in producing TPC response. Interaction plot of this study is shown in Figure 4.The first is interaction of ethanol concentration and the ratio of solvent to solid; the black line shows the concentration of ethanol 0% (level -1), the red dot indicates the concentration of ethanol is 48% and the ratio of solid and solvent of 10 (level 0), the green line shows the concentration of ethanol 96% (level 1). At the concentration of ethanol 0% (level -1) and 96% (level 1), TPC decreased with the increasein the solvent to solid ratio of 5:1 (level -1) until 15:1 (level 1). At a concentration of 48% ethanol and solvent to solid ratio of 10:1, the value of TPC was greater than that of 0% ethanol concentration and lower than that of 96% ethanol concentrationfor the same solvent to solid ratio.

For the second interaction, i.e. ethanol concentration and irradiation time, the black line shows the concentration of ethanol 0% (level -1), the red dot indicates 48% ethanol concentration and irradiation time of 90 seconds (level 0), the green line shows the concentration of ethanol 96% (level 1). At 0%ethanol concentration (level -1) and 96% (level 1),the value of TPC increased with the increase in irradiation time from 30 seconds (level -1) to 150 seconds (level 1). Underthe following DMME extraction condition, i.e. ethanol concentration of 48% with 90 s irradiation time, peanut shell extract has a greater value of TPC compared to that of 0% ethanol concentration and has lower TPC value for 96% ethanol concentration using the same irradiation time.

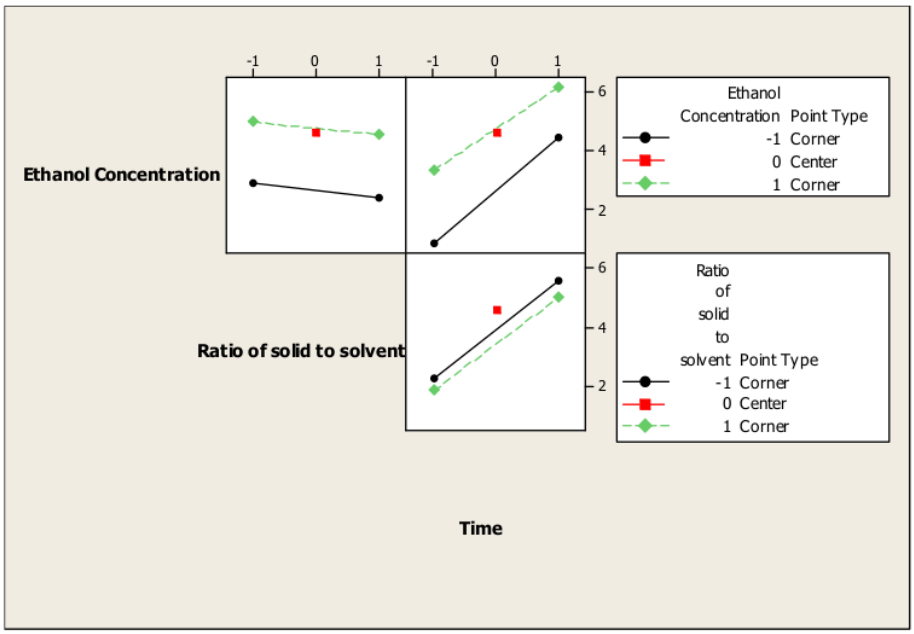

Figure 4. Interactions plot on TPC response

Conclusion

An optimized domestic microwave maceration extraction method (DMME) of phenolic compounds from peanut shells has been developed. DMME proved to be an attractive alternative to conventional extraction methods, such as solid–liquid extraction, to obtain phenolic compounds from peanut shells. DMME showed obvious advantages in terms of higher extraction efficiency, less solvent, savings of energy, and shorter extraction time. The results demonstrated that DMME could be a fast and reliable method for phenolic compounds extraction from peanut shells. The present study confirmed the advantages of design of experiment (DOE) in optimising the extraction conditions for phenolic compounds as antioxidants from peanut shells. The results from DOE showed that TPC of peanut shells were most affected by irradiation time followed by ethanol concentration and solvent to solid ratio. Using the DMME method, the optimum conditions for maximum TPC were as follows: ethanol concentration of 96%, irradiation time of 150 second, and solvent to solid ratio of 10:1. Under the mentioned conditions, the experimental value for TPC was 7.7901 g GAE/100 g extract, which was reasonably close to the predicted DOE value (6.3898 g GAE/100 g extract). Further works on isolation and characterization of extract obtained under the optimum conditions may be needed to elucidate the identity of phenolic compounds responsible for the antioxidant properties of peanut shells.

Acknowledgements

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