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# Experimental design for solid-liquid extraction from peanut kernel: Optimization through variability in antioxidant potential

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## ABSTRACT

Extraction is a critical step in determining the actual content and related bioactivity potential of medicinal plants. This study aimed to develop optimized conditions for solid-liquid extraction (SLE) for the efficient isolating of phenolic substances and to accurately determine the antioxidant potential of peanut kernel. Relevant variables were studied through response surface methodology. During the initial screening step, 2-level full factorial designs were conducted. Independent variables included the percentage of ethanol content, solvent volume, temperature, and time for extraction significantly influenced SLE from the peanut kernels. Subsequently, the four significant factors were optimized by 2-level center-faced central composite design. Response surface plots showed significant correlation between independent variables and response variables. An increase in independent factor levels increased total phenolic content and antioxidant activity in a quadratic manner. The optimal conditions comprising 30% v/v ethanol content, 68 mL of extraction solvent, extraction temperature of 78°C, and extraction time of 153 min, were located at maximum antioxidant activity. The validity of the generated models was confirmed by the highest predicted R-square.

**Keywords:** antioxidant potential; central composite design; full factorial design; solid-liquid extraction; peanut kernel

## 1. INTRODUCTION

Peanut (*Arachis hypogaea*) is a common Thai snack as well as an ingredient in traditional Thai food. Besides having a high nutritional value, they also contain a high content of substances beneficial to health, such as phenolics (Limmongkon et al., 2017), flavonoids (Chukwumah et al., 2012), and tannins (Attree et al., 2015). Exploration of the bioactive compounds of Thai peanuts and their potency can further reveal the benefits of ordinary peanuts that are native to Thailand. Extraction is a critical step in determining the content and related bioactivity potential of medicinal plants. Owing to its simplicity, efficiency and wide applicability, solid-liquid extraction (SLE) is a conventional extraction method for the extraction of phytochemicals from natural sources (Dai and Mumper, 2010). SLE involves softening and breaking a plant's cell wall, before releasing soluble bioactive compounds into an extraction medium (Azwanida, 2015). Various factors such as solvent types, solvent volume, extraction temperature and extraction time influence the extraction process (Karacabey and Mazza, 2008). Inappropriate extraction conditions may cause incomplete extraction and reduce the efficiency of isolating the expected compounds. Evaluation of experimental designs and multivariate statistical techniques have been

simultaneously used to investigate the effect of several variables on the extraction of bioactive compounds (Ali et al., 2018). Generally, full factorial designs (FFD) are chosen for screening and identifying the most influential factors whereas central composite designs (CCD) are widely used in the optimization process to determine the optimal conditions (Dejaegher and Heyden, 2011; Zhang et al., 2014). Use of a combination of experimental designs in the extraction process has successfully improved extraction efficiency (Suphamityotin, 2011).

The predominant bioactivity of peanut extracts is antioxidant activity, which plays a major role in preventing many degenerative diseases and cancers. The antioxidant activity of peanut extracts is based on both reducing mechanisms (Chuenchom et al., 2016) and radical scavenging (Ballard et al., 2009). Various types of antioxidant tests are used to examine different mechanisms. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) are antioxidant tests that measure the anti-radical activity of bioactive compounds whereas the ferric reducing antioxidant power (FRAP) assay measures the reducing ability of compounds to metals or ion complexes (Jones et al., 2017). The oxygen radical absorbance capacity (ORAC) assay measures the anti-radical activity involving transferring a hydrogen atom from peroxyl radicals.

The total phenolic content (TPC) and antioxidant activities of peanuts have been reported in different sources and cultivars (Talcott et al., 2005; Lee et al., 2004), different parts of the peanut (Yu et al., 2005; Limmongkon et al., 2017; Swatsitang et al., 2011), or different solvents (Lee et al., 2004; Swatsitang et al., 2011; Talcott et al., 2005). However, all studies based on unoptimized extraction methods, which might not reflect the actual values. Therefore, this study determined the optimal extraction conditions that would provide the highest antioxidant potential and reveal the correct antioxidant capacity of Thai peanut kernels, using experimental designs. In the screening step, FFD were used for screening significant factors among solvent volume, ethanol percentage in extraction solvent, extraction time, and extraction temperature with TPC as a dependent variable since it is positively correlated with antioxidant activity of the extract (Diaz et al., 2012; Marrassini et al., 2018). The significant factors were then optimized by center-faced central composite design using the antioxidant capacity of the extracts as responses and quantifying TPC at optimized conditions.

## 2. MATERIALS AND METHODS

### 2.1 Materials

ABTS and Trolox® were purchased from Sigma-Aldrich, USA. Gallic acid, Folin–Ciocalteu's (FC) reagent, DPPH, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2-2'-azobis(2-amino-propane) dihydrochloride (AAPH), and fluorescein sodium were procured from Sisco Research Laboratories, India. Sodium carbonate ($Na_2CO_3$), potassium persulphate ($K_2S_2O_8$), sodium dihydrogen orthophosphate, di-sodium hydrogen orthophosphate, sodium acetate trihydrate and iron(III) chloride hexahydrate ($FeCl_3.6H_2O$) were purchased from Ajax Finechem, Australia. A Victor Nivo (PerkinElmer®, UK) microplate reader was used.

### 2.2 Preparation of samples

Peanuts (cultivar Kalasin-I) were purchased from a local market at Phutthamonthon Sai 2 Road, Bangkok, Thailand. They were cleaned and unshelled. Raw kernels were blended and dried at 60°C. Coarse powder was ground into fine powder by mortar and pestle. The dry peanut powder was kept at 4-8°C until extraction.

### 2.3 SLE

A mass of 1 g of peanut powder was extracted in a temperature-controlled water bath (J.P. Selecta, Spain) by assigning the independent variables, including percentage of ethanol content, extraction solvent volume, temperature, and extraction time according to the experimental design table (Table 1 in screening step and Table 2 in optimization step). Crude extracts were filtered and dried under a rotary evaporator. The test solution for the subsequent tests was prepared by dissolving the extract in 10 mL of methanol.

### 2.4 Determination of TPC

The test solution was diluted (5 times) with water before 20 µL of assay preparation was reacted with 20 µL of FC's reagent on a 96-well plate for 5 min. Then, 160 µL of 5% w/v $Na_2CO_3$ solution was added and the mixture kept at 40°C for 20 min. Absorbance was measured at 700 nm and TPC was calculated as milligram gallic acid equivalent per gram of dried sample (mgGAE/g).

### 2.5 DPPH anti-radical activity determination

The test solution was diluted (10 times) with methanol before 100 µL of the diluted solution was reacted with 100 µL of 0.2 mM DPPH solution on a 96-well plate. After 30 min, absorbance was measured at 515 nm. DPPH anti-radical activity was calculated as milligram Trolox® equivalent per gram dry sample (mgTE/g) by comparing the absorbance values at 515 nm.

### 2.6 ABTS anti-radical activity determination

The test solution was diluted 50-times with methanol. $ABTS^+$ working solution was derived from diluting the 14 h-aged mixture of 2.4 mM $K_2S_2O_8$ solution and 7 mM ABTS until the absorbance value at 734 nm was 0.8 ± 0.02 AU. A volume of 50 µL of the diluted test solution was reacted with 100 µL of $ABTS^+$ working solution on a 96-well plate and kept for 30 min. ABTS anti-radical activity was calculated as milligram Trolox® equivalent per gram dry sample (mgTE/g) by comparing the absorbance values at 734 nm.

### 2.7 FRAP determination

The FRAP reagent was prepared by mixing 20 mM $FeCl_3$, 10 mM TPTZ solution, UPW and 300 mM acetate buffer, pH 3.6 (1:1:1.1:10) at 37°C. The test solution was diluted (10 times) with UPW before 50 µL of the diluted test solution was mixed with 150 µL of FRAP reagent on a 96-well plate and incubated for 30 min. The results were calculated as milligram Trolox® equivalent per gram dry sample (mgTE/g) by comparing the absorbance values at 593 nm.

### 2.8 ORAC determination

The test solution was diluted (100 times) with 75 mM phosphate buffer, pH 7.4 before 25 µL of diluted test solution was mixed with 150 µL of 0.084 µM fluorescein solution in an opaque 96-well plate and incubated at 37°C. After 15 min, 25 µL of 153 mM AAPH solution was pipetted

and fluorescence values were measured at Ex.485/Em.535 nm, 37°C every minute for 60 cycles. Area under the decay curve was compared between samples and Trolox® standard solution and reported as milligram Trolox® equivalent per gram dry sample (mgTE/g).

## 2.9 Experimental design

### 2.9.1 FFD

FFD were performed for the screening of four significant variables, each at two levels with 16 run orders including three replicates at center points. The independent variables included solvent volume ($X_1$; 10, 50 mL), percentage of ethanol in extraction solvent ($X_2$; 20%, 80%), extraction temperature ($X_3$; 30, 70°C), and extraction time ($X_4$; 30, 120 min). The center point was set at 30 mL of solvent volume, 50% ethanol, 50°C and 75 min extraction time. The dependent variables were TPC ($Y$). The range and levels of independent variables in the un-coded unit as well as the experimental values for each run order are shown in Table 1.

Analysis of variance (ANOVA) was performed using the Minitab18™ statistical software. Multiple regression method was used to generate the mathematical models for each response. A linear polynomial regression model, given by Equation (1) below, was fitted to the experimental data:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i<j=1}^{k} \beta_{ij} x_i x_j \quad (1)$$

where, $Y$ is the dependent variable; $\beta_0$ is the constant term; $k$ number of variables; $\beta_i$ is the coefficients of linear terms; $\beta_{ij}$ is the coefficients of interaction terms.

The significance of each independent variable for each response was evaluated by the F-test and $p$<0.05 was considered significant. Factors with significant effect on the response variable were included in the optimization step.

**Table 1.** Solid-liquid extraction full factorial experimental design and its response

| Run order | Independent variable | | | | Response |
|---|---|---|---|---|---|
| | Solvent volume (mL) | Ethanol (%) | Temperature (°C) | Time (min) | Total phenolic content (mgGAE/g) |
| 1 | 10 | 80 | 70 | 30 | 0.8688 |
| 2 | 50 | 80 | 30 | 30 | 0.3443 |
| 3 | 50 | 80 | 70 | 30 | 1.0871 |
| 4 | 10 | 80 | 70 | 120 | 1.0562 |
| 5 | 50 | 20 | 30 | 30 | 1.0069 |
| 6 | 30 | 50 | 50 | 75 | 1.6410 |
| 7 | 50 | 20 | 30 | 120 | 1.2033 |
| 8 | 10 | 20 | 30 | 120 | 0.8244 |
| 9 | 10 | 80 | 30 | 30 | 0.1476 |
| 10 | 10 | 20 | 30 | 30 | 0.5367 |
| 11 | 50 | 80 | 70 | 120 | 1.3051 |
| 12 | 50 | 20 | 70 | 30 | 2.6081 |
| 13 | 50 | 80 | 30 | 120 | 0.3658 |
| 14 | 30 | 50 | 50 | 75 | 1.3436 |
| 15 | 30 | 50 | 50 | 75 | 1.5976 |
| 16 | 10 | 20 | 70 | 30 | 1.7399 |
| 17 | 10 | 20 | 70 | 120 | 1.7508 |
| 18 | 50 | 20 | 70 | 120 | 2.4762 |
| 19 | 10 | 80 | 30 | 120 | 0.4955 |

### 2.9.2 CCD

The independent variables were the same as those in the FFD study as all of them had significant effect. There were 31 total experimental runs of center-faced CCD, which comprised four factors, and two levels with seven center points. The dependent variables included TPC ($Z_1$), DPPH ($Z_2$), ABTS ($Z_3$), FRAP ($Z_4$), and ORAC ($Z_5$) antioxidant capacity. The un-coded range and levels of independent variables including experimental values are presented in Table 2. The dependent variables were elaborated to a given second-order polynomial model, Equation (2):

$$Z_i = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j=1}^{k} \beta_{ij} x_i x_j \quad (2)$$

where, $Z_i$ is the dependent variable; $\beta_0$ is the constant term; $k$ number of variables; $\beta_i$ is the coefficients of linear terms; $\beta_{ii}$ is the coefficients of quadratic terms; $\beta_{ij}$ is the coefficients of interaction terms.

Multiple regression analysis was conducted using Minitab18. The extraction condition giving the highest antioxidant activity was regarded as the optimum.

## 2.10 Statistical analysis

The significance, validity and reliability of the proposed model, were assessed by ANOVA. The model variance was determined by multiple coefficients of determination ($R^2$). The influences of factors on the response variables were examined by the standardized main effect plots and on Pareto's charts, with significance level of 0.05. Correlations between each pair of responses were analyzed by Pearson's method.

**Table 2.** Solid-liquid extraction central composite experimental design and its responses

| Run order | Independent variable | | | | Response | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Solvent volume (mL) | Ethanol (%) | Tempera-ture (°C) | Time (min) | TPC (mgGAE/g) | FRAP (mgTE/g) | DPPH (mgTE/g) | ABTS (mgTE/g) | ORAC (mgTE/g) |
| 1 | 75 | 10 | 80 | 60 | 3.4730 | 1.5493 | 0.9802 | 6.4038 | 14.3965 |
| 2 | 25 | 10 | 80 | 180 | 2.9488 | 1.3658 | 0.8769 | 6.2069 | 12.9682 |
| 3 | 75 | 10 | 80 | 180 | 3.4499 | 1.5814 | 1.0768 | 6.5382 | 15.7068 |
| 4 | 25 | 10 | 60 | 60 | 2.8738 | 1.1185 | 0.7657 | 6.0205 | 11.8995 |
| 5 | 75 | 30 | 60 | 60 | 2.9017 | 1.3205 | 1.0548 | 6.4601 | 11.7425 |
| 6 | 25 | 30 | 80 | 60 | 3.0793 | 1.3969 | 1.0860 | 6.5269 | 14.5162 |
| 7 | 50 | 30 | 70 | 120 | 3.3343 | 1.6419 | 1.2582 | 6.7509 | 17.5218 |
| 8 | 25 | 10 | 60 | 180 | 3.0441 | 1.2560 | 0.8394 | 6.1413 | 12.9554 |
| 9 | 75 | 30 | 80 | 180 | 3.3995 | 1.8235 | 1.3664 | 6.8888 | 17.7787 |
| 10 | 75 | 30 | 80 | 60 | 3.6934 | 1.6691 | 1.2878 | 6.6458 | 15.0488 |
| 11 | 50 | 10 | 70 | 120 | 3.5947 | 1.5984 | 0.9870 | 6.6959 | 15.1708 |
| 12 | 50 | 20 | 70 | 120 | 3.4584 | 1.5464 | 1.0696 | 6.7227 | 13.9065 |
| 13 | 50 | 20 | 70 | 120 | 3.4465 | 1.5505 | 1.1438 | 6.7227 | 14.6275 |
| 14 | 25 | 30 | 60 | 60 | 2.7433 | 1.2654 | 0.9338 | 6.3805 | 13.5717 |
| 15 | 50 | 20 | 80 | 120 | 3.1759 | 1.5804 | 1.1671 | 6.6195 | 15.0847 |
| 16 | 25 | 10 | 80 | 60 | 3.0810 | 1.3193 | 0.8431 | 6.1205 | 15.4365 |
| 17 | 50 | 20 | 70 | 120 | 3.6179 | 1.5721 | 1.0647 | 6.5632 | 14.5516 |
| 18 | 25 | 30 | 60 | 180 | 3.1356 | 1.4048 | 1.0180 | 6.2102 | 13.7678 |
| 19 | 75 | 10 | 60 | 60 | 2.9344 | 1.4210 | 0.9238 | 6.4258 | 9.9260 |
| 20 | 50 | 20 | 70 | 60 | 3.4166 | 1.5304 | 1.1082 | 6.5506 | 11.2460 |
| 21 | 50 | 20 | 70 | 180 | 3.3820 | 1.5577 | 1.1318 | 6.7359 | 12.9469 |
| 22 | 50 | 20 | 70 | 120 | 3.5894 | 1.6146 | 1.0872 | 6.7415 | 12.9076 |
| 23 | 50 | 20 | 70 | 120 | 3.4613 | 1.5836 | 1.1103 | 6.8078 | 12.7981 |
| 24 | 50 | 20 | 70 | 120 | 3.6021 | 1.6391 | 1.1296 | 6.5846 | 12.2018 |
| 25 | 25 | 30 | 80 | 180 | 3.1541 | 1.5124 | 1.1396 | 6.4362 | 14.9876 |
| 26 | 75 | 30 | 60 | 180 | 3.5484 | 1.5220 | 1.1156 | 6.3926 | 12.3682 |
| 27 | 25 | 20 | 70 | 120 | 3.1555 | 1.4562 | 0.9597 | 6.5656 | 10.6331 |
| 28 | 75 | 20 | 70 | 120 | 3.7378 | 1.6281 | 1.1163 | 6.9521 | 11.9614 |
| 29 | 75 | 10 | 60 | 180 | 3.5449 | 1.5772 | 0.9355 | 6.2065 | 11.2126 |
| 30 | 50 | 20 | 70 | 120 | 3.4526 | 1.5417 | 1.0572 | 6.5221 | 11.0186 |
| 31 | 50 | 20 | 60 | 120 | 3.2364 | 1.5164 | 1.0035 | 6.3781 | 9.4519 |

Note: TPC = total phenolic content, FRAP = ferric reducing antioxidant power, DPPH = 2,2-diphenyl-1-picrylhydrazyl, ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), ORAC = oxygen radical absorbance capacity

## 3. RESULTS AND DISCUSSION

### 3.1 Screening design

High variation in TPC from inappropriate conditions is shown in Table 1. Pareto charts and main effect plots of dependent variables in Figure 1 indicate that all factors (solvent volume ($X_1$), % ethanol content ($X_2$), extraction temperature ($X_3$), and extraction time ($X_4$)) had significant effects on SLE ($p$<0.05) on TPC ($Z_1$). From Pareto charts, the most influential variable on TPC level was extraction temperature, followed by ethanol percentage content, solvent volume and extraction time, respectively. The relationship of independent variables and TPC, as shown by the main effect plots, was found to be in a positive direction, except for ethanol percentage content. These findings implied that, within the experimental range, an increase in extraction temperature, solvent volume and extraction time resulted in higher TPC levels in the extracts. These findings are attributed to the fact that an increase in extraction temperature decrease the viscosity of the extraction solvent, promoting molecular motions, which increase the solubility of the solute (Yothipitak et al., 2008). Increasing the solvent volume gave rise to an increase in the contact area between sample and extraction solvent, which enhanced the solubility of bioactive compounds in plant cells (Yothipitak et al., 2008). The observed positive correlation between extraction time and values of the response variables is not surprising as long extraction time increased the contact time between the solvent and the solute (Nepote et al., 2005). Although increasing the extraction time increased the extracted TPC level, this factor showed a smaller effect than other factors as indicated by the less-steep slope of extraction time in the main effect plot. However, increasing the percentage of ethanol content reduced the level of TPC extracted. This is expected because higher percentage of ethanol content in aqueous solution resulted in lower solvent polarity, which might not be suitable for extraction of polar phenolic acids (Nawaz et al., 2018). This finding is supported by that of a previous study in which high ethanol concentration in extraction solvent lead to a decrease in extracted total polyphenols (Diaz et al., 2012).

The optimum conditions that provided the highest TPC in the FFD study consisted of a solvent volume of 50 mL, 20% v/v ethanol content, extraction temperature of 70°C, and extraction time of 120 min, which were applied as the zero level in the optimization study.

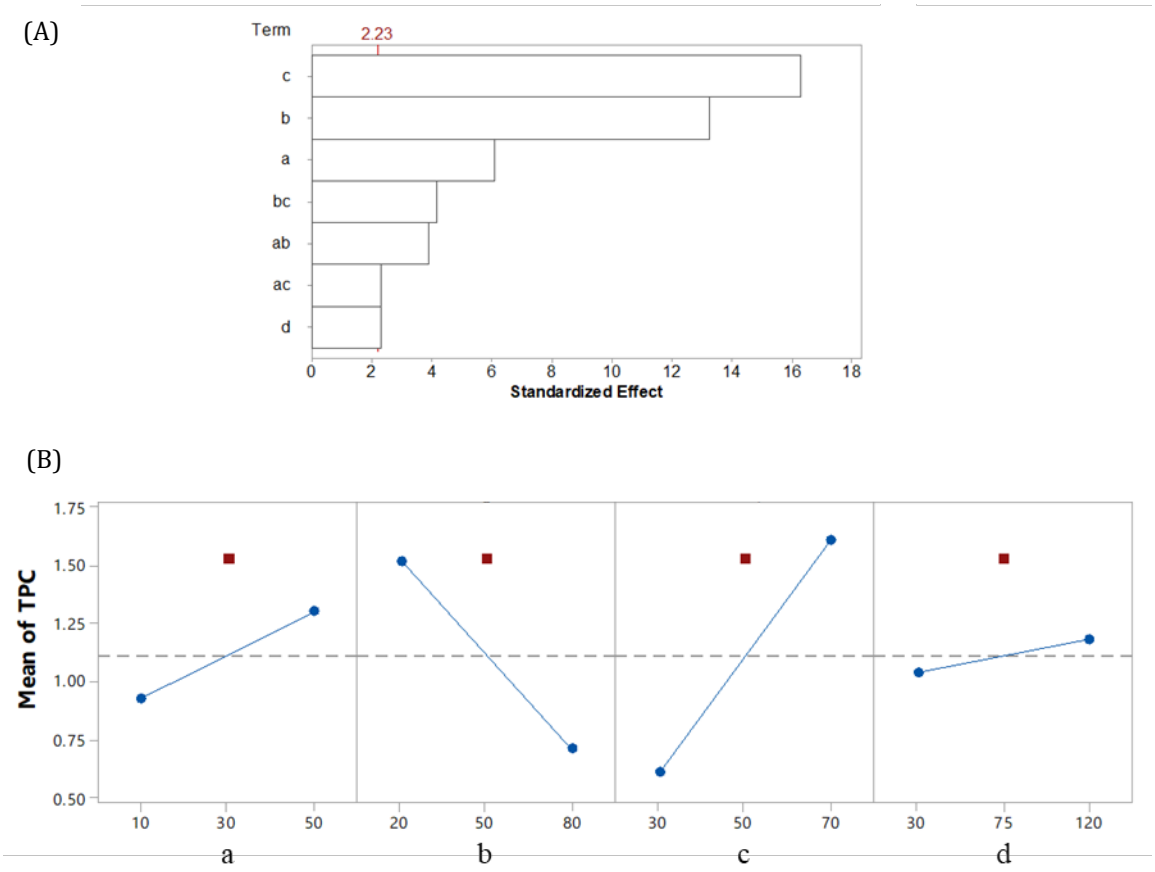


**Figure 1.** (A) Pareto charts and (B) main effect plots for total phenolic content
Note: a = solvent volume (mL), b = ethanol content (%), c = extraction temperature (°C), d = extraction time (min)

## 3.2 Optimization study

### 3.2.1 Model fitting and statistical analysis

The significant variables from the FFD study were newly assigned ranges and levels that conformed to the center-faced CCD study. Table 2 presents experimental data and un-coded values of the independent variables including solvent volume ($X_1$; 25, 75 mL), ethanol percentage ($X_2$; 10%, 30%), extraction temperature ($X_3$; 60°C, 80°C), and extraction time ($X_4$; 60, 180 min). The quadratic mathematical models of each response obtained from multiple regression analysis are shown in the form of un-coded values as follows:

TPC = −12.01 + 0.00025 %EtOH + 0.00771 Volume + 0.3850 Temp + 0.02106 Time − 0.002495 Temp* Temp − 0.000016 Time*Time − 0.000229 Temp*Time

DPPH = −0.468 − 0.00818 %EtOH + 0.00890 Volume + 0.0270 Temp + 0.000478 Time − 0.000109 Volume*Volume − 0.000210 Temp*Temp + 0.000278 %EtOH*Temp + 0.000073 Volume*Temp

ABTS = −9.02 + 0.01074 %EtOH + 0.00512 Volume + 0.4254 Temp − 0.002968 Temp*Temp

FRAP = −1.85 − 0.0236 %EtOH + 0.01359 Volume + 0.0735 Temp + 0.00462 Time + 0.000208 %EtOH*%EtOH − 0.000092 Volume*Volume − 0.000509 Temp*Temp − 0.000015 Time*Time + 0.000279 %EtOH*Temp

ORAC = 14.54 − 1.266 %EtOH + 0.032 Volume + 0.0117 Temp + 0.0541 Time + 0.03325 %EtOH*%EtOH − 0.002758 Volume*Volume - 0.000257 Time*Time + 0.00299 Volume*Temp + 0.000279 Volume*Time

The ANOVA tables of all experimental data for TPC and antioxidant response are summarized in Tables 3 and 4, respectively. The adjusted sums of squares and adjusted mean square that reflect the variation for different components of the model and are necessary for calculation the *p*-value are also shown. The significance of the effects of variables was considered from the F-value and *p*-value. The greater F-value and the smaller *p*-value indicate greater significance of effects of the variables (Zhang et al., 2014). The computed models of all dependent variables were significant ($p<0.05$), suggesting that the regression model fitted well with the data.

All linear terms were statistically significant for the DPPH and FRAP assays ($p<0.05$) whereas the effects of percentage ethanol content on TPC, extraction time on

ABTS test and solvent volume and extraction time on ORAC assays were non-significant ($p$>0.05). Some second-order terms (Time*Time, Temp*Temp, and %EtOH*%EtOH) were also non-significant ($p$>0.05), which indicated that a linear model is more suitable relationship between the independent variables and the response variables. However, the second-order terms were included in the models as they were important in predicting values of the response variables. The $R^2$(adj) values of computed models for TPC and all antioxidant tests ranged from 74.08% to 96.13%, which demonstrated that the models explained moderate to high proportions of the variations in the response variables. Meanwhile, the $R^2$(pred) values in the range of 66.71%-95.14% also implied a moderate to high degree of correlation between experimental values and predicted values (Zulueta et al., 2009). The DPPH assay values showed the best fit to the mathematical model. The lowest values of $R^2$(adj) and $R^2$(pred) obtained for the ORAC assay show that the model poorly explains variation in ORAC assay values. A large data variability has been mentioned as one of the disadvantages of the ORAC assay.

**Table 3.** ANOVA tables of predicted models of total phenolic content response

| Response | Source | Adj SS[a] | Adj MS[b] | F-value | $p$-value |
|---|---|---|---|---|---|
| TPC | Model | 1.85279 | 0.264684 | 19.54 | 0.000 |
| | %EtOH | 0.00011 | 0.000112 | 0.01 | 0.928 |
| | Volume | 0.66798 | 0.667975 | 49.31 | 0.000 |
| | Temp | 0.12372 | 0.123720 | 9.13 | 0.006 |
| | Time | 0.11058 | 0.110575 | 8.16 | 0.009 |
| | Temp*Temp | 0.21593 | 0.215933 | 15.94 | 0.001 |
| | Time*Time | 0.01100 | 0.011000 | 0.81 | 0.377 |
| | Temp*Time | 0.30091 | 0.300907 | 22.21 | 0.000 |
| | Lack-of-fit | 0.27326 | 0.016074 | 2.52 | 0.129 |
| | $R^2$(adj) = 81.22% | | $R^2$(pred) = 71.63% | | |

Note: [a] = adjusted sums of squares, [b] = adjusted mean square, TPC = total phenolic content

### 3.2.2 Analysis of response surfaces and model verification

The response surface plots of significant variables were selected to evaluate the power of two independent variables on antioxidant activity, while the other two independent variables were fixed at zero level (Figure 2).

The influence of all factors on TPC and antioxidant activities by DPPH, ABTS and FRAP assays, in both linear and quadratic models, was investigated. For TPC, the ethanol content of 10%-30% was not effective. Increasing the solvent volume increased TPC level in a linear direction, whereas increasing extraction temperature and time increased TPC level in a quadratic direction, which indicated that extraction temperature and time could be increased only to a specific point beyond which TPC values would begin to decrease (Figure 2A). This finding is similar to that of Ali et al. (2018). With respect to antioxidant capacities in the range studied, increasing the percentage ethanol content, solvent volume, extraction temperature and extraction time, often increased the antioxidant activity of the extracts by DPPH, ABTS and FRAP assays (Figure 2B-D). In contrast, ORAC antioxidant activity decreased when the percentage of ethanol content was increased from 10% to 20%, then the activity was increased when the ethanol content was higher than 20%. (Figure 2E). The optimal conditions for obtaining the highest antioxidant capacity by DPPH, ABTS, FRAP and ORAC assays were found to be the following: 30% v/v ethanol content, extraction solvent volume of 68 mL, extraction temperature of 78°C, and extraction time of 153 min.

The validity of the models was tested through six replications performed at the optimization point, and the experimental values within the prediction ranges. The predicted values, actual values and prediction intervals for TPC and all antioxidant tests are presented in Table 5. The experimental values of all responses were within predicted values (95% PI). This indicated the validity of the computed models in the prediction of those responses. The levels of TPC extracted under the optimum conditions in this study were nearly three-times higher than those found in the antioxidant activity by ORAC assay was higher than that of Talcott et al. (2005) who used a different solvent and no experimental design. Our findings highlighted the advantage of using experimental design in optimization conditions for SLE of peanut kernel. Moreover, the highest antioxidant activities of peanut extracted from the ORAC assay might be caused by the fact that the ORAC assay is the only method that measures both inhibition time and degree of inhibition. This was the special characteristic result in a complete measurement whereas the other assays measured the activity only at a specific time (Tabart et al., 2009). The antioxidant value obtained by the ABTS assay was greater than that of the DPPH assay, which agreed with the findings of previous studies of Chuenchom et al. (2016). The reason for this might be $p$-coumaric acid, which is dominantly available in peanuts and possesses a significant scavenging capacity of ABTS radicals rather than DPPH radicals (Chuenchom et al., 2016).

### 3.3 Pearson's correlation analysis between TPC and antioxidant capacities

The values of each experimental run of TPC and antioxidant activity tests were used to examine the correlation of the data by Pearson's correlation analysis. The results showed a significant positive correlation between TPC and antioxidant activity of the extracts. They showed a high positive correlation between TPC and FRAP (Pearson's correlation = 0.813), and moderate positive correlation with DPPH and ABTS tests (Pearson's correlation = 0.543 and 0.603, respectively) (Table 6). These results corroborate those of previous studies (Lou et al., 2014; Rajurkar and Hande, 2011). These results imply that phenolic acids contributed to antioxidant activity of peanut extracts. The ORAC assay exhibited a non-significant correlation with TPC and other

antioxidant tests except for DPPH, which showed a mild positive correlation (Pearson's correlation = 0.473). This discrepancy could be attributed to the difference in principles of determination whereby the ORAC assay is the only method that measures the response kinetically whereas the others measure only at specific time point.

**Table 4.** ANOVA table of predicted models of antioxidant response

| Response | Source | Adj SS[a] | Adj MS[b] | F-value | *p*-value |
|---|---|---|---|---|---|
| DPPH | Model | 0.508938 | 0.063617 | 94.23 | 0.000 |
| | %EtOH | 0.229345 | 0.229345 | 339.72 | 0.000 |
| | Volume | 0.108113 | 0.108113 | 160.14 | 0.000 |
| | Temp | 0.084570 | 0.084570 | 125.27 | 0.000 |
| | Time | 0.014826 | 0.014826 | 21.96 | 0.000 |
| | Volume*Volume | 0.016166 | 0.016166 | 23.95 | 0.000 |
| | Temp*Temp | 0.001524 | 0.001524 | 2.26 | 0.147 |
| | %EtOH*Temp | 0.012377 | 0.012377 | 18.33 | 0.000 |
| | Volume*Temp | 0.005358 | 0.005358 | 7.94 | 0.010 |
| | Lack-of-Fit | 0.007987 | 0.000499 | 0.44 | 0.913 |
| | $R^2$(adj) = 96.13% | | $R^2$(pred) = 95.14% | | |
| ABTS | Model | 1.34190 | 0.33547 | 29.75 | 0.000 |
| | %EtOH | 0.20750 | 0.20750 | 18.40 | 0.000 |
| | Volume | 0.29519 | 0.29519 | 26.17 | 0.000 |
| | Temp | 0.17425 | 0.17425 | 15.45 | 0.001 |
| | Temp*Temp | 0.66496 | 0.66496 | 58.96 | 0.000 |
| | Lack-of-Fit | 0.22309 | 0.01115 | 0.95 | 0.575 |
| | $R^2$(adj) = 79.31% | | $R^2$(pred) = 74.38% | | |
| FRAP | Model | 0.563499 | 0.062611 | 20.63 | 0.000 |
| | %EtOH | 0.032905 | 0.032905 | 10.84 | 0.003 |
| | Volume | 0.221512 | 0.221512 | 72.97 | 0.000 |
| | Temp | 0.108314 | 0.108314 | 35.68 | 0.000 |
| | Time | 0.056717 | 0.056717 | 18.68 | 0.000 |
| | %EtOH*%EtOH | 0.001123 | 0.001123 | 0.37 | 0.550 |
| | Volume*Volume | 0.008491 | 0.008491 | 2.80 | 0.109 |
| | Temp*Temp | 0.006736 | 0.006736 | 2.22 | 0.151 |
| | Time*Time | 0.007936 | 0.007936 | 2.61 | 0.121 |
| | %EtOH*Temp | 0.012438 | 0.012438 | 4.10 | 0.056 |
| | Lack-of-Fit | 0.055535 | 0.003702 | 2.71 | 0.113 |
| | $R^2$(adj) = 85.48% | | $R^2$(pred) = 75.45% | | |
| ORAC | Model | 101.067 | 11.2296 | 10.53 | 0.000 |
| | %EtOH | 7.516 | 7.5156 | 7.05 | 0.015 |
| | Volume | 0.020 | 0.0196 | 0.02 | 0.893 |
| | Temp | 46.814 | 46.8138 | 43.88 | 0.000 |
| | Time | 2.652 | 2.6515 | 2.49 | 0.130 |
| | %EtOH*%EtOH | 31.485 | 31.4852 | 29.51 | 0.000 |
| | Volume*Volume | 8.460 | 8.4597 | 7.93 | 0.010 |
| | Time*Time | 2.434 | 2.4336 | 2.28 | 0.146 |
| | Volume*Temp | 8.951 | 8.9512 | 8.39 | 0.009 |
| | Volume*Temp | 2.803 | 2.8034 | 2.63 | 0.120 |
| | Lack-of-Fit | 12.058 | 0.8039 | 0.47 | 0.892 |
| | $R^2$(adj) = 74.08% | | $R^2$(pred) = 66.71% | | |

Note: [a] = adjusted sums of squares, [b] = adjusted mean square, DPPH = 2,2-diphenyl-1-picrylhydrazyl, ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), FRAP = ferric reducing antioxidant power, ORAC = oxygen radical absorbance capacity

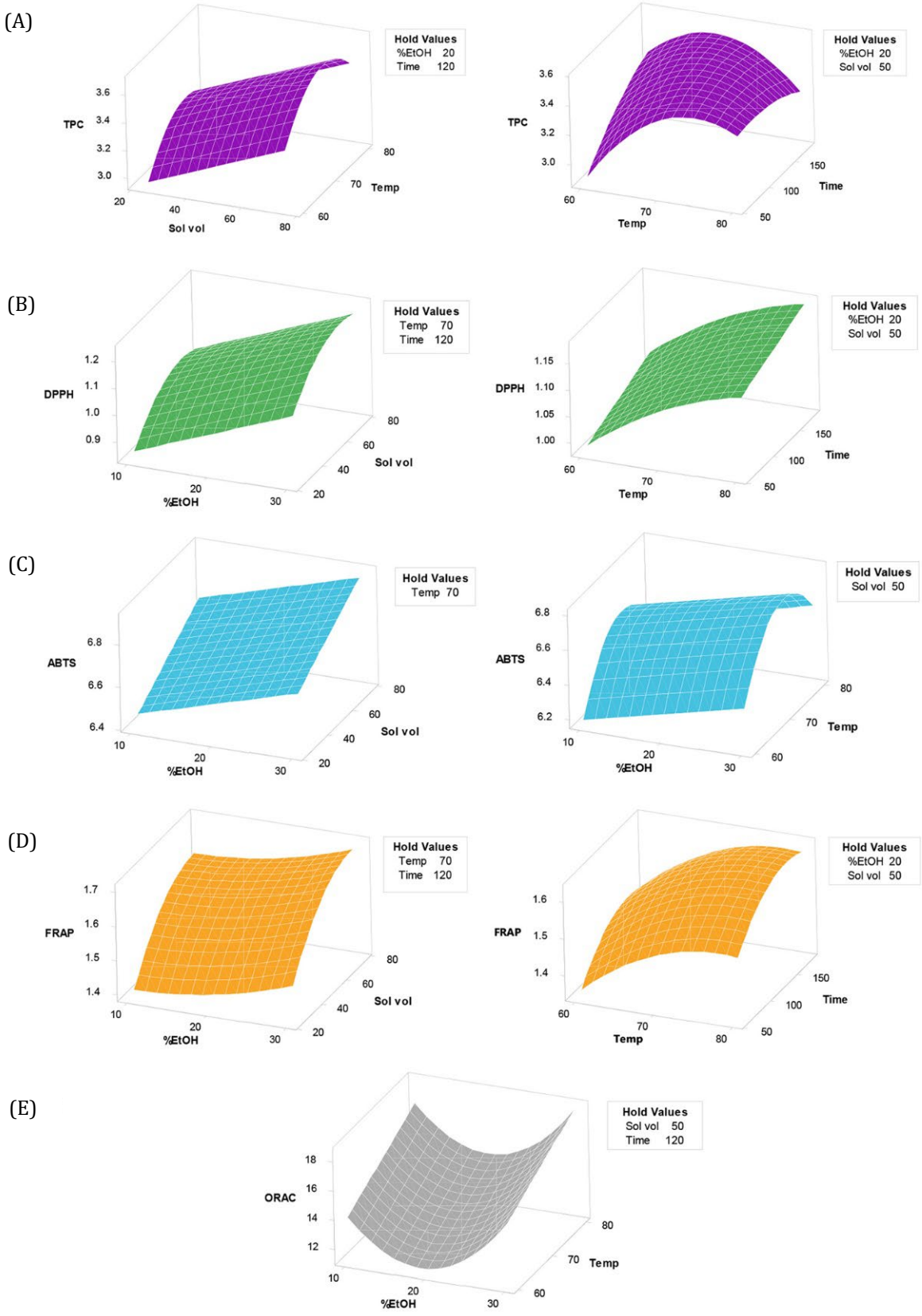


**Figure 2.** Response surface plots of significant variables for different antioxidant assays: TPC (A), DPPH (B), ABTS (C), FRAP (D), and ORAC (E)
Note: TPC = total phenolic content, FRAP = ferric reducing antioxidant power, DPPH = 2,2-diphenyl-1-picrylhydrazyl, ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), ORAC = oxygen radical absorbance capacity

**Table 5.** Prediction values and actual values obtained from optimized conditions

| Response | Predicted | Actual (±SD) | 95% Prediction intervals |
|---|---|---|---|
| TPC (mgGAE/g) | 3.5030 | 3.5915 (±0.0720) | (3.2380, 3.7680) |
| ORAC (mgTE/g) | 17.742 | 17.957 (±0.954) | (15.275, 20.209) |
| FRAP (mgTE/g) | 1.7663 | 1.7669 (±0.0676) | (1.6343, 1.8983) |
| ABTS (mgTE/g) | 6.7914 | 6.7708 (±0.9540) | (6.5575, 7.0252) |
| DPPH (mgTE/g) | 1.3244 | 1.3469 (±0.0244) | (1.2649, 1.3839) |

Note: TPC = total phenolic content, FRAP = ferric reducing antioxidant power, DPPH = 2,2-diphenyl-1-picrylhydrazyl, ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), ORAC = oxygen radical absorbance capacity

**Table 6.** Pearson's correlation of TPC, DPPH, ABTS, FRAP and ORAC values

| | TPC | DPPH | ABTS | FRAP |
|---|---|---|---|---|
| DPPH | 0.543* | | | |
| ABTS | 0.603* | 0.784* | | |
| FRAP | 0.813* | 0.818* | 0.780* | |
| ORAC | 0.148 | 0.473* | 0.287 | 0.340 |

Note: *$p$<0.01, TPC = total phenolic content, FRAP = ferric reducing antioxidant power, DPPH = 2,2-diphenyl-1-picrylhydrazyl, ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), ORAC = oxygen radical absorbance capacity

## 4. CONCLUSION

The optimum conditions for SLE from peanut kernel that provided the most effective isolation and gave the highest level of antioxidant activity were successfully determined. Moreover, extraction under the optimum conditions prevented wasting extraction time and resources. The generated response surface models were valid and reliable in the prediction of dependent variables. The optimized conditions with the highest antioxidant capacities of DPPH, ABTS, FRAP, and ORAC assays were at 30% v/v ethanol content, extraction solvent of 68 mL, extraction temperature of 78°C, and extraction time of 153 min. The effect of extraction conditions on response variables was explained by response surface plots, which showed that increasing of all factor levels often resulted in increasing TPC and antioxidant values. At optimum conditions, higher levels of TPC were obtained, indicating the advantage of using experimental designs for optimization. Additionally, there was a moderate to strong positive relationship between TPC and antioxidant activity of the extracts. Finally, the full realization of the anti-oxidizing potential of Thai peanuts could be realized from the determination of the optimum conditions for isolating anti-oxidizing agents through the experimental design strategy.

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