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Optimizing the Extraction of Phenolic Antioxidants from Peanut Skins Using Response Surface Methodology

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Peanut skins are a byproduct of peanut blanching operations and contain high levels of phenolic antioxidants. The effect of solvent type (methanol MeOH, ethanol EtOH, and water), concentration (0, 30, 60, 90%), temperature (30, 45, 60 °C), and time (10, 20, 30 min) on total phenolic content (TPC), oxygen radical absorbance capacity (ORAC) level, and resveratrol content of peanut skins was investigated. Response surface methodology was used to estimate the optimum extraction conditions for each solvent. EtOH extracts had the highest TPC followed by MeOH and water. The maximum predicted TPC under the optimized conditions (30.8%, 30.9 °C, 12 min) was 118 mg of gallic acid equivalents (GAE)/g of skins. MeOH extracts had the highest ORAC activity of 2149 μmol of TE/g followed by EtOH and water under the optimized conditions of 30% MeOH, 52.9 °C and 30 min. Resveratrol was identified in MeOH extracts but was not found in samples extracted with EtOH or water.

KEYWORDS: Polyphenols; antioxidants; peanut skins; resveratrol; ORAC; extraction; response surface methodology

INTRODUCTION

Peanut skins are a low-value byproduct of the peanut processing industry. The world production of peanut skins can be estimated at over 750 000 tons annually (*1*). Currently, this agricultural waste material is either incinerated or sold as animal feed for less than one cent per pound (*2*). However, peanut skins contain high concentrations of natural antioxidants, namely, polyphenolic compounds that can be extracted and potentially utilized in a variety of food and pharmaceutical applications. Peanut skins have been used for centuries in traditional Chinese medicine to treat chronic hemorrhage and bronchitis (*3*). Despite these beneficial properties, peanut skins remain an underutilized natural resource. More research is needed to develop efficient extraction procedures for the removal of these health-promoting compounds from peanut skins.

Phenolics derived from other natural sources such as grapes, wine, and tea have been widely studied by a number of researchers. Grape skins, for example, have been shown to contain high concentrations of resveratrol (*4*, *5*). Resveratrol is a stilbene phytoalexin that is produced by plants in response to a microbial challenge or some other exogenous stimuli (*6*). It is reported to have a wide range of beneficial effects including blocking platelet aggregation (*7*), vasodilatation (*8, 9*), and prevention of cancer (*10*). It is believed that resveratrol is the compound primarily responsible for the "French Paradox", which refers to the French having lower incidences of cardiovascular disease despite consuming a diet high in saturated fats due to their frequent consumption of red wine (*11*). Sobolev and Cole (*6*) reported that commercial peanuts and peanut products contained resveratrol at levels ranging from 0.06 ppm in roasted peanuts to 5.1 ppm in boiled peanuts.

The early work of Karchesy and Hemingway (*12*) employed several techniques (NMR, TLC, and HPLC) to separate and identify procyanidin compounds in peanut skin extracts. Among the compounds identified were the flavan-3-ols catechin, epicatechin, and epicatechin-(4β→8; 2β→O→7)-catechin. Lou et al. (*13*) reported the presence of six A-type proanthocyanidins from the water-soluble fraction of peanut skin extracts. Of those six compounds, three were deemed new and were identified as epicatechin-(2β→O→7, 4β→6)-catechin, epicatechin-(2β→O→7, 4β→6)-*ent*-catechin and epicatechin-(2β→O→7, 4β→6)-*ent*-epicatechin. Yu et al. (*14*) observed three classes of compounds in peanut skin extracts: (1) phenolic acids including chlorogenic and caffeic acids; (2) flavonoids including epigallocatechin (EGC) and epicatechin; and (3) stilbene (resveratrol). The presence of other flavonoids including quercetin and luteolin in peanuts and peanut byproducts has also been reported (*15*−*17*).

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Table 1. The Coded Values and Corresponding Actual Values of the Optimization Parameters Used in the Response Surface Analysis

code	solvent concentration (% v/v)	temperature (°C)	time (min)
	MeOH and EtOH		
−1	30	30	10
0	60	45	20
1	90	60	30
	water		
−1		30	10
0		45	20
1		60	30

Table 2. Experimental Design for Response Surface Analysis of Peanut Skins Extracted Using MeOH and EtOH in Terms of Coded Values

experiment no.	solvent concentration (% v/v)	temperature (°C)	time (min)
1	−1	−1	1
2	−1	1	1
3	0	0	−1
4	0	0	0
5	0	0	0
6	0	−1	0
7	1	−1	−1
8	0	0	0
9	1	−1	1
10	1	1	1
11	1	0	0
12	1	1	−1
13	−1	−1	−1
14	0	0	0
15	0	0	0
16	−1	1	−1
17	−1	0	0
18	0	0	1
19	0	1	0
20	0	0	0

Although a number of compounds in peanut skins have been identified, many remain unknown. In addition, there have been few studies to determine the best extraction conditions for the removal of antioxidant compounds from peanut skins. Nepote et al. (*18*) investigated the effects of several parameters including ethanol concentration in water, particle size, and number of extraction stages on the extraction of phenolic compounds from peanut skins. In that study, optimum extraction conditions were solely based on the quantity of total phenolic compounds extracted as determined by Folin-Ciocalteu reagent, and no identification of phenolics were reported. The maximum yield of total phenols observed under the optimum conditions was 118 mg of total phenols/g of peanut skins. Yu et al. (*14*) studied the effect of three skin removal methods (direct peeling, blanching, and roasting) and extraction solvent (water, ethanol, and methanol) on total phenolics and antioxidant activity of peanut skin extracts. The authors reported a total phenolics content of 90−125 mg/g of dry skin.

Response surface methodology (RSM) was first introduced by Box and Wilson (*19*) as a useful method to evaluate the effects of multiple factors and their interactions on one or more response variables. RSM can be effectively used to find the combination of factor levels that produce an optimum response. One of the main advantages of this method is that it generally requires fewer experimental runs than what is needed in traditional full factorial designs, while providing statistically acceptable results (*20*). The central composite design (CCD) is the most popular form of RSM as it has been utilized by a number of researchers to optimize cell culture conditions (*21*, *22*) and for the optimization of various food processing methods such as milling (*23*), extraction (*24*−*26*), and fermentation (*27*).

Table 3. Experimental Design for Response Surface Analysis of Peanut Skins Extracted Using Water in Terms of Coded Values

experiment no.	temperature (°C)	time (min)
1	−1	1
2	1	1
3	0	−1
4	0	0
5	0	0
6	−1	0
7	−1	−1
8	0	0
9	−1	1
10	1	1
11	0	0
12	1	−1
13	−1	−1

The objectives of this study were to use RSM to determine optimal conditions for the extraction of phenolic antioxidant compounds from peanut skins, to identify resveratrol using high performance liquid chromatography, and to measure the antioxidant activity of peanut skin extracts using the oxygen radical absorbance capacity (ORAC) assay. Optimization parameters tested were solvent type, solvent concentration, temperature, and time.

MATERIALS AND METHODS

Materials. Peanut skins obtained from blanching were a gift from Tidewater Blanching Company (Suffolk, VA). The skins were stored in a freezer at −4 °C in sealed plastic bags until analysis. Food grade ethanol (95% Everclear, Luxco Distilling Co., St. Louis, MO) was obtained from a local distributor. Methanol and was obtained from Fisher Scientific (Fair Lawn, NJ). A *trans*-resveratrol standard, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and fluorescein (FL) were purchased from Sigma-Aldrich Co. (St. Louis, MO). 2−2′-azobis(2-amino-propane) dihydrochloride (AAPH) was obtained from Wako Chemicals (Richmond, VA).

Extraction Procedure. The phenolic compounds were extracted using 30, 60, and 90% (v/v) methanol (MeOH) and ethanol (EtOH) in water or 100% distilled water. 50 mL of solvent was added to 2.5 g of peanut skins (20 mL of solvent/g of skins) and placed in a reciprocal shaking water bath (Model 50, Precision Scientific, Chicago, IL) at a speed of 150 rpm. Samples were heated to temperatures of 30, 45, and 60 °C for 10, 20, and 30 min. The crude extracts were allowed to cool to room temperature before centrifugation at 5000 rpm for 10−30 min. The supernatant was collected, placed in 15 mL glass centrifuge tubes, and flushed with nitrogen gas. Crude extracts were stored at −70 °C until analysis. Each solvent extraction was run in triplicate.

Total Phenolics Determination. The total phenolic content (TPC) of the extracts was determined spectrophotometrically using the Folin-Ciocalteu total phenol procedure outlined by Spanos and Wrolstad (*28*) with slight modifications. Gallic acid standard solutions were prepared at 0.0, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL. The extracts (0.1 mL) and the gallic acid standards (0.1 mL) were transferred to 15 mL test tubes. 3.0 mL of 0.2 N Folin-Ciocalteu reagent (Sigma-Aldrich Co., St. Louis, MO) was added to each test tube and mixed using a vortex mixer. After 1 min, 2.0 mL of 9.0% (w/v) Na_2CO_3 in water was added and mixed. The absorbance at 765 nm was determined using a Spectronic 21 spectrophotometer (Bausch and Lomb, USA) after 2 h at room temperature. The concentration of total phenolic compounds in the extracts was determined by comparing the absorbance of the extract samples to that of the gallic acid standard solutions. All samples were assayed in duplicate. TPC was expressed as mg of gallic acid equivalents (GAE)/g of dry peanut skins.

Oxygen Radical Absorbance Capacity (ORAC) Assay. Antioxidant activity (AOA) was determined using an ORAC procedure outlined by Zhou et al. (*29*). Trolox standard solutions were prepared at 20, 40,

Table 4. ANOVA for the Effect of Solvent Concentration, Temperature, and Time on TPC for EtOH, MeOH, and Water Using a Quadratic Response Surface Model[a]

	EtOH				MeOH			
source	SS[b]	DF	*F*-value	prob > F	SS	DF	*F*-value	prob > F
model	3165.99	9	12.81	0.0002	9.755×10^{-4}	9	5.93	0.0051
A	2316.48	1	84.33	<0.0001	3.261×10^{-7}	1	0.018	0.8964
B	75.63	1	2.75	0.1281	1.550×10^{-4}	1	8.47	0.0156
C	16.13	1	0.59	0.4612	1.929×10^{-5}	1	1.05	0.3287
AB	185.28	1	6.75	0.0266	6.804×10^{-5}	1	3.72	0.0826
AC	67.86	1	2.47	0.1471	1.956×10^{-6}	1	0.11	0.7504
BC	13.78	1	0.5	0.4949	9.479×10^{-6}	1	0.52	0.4881
A^2	60.98	1	2.22	0.1671	2.262×10^{-4}	1	12.36	0.0056
B^2	93.82	1	3.42	0.0943	1.750×10^{-5}	1	0.96	0.3511
C^2	240.88	1	8.77	0.0143	8.894×10^{-6}	1	0.49	0.5015
residual	274.68	10			1.829×10^{-4}	10		
lack-of-fit	20.95	5	0.083	0.9920	8.615×10^{-5}	5	0.89	0.5492
R^2	0.9202				0.8421			

	water			
source	SS	DF	*F*-value	Prob > F
model	3.605×10^{-4}	5	22.41	0.0004
A	1.257×10^{-4}	1	39.06	0.0004
B	3.015×10^{-7}	1	0.094	0.7684
AB	4.589×10^{-5}	1	14.26	0.0069
A^2	1.830×10^{-4}	1	56.88	0.0001
B^2	8.770×10^{-6}	1	2.73	0.1427
residual	2.252×10^{-5}	7		
lack-of-fit	1.954×10^{-6}	3	0.13	0.9394
R^2	0.9412			

[a] All values represent mean ± SD of three values. [b] SS − sum of square.

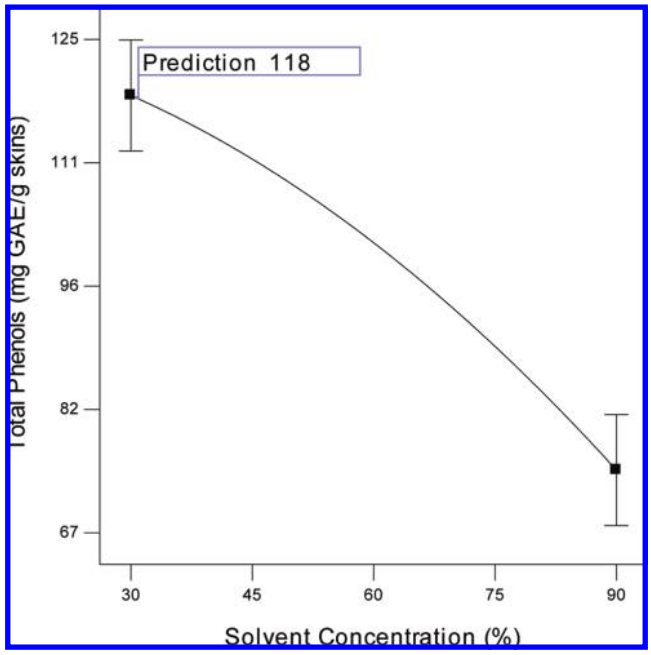

Figure 1. Effect of EtOH concentration on TPC of peanut skin extracts.

80, 200, and 400 μM. The Trolox standards (40 μL), crude extracts (40 μL), and blanks containing only extraction solvent (40 μL) were added to appropriate wells of a 96-well plate. 200 μL of a 100 nM FL solution was added to each well and the plates were covered and incubated for 20 min in a Victor (*3*) multilabel plate reader (Perkin-Elmer, Turku, Finland) preheated to 37 °C. A solution of 0.36 M AAPH (35 μL) was added to each of the wells to generate the peroxyl radicals. Fluorescence readings of the plate were taken every minute using an excitation wavelength of 485 nm and an emission wavelength of 535 nm until all fluorescence readings declined to less than 5% of the initial values. Samples and standards were run in duplicate.

Table 5. Optimum Conditions for the Extraction of Phenolic Compounds from Peanut Skins Based on Total Phenolic Content (TPC) and ORAC Activity

	TPC			
solvent	solvent concentration (% v/v)	temperature (°C)	time (min)	TPC (mg of GAE/g of dry skins)
EtOH	30.8	30.9	12.2	118
MeOH	63.8	57.3	21.6	112
water	100	50.4	10.1	81.0

	ORAC Activity			
solvent	solvent concentration (% v/v)	temperature (°C)	time (min)	ORAC activity (μmol of TE/g of dry skins)
EtOH	30.0	60.0	16.4	2049.7
MeOH	30.0	52.9	30.0	2149.0
water	0	60.0	24.9	612.0

HPLC Analysis. Identification of resveratrol was performed using an Agilent 1200 Series HPLC (Santa Clara, CA) system with a diode array detector. The column used was a Phenomenex Luna C_{18} (250 mm × 4.6 mm, 5 μm particle size) with a guard column. The binary mobile phase consisted of solvent (A) 0.5% aqueous acetic acid and solvent (B) 0.5% acetic acid in methanol. The flow rate of the mobile phase was 0.8 mL/min. The elution gradient started with 100% A and 0% B. A decreased to 95.3% and B increased to 4.7% in 4 min. Over the next 38 min, solvent A was decreased to 25.3% while solvent B was increased to 74.7%. A was decreased to 5% at 54.5 min before increasing to 100% from 55 to 65 min. All ramps were linear. Absorbance was measured by a UV−vis diode array detector at a wavelength of 280 nm. Resveratrol in the peanut skin extract (PSE) was identified by comparing the retention time and UV-spectra of the

PSE samples to a *t*-resveratrol standard analyzed under the same chromatographic conditions. Identification was confirmed by using liquid chromatography mass spectrometry on collected peaks.

Statistical Analysis. RSM was used to determine the optimal conditions for extraction. RSM was performed using the Design Expert Version 7.1.3 software (Stat-Ease, Inc., Minneapolis, MN) program. A central composite design was used to investigate the effects of three independent variables (solvent concentration, extraction temperature, and extraction time) at three levels on the dependent variables (TPC, ORAC activity, and resveratrol concentration). CCD uses the method of least-squares regression to fit the data to a quadratic model. The quadratic model for each response was as follows:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \sum \beta_{ij} X_i X_j$$

where Y is the predicted response; β_0 is a constant; β_i is the linear coefficient; β_{ii} is the quadratic coefficient, β_{ij} is the interaction coefficient of variables i and j; and X_i and X_j are independent variables. The adequacy of the model was determined by evaluating the lack of fit, coefficient of determination (R^2) and the Fisher test value (F-value) obtained from the analysis of variance (ANOVA) that was generated by the software. Statistical significance of the model and model variables was determined at the 5% probability level ($\alpha = 0.05$). The software uses the quadratic model equation shown above to build response surfaces. Three-dimensional response surface plots and contour plots were generated by keeping one response variable at its optimal level and plotting that against two factors (independent variables). Response surface plots were determined for each solvent. The coded values of the experimental factors and factor levels used in the response surface analysis are given in **Table 1**. The complete design using aqueous MeOH and EtOH as extraction solvents consisted of 20 experimental points including six replications of the center point. The complete design using water as the extraction solvent consisted of 13 experimental points with five replications of the center point. The coded values for the experimental designs are given in **Tables 2** and **3**.

RESULTS AND DISCUSSION

Model Fitting. The ANOVA of the quadratic regression models for EtOH, MeOH, and water showed that the models were significant ($p < 0.05$) with F-values of 12.81, 5.93, and 22.41, respectively (**Table 4**). The R^2 values of the models were 0.92, 0.84, and 0.94 for EtOH, MeOH, and water, respectively, and there was no significance in the lack of fit ($p > 0.05$) in each of the three models. This indicated that the models could be used to predict the responses. The software generated the following regression equation which demonstrates the empirical relationship between solvent concentration (A), temperature (B), time (C), and TPC (Y) for each solvent in terms of coded units:

EtOH

$$Y = +106.10 - 15.22A + 2.75B + 1.27C + 4.81AB + 2.91AC - 1.31BC - 4.71A^2 + 5.84B^2 - 9.36C^2$$

MeOH

$$Y = 0.11 - 1.806 \times 10^{-4}A + 3.936 \times 10^{-3}B + 1.389 \times 10^{-3}C + 2.916 \times 10^{-3}AB + 4.945 \times 10^{-4} - 1.089 \times 10^{-3}BC - 9.068 \times 10^{-3}A^2 - 2.522 \times 10^{-3}B^2 - 1.798 \times 10^{-3}C2$$

Water

$$Y = +0.078 + 4.577 \times 10^{-3}A + 2.242 \times 10^{-4}B - 3.387 \times 10^{-3}AB - 8.140 \times 10^{-3}A^2 + 1.782 \times 10^{-3}B^2$$

Optimum Extraction Conditions Based on TPC. *EtOH.* RSM was used to determine the optimum extraction conditions for each solvent. In this study, the independent variables were allowed to assume any value within the range of levels tested in order to determine the optimum value. The main effect, solvent concentration, and the treatment interaction of solvent concentration and temperature had significant ($p < 0.05$) effects on the TPC of samples extracted using EtOH. TPC of the extracts decreased by 37.3% when going from 30% (118 mg/g) to 90% EtOH concentration (74 mg/g) (**Figure 1**). The maximum predicted TPC of 118 mg of GAE/g of skins was obtained under the optimum extraction conditions of 30.8%, 30.9 °C and 12 min for EtOH concentration, temperature and time, respectively (**Table 5**). An optimum extraction temperature of 31 °C was somewhat surprising as we expected that higher temperatures would result in greater extraction. At present, we cannot be certain of why this occurred. More work is needed to obtain the exact chemical composition of the extracts such that we can determine which compounds are preferentially extracted at the lower temperatures and what impact individual polyphenols have on the Folin phenol and ORAC assays.

The treatment interaction of solvent concentration and temperature had a significant ($p < 0.05$) impact on TPC of the extracts

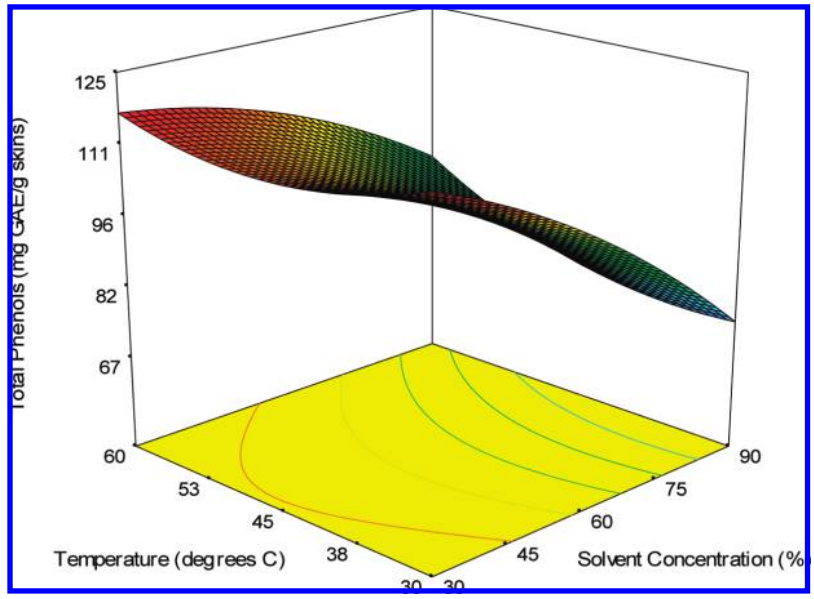

Figure 2. Response surface of the effect of EtOH concentration and temperature on TPC of peanut skin extracts.

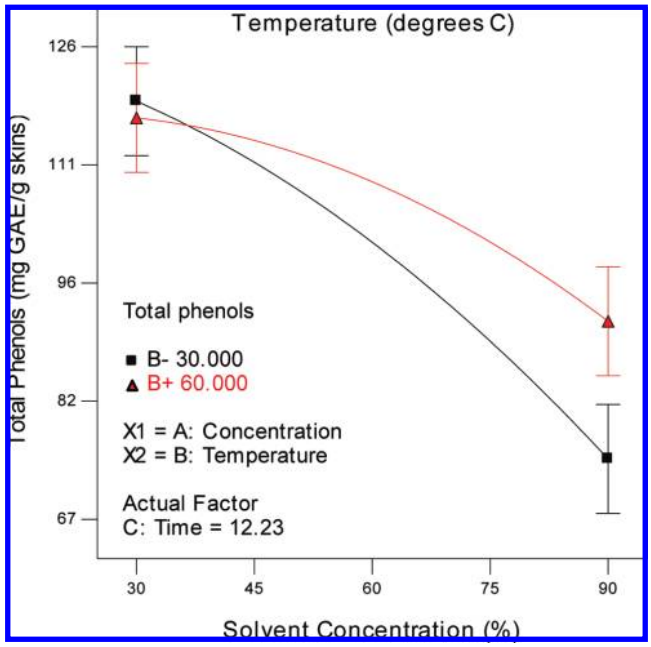

Figure 3. Effect of EtOH concentration and temperature on TPC of peanut skin extracts.

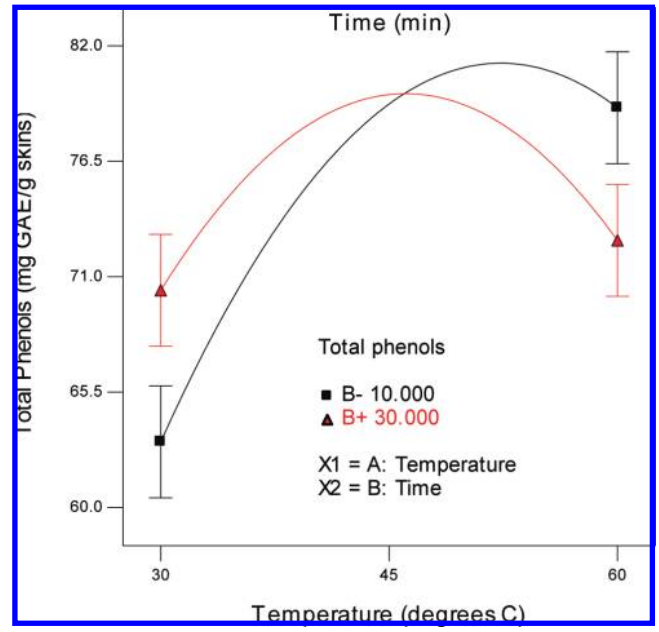

Figure 5. Effect of the treatment interaction of temperature and time on TPC of peanut skin extracts using water as the extraction solvent.

(**Figure 2**). At an EtOH concentration of 30%, the TPC at 30 and 60 °C was 119 mg/g and 117 mg/g, respectively (**Figure 3**). In comparison, the TPC of the extracts at 30 and 60 °C using 90% EtOH was approximately 75 and 92 mg/g, respectively. The samples extracted at 30 and 60 °C using 90% EtOH were significantly different. An increase in temperature from 30 to 60 °C caused a 22.6% increase in TPC when extracting with 90% EtOH (**Figure 3**). No significant difference in TPC was found between skins extracted at 30 and 60 °C using 30% EtOH. Although extraction time was not shown to be a significant factor in regards to TPC, it was observed that the TPC of the extracts initially increased with increasing time until reaching a maximum, after which the TPC decreased with increasing time. Degradation of some of the thermolabile phenolic compounds may have occurred after the optimum extraction time was reached, thereby leading to a lower concentration of phenolic compounds. Romero-Pérez et al. (*30*) investigated the effects of solvent type, time, and temperature on the extraction of resveratrol from grape skins. The authors reported degradation of resveratrol at temperatures and times higher than the determined optimum values.

MeOH. Temperature had a significant ($p < 0.05$) effect on TPC of peanut skins extracted with aqueous MeOH. The response surface shown in **Figure 4** indicates that TPC of the extracts increased with increasing temperature. The squared term of solvent concentration had a significant impact on the model. TPC of extracts using 30% MeOH as the extraction solvent increased up to a maximum MeOH concentration of approximately 64% before declining (**Figure 4**). The optimum extraction conditions were estimated as 63.8%, 57.3 °C, and 21.6 min for MeOH concentration, temperature, and time, respectively (**Table 5**). The maximum predicted TPC obtained under these optimized conditions was 112 mg/g. In the case of MeOH, higher temperatures did result in increased extraction

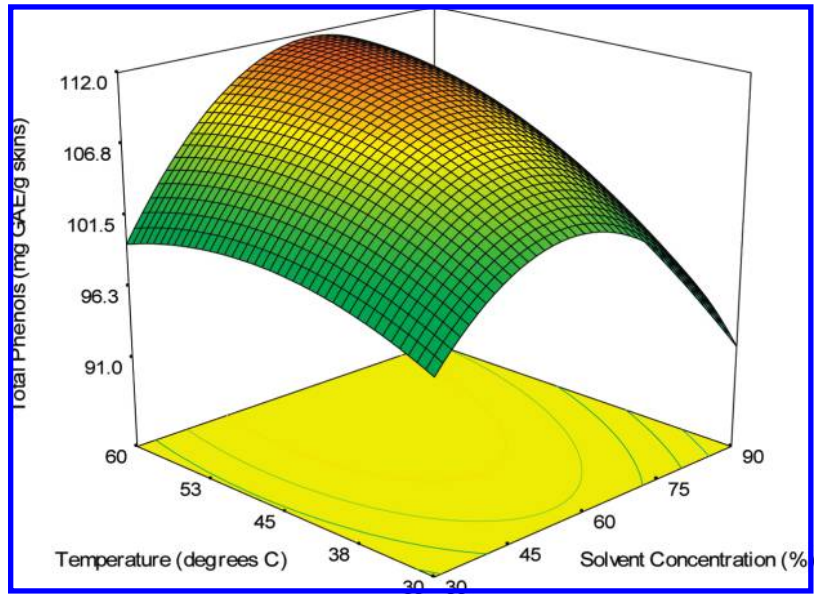

Figure 4. Response surface of the effect of MeOH concentration and temperature on TPC of peanut skin extracts.

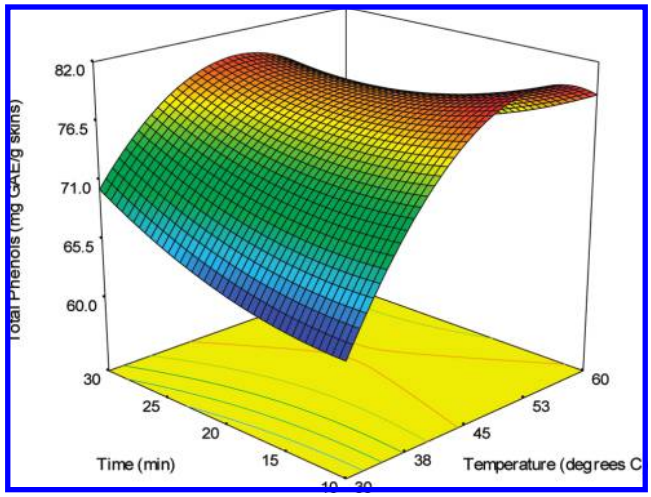

Figure 6. Response surface of the effect of temperature and time on TPC of peanut skin extracts using water as the extraction solvent.

Table 6. Comparison of Optimized Conditions for the Extraction of Phenolic Compounds from Peanut Skins Reported in the Literature

study[a]	EtOH concentration (% v/v)	temperature (°C)	solvent volume/ skins (mL/g)	time	TPC (mg of GAE/g of dry skins)
1	50	25	15	overnight	97
2	80	25	80	overnight	90−125
3	70	25	20	10 min	118
4	30.8	30.9	20	12 min	118

[a] Study (1) Wang et al. (*3*); (2) Yu et al. (*14*); (3) Nepote et al. (*18*); (4) present work.

of phenolics, which was not seen with EtOH. It is highly likely that under different conditions of extraction different compounds are preferentially extracted.

Water. Extraction temperature and the treatment interaction of temperature and time had significant effects ($p < 0.05$) on TPC of samples extracted with water. The TPC of the skins increased with increasing temperature (**Figure 6**). In the case of the treatment interaction of temperature and time, TPC of the peanut skin extracts increased by 20.2% when going from 30 °C (63.1 mg/g) to 60 °C (79.1 mg/g) at an extraction time of 10 min (**Figure 5**). A smaller increase of about 3.4% in TPC occurred at 30 min extraction as temperature increased from 30 to 60 °C. The estimated optimum extraction temperature (50.4 °C) and time (10.1 min) yielded a predicted maximum TPC of 81 mg/g (**Table 5**).

In the current study, EtOH (30% v/v) was found to produce the highest yields of phenolic compounds followed by MeOH and water. Yu et al. (*14*) used 80% EtOH, 80% MeOH, and water as solvents for the extraction of phenolics from peanut skins. They found that extracting the skins overnight with 80% EtOH at room temperature and a solvent/mass ratio of 80 mL/g yielded the highest TPC recovery of 90−125 mg of GAE/g of peanut skins (**Table 6**). In our work, it was shown that extraction of peanut skins using just 30% EtOH at 31 °C and a solvent/mass ratio of 20 mL/g for 12 min could be used to extract a maximum of 118 mg of GAE/g of peanut skins. Hence, we were able to extract comparable levels of phenolic compounds using less solvent and in a much shorter time than that reported by Yu et al. (*14*). Nepote et al. (*18*) observed a maximum TPC of 118 mg/g under the optimum extraction conditions of 70% (v/v) EtOH in water, shaking for 10 min at room temperature,

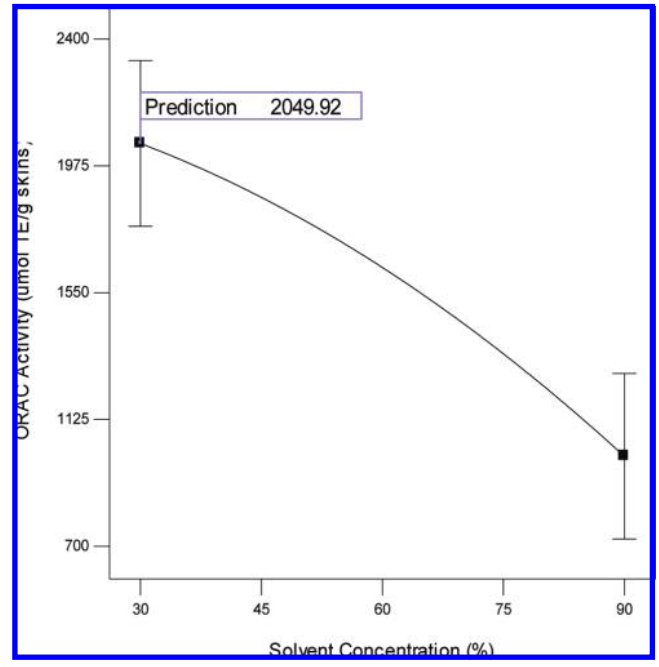

Figure 7. Effect of EtOH concentration on the ORAC activity of peanut skin extracts.

a solvent/mass ratio of 20 mL/g, and three extraction stages. The optimization procedure employed in this study resulted in a similar recovery of phenolic compounds using just one extraction stage, thereby allowing for a reduction in solvent consumption and time required for extraction.

MeOH was also shown to be an effective solvent, although higher extraction temperatures were required when compared to EtOH. The optimum temperature for extraction with MeOH was about 57 °C compared to just 30 °C for EtOH, with corresponding predicted maximum TPC recoveries of 118 and 112 mg/g, respectively (**Table 3**). Additionally, a higher MeOH concentration (approximately 64%) was required to reach TPC levels comparable to that of samples extracted with EtOH. When considering the cost associated with higher extraction temperatures in addition to potential toxicity issues related to the use of MeOH in food applications, EtOH may be preferred over MeOH. Both MeOH and EtOH proved to be more efficient at extracting phenolic compounds than pure water. Nepote et al. (*31*) also found water to be less effective in extracting phenolics from peanut skins, with skins extracted using water containing approximately 39% less phenolic compounds than EtOH extracts.

Optimum Extraction Conditions Based on ORAC Activity. ORAC was used to measure the peroxyl radical scavenging ability of peanut skin extract using Trolox (water-soluble vitamin E analogue) as an antioxidant standard. A major advantage of the ORAC assay in measuring AOA is that it provides a controllable source of peroxyl radicals that model the reaction of antioxidants with lipids in both food and physiological systems (*32*). The ORAC values were expressed as micromoles of Trolox equivalents (TE) per gram of dry peanut skins.

Model Fitting. The results of the ANOVA for the quadratic regression model describing ORAC activity was significant ($p < 0.05$) when EtOH was used as the extraction solvent. The quadratic models generated for MeOH and water were not found to be significant ($p > 0.05$). The software generated the following quadratic equation to describe the empirical relationship between

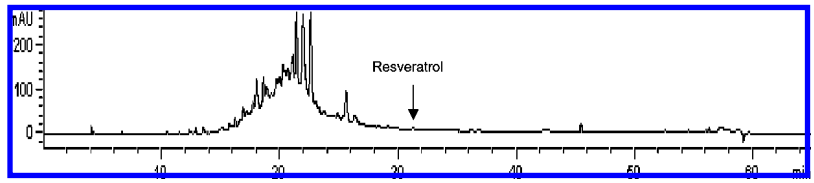

Figure 8. HPLC chromatograph of PSE extracted using 30% MeOH at 60 °C and 30 min.

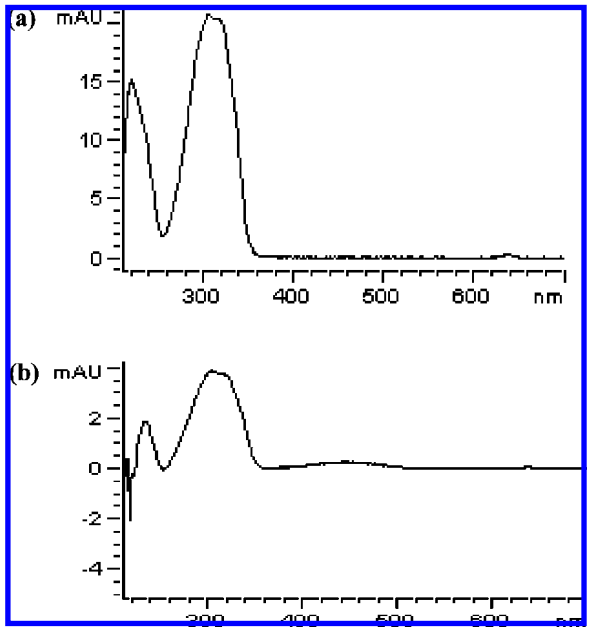

Figure 9. UV−vis spectra of (a) resveratrol standard and (b) peak with matching retention time in PSE sample extracted with 30% MeOH at 60 °C and 30 min.

the independent variables and ORAC activity using EtOH as the extraction solvent:

$$Y = +60.37 - 14.05A + 7.22B + 0.11C - 2.54AB + 4.69AC - 5.91BC - 6.75A^2 + 4.90B^2 + 3.65C^2$$

EtOH. Solvent concentration had a significant effect ($p < 0.05$) on the ORAC activity of peanut skins extracted with aqueous EtOH. As the EtOH concentration increased from 30% (2049.7 μmol of TE/g of skins) to 90% (1001.5 μmol of TE/g of skins), ORAC activity decreased by about 51% (**Figure 7**). The significant decrease in ORAC values with increased EtOH content was unexpected, but it is consistent with the previous finding that fewer phenolic compounds were extracted at higher EtOH concentrations. The Folin and ORAC assays measure different attributes of the polyphenols in the extracts. While there is likely some correlation between the two assays, we do not have data to unequivocally prove this. Nevertheless, our results suggest that higher EtOH concentrations significantly reduce the extraction of those phenolic compounds that can effectively scavenge peroxyl radicals.

The optimum extraction conditions that provided maximum ORAC activity (2049.7 μmol of TE/g) were predicted as 30%, 60 °C, and 16.4 min for EtOH concentration, extraction temperature, and time, respectively (**Table 5**). Upon comparison of the optimum parameters for total phenolic recovery and ORAC activity, the estimated maximum TPC and ORAC activity both occurred at an EtOH concentration of about 30% (**Table 5**). The optimal extraction time was 12.2 min for total phenolic activity and 16.4 min for ORAC activity. A major difference observed between optimum conditions based on TPC and ORAC activity was extraction temperature. The optimum temperatures were 30.9 and 60 °C for TPC and ORAC activity, respectively. It appears that higher temperatures were required for the extraction of those compounds that increased ORAC activity.

MeOH. The independent variables did not have significant effects ($p > 0.05$) on the ORAC activity of peanut skins extracted with MeOH. However, under the optimized extraction conditions (30% MeOH concentration, 52.9 °C and 30 min), the maximum predicted value for ORAC activity was 2149 μmol of TE/g, which was slightly higher than that found under the optimum conditions determined for EtOH (2049.7 μmol of TE/g) (**Tables 3** and **4**). Despite EtOH extracts having a higher TPC, they did not have a higher ORAC activity than the MeOH extracts. Yu et al. (*14*) also found that peanut skins extracted with MeOH had greater total antioxidant activity than samples extracted with EtOH, despite the fact that EtOH extracts had higher yields of total phenols. O'Keefe and Wang (*33*) used MeOH to extract phenolic compounds from blanched peanut skins, and it was noted that extracts in which MeOH was used as the extraction solvent showed greater AOA in meat systems than those extracted with 95% EtOH. However, the authors did point out that no optimization of extraction conditions were performed in their study. Contradictory results were reported by Huang et al. (*34*) in which it was found that peanut skins extracted with EtOH had the highest yield of total phenolics and the strongest AOA of five tested solvents including MeOH. This discrepancy may be related to the fact that each study assessed the AOA of the extracts in different systems. For example, O'Keefe and Wang (*33*) used a meat model system while Huang et al. (*34*) assessed AOA in a linoleic acid system. Antioxidants behave differently depending on the type of system and the type of lipid substrate involved, thereby making it difficult to compare results.

Water. The independent variables did not have significant effects ($p > 0.05$) on the ORAC activity of peanut skins extracted using water. The optimum extraction conditions were 60 °C and 24.9 min for temperature and time, respectively. The maximum predicted ORAC activity under the given optimum conditions was 612 μmol of TE/g, which represents a 69.2% and 71.5% reduction in ORAC activity when compared to extract obtained from EtOH and MeOH, respectively. Extraction of phenolic antioxidants from peanut skins using water proved to be less efficient in terms of increasing total phenolic yield and ORAC activity when compared to EtOH and MeOH. Other authors (*14*, *35*) have also reported water to be a less efficient solvent for the extraction of phenolic compounds from a number of plant materials including peanut skins.

To our knowledge, there have been no previous reports of ORAC activity of peanut skin extracts in the literature. ORAC values have, however, been reported for some common fruits, vegetables, nuts, and spices (*36*, *37*). ORAC values of cranberries and blueberries were determined as 93 and 92 μmol of TE/g, respectively, compared to 42.3 μmol of TE/g for Red Delicious apples (*37*). Wu et al. (*37*) reported that spices such as ground cinnamon (2641 μmol TE/g), ground cloves (1533 μmol of TE/g), and oregano leaves (1831 μmol of TE/g) had

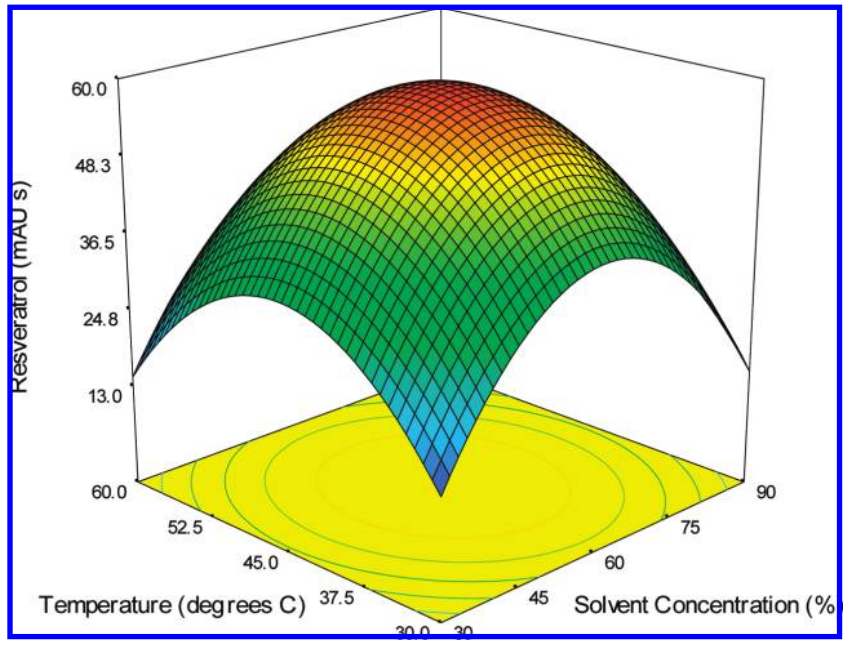

Figure 10. Response surface of the effect of temperature and MeOH concentration on resveratrol content.

relatively high ORAC activity when compared to fresh fruits and vegetables. These products, in addition to the peanut skins used in this study, have much lower water contents than fresh fruits and vegetables. Yilmaz et al. (*35*) investigated the antioxidant capacities of three different types of grape seeds based on the ORAC assay. It was found that grapes seeds from Chardonnay had an ORAC value of 638 μmol of TE/g. In our study, the maximum predicted ORAC activity was 2149 μmol of TE/g, which is more than three times higher than the reported ORAC value of grape seeds from Chardonnay (*35*) and nearly 25 times that reported for blueberries (*37*). PSE contain antioxidants that have excellent peroxyl radical scavenging properties in vitro, thereby potentially making peanut skins a viable source of natural antioxidant compounds for food and pharmaceutical applications.

Identification of Resveratrol. The HPLC chromatogram of a PSE sample extracted with MeOH is shown in **Figure 8**. We were able to identify resveratrol in the MeOH extracts based on matching retention times and UV spectra of the PSE sample compared to the resveratrol standard (**Figure 9**) analyzed under the same chromatographic conditions. The resveratrol peak in the PSE was also confirmed by LC-MS (data not shown). However, resveratrol levels in the extracts were extremely low and were therefore, not likely to have a significant impact on overall AOA of the extracts. We chose to focus on resveratrol in this study due its suggested health benefits and prior work (*6*, *14*, *38*) identifying its presence in peanut products. The quadratic model for resveratrol concentration (as determined by the peak area) of peanut skins extracted using MeOH was not significant ($p = 0.0523$) based on the significance level used in this study. Therefore, the optimum conditions for extraction of resveratrol using MeOH could not be determined. However, a response surface showing the relationships between the independent variables when extracting with MeOH is shown in **Figure 10**.

Resveratrol could not be identified in PSE samples extracted using aqueous EtOH. In some of the EtOH samples, there were peaks occurring at the same retention time as the resveratrol standard, but the UV spectra did not match. In a large number of the EtOH samples, there was no detectable peak at the retention time of the standard. These findings are not consistent with other studies (*14*, *38*) which have shown resveratrol to be present in PSE extracted with EtOH. Yu et al. (*38*) found that PSE extracted with 80% EtOH contained resveratrol, although at relatively very low concentrations compared to the major identified compounds (A- and B-type procyanidin dimers and trimers). The inability to identify resveratrol in EtOH extracts in this study may be due to the detection threshold of our HPLC system, which was found to be about 805 ng/mL. It is worth noting that the data generated by the software showed that samples extracted with 90% EtOH had fewer detectable peaks than those samples extracted with 30% EtOH, although this was not visually noticeable when comparing the two chromatograms. The number of peaks detected using 30% EtOH was 61 compared to just 45 peaks using 90% EtOH as the extraction solvent. Resveratrol could not be identified in samples extracted with water. As with EtOH extracts, skins extracted with water contained peaks at the same retention time as the standard, but the UV spectra were not the same.

In our study, we were able to extract similar levels of phenolic compounds from peanut skins as that previously reported in the literature while using less solvent and reducing extraction time. Although more identification work is needed to obtain a more complete phenolic profile of the extracts, this work clearly shows that the extraction of natural antioxidants from peanut skins can be improved by optimizing several key extraction parameters. Perhaps this research will renew interest in utilizing peanut skins as an inexpensive source of natural antioxidants with the extracts having the potential to be developed into functional food ingredients and dietary supplements.

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