

## Valorization of agricultural by-product: Optimization of alcohol-based extraction of polyphenols from chestnut peel using Box-Behnken Design

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### Abstract

In the present work, solvent concentration, extraction time, and solid to solvent (S/S) ratio were evaluated in order to recover the majority of polyphenols from chestnut peel waste using ethanol and methanol. Extraction method for polyphenols from peel pre-treated by ohmic heating (OH) was optimized using response surface methodology (RSM). The effect of these independent variables on total phenolic content (TPC) and antioxidant capacity (AC) was studied using Box-Behnken Design (BBD). A second-order polynomial model provided a satisfactory fit to the experimental data with a high coefficient of determination ( $R^2$ ) value. Results showed that S/S ratio and solvent concentration were generally significant variables during extraction in terms of TPC and AC. The optimum extraction conditions were obtained as 1/10 of S/S ratio and 60% of solvent concentration for both solvents. As the optimum extraction time, 82.41 min for ethanolic extraction and 116.97 min for methanolic extraction were selected. Under these optimal conditions, TPC values of the ethanolic and methanolic extracts were found to be 39.02 and 38.79 mg gallic acid equivalents per gram of dry matter (mg GAE/g DM), respectively, thus indicating highly close agreement to the predicted values. Consequently, the effectiveness of the solvents used was found to be very similar to each other. The OH pre-treatment appeared to be a promising technique for polyphenolic extraction from industrial wastes.

### Keywords

chestnut peel, extraction, optimization, polyphenols, response surface methodology

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## Introduction

The food industry produces a large amount of agricultural residues (Moorthy *et al.*, 2015) which could be excellent sources of bioactive compounds providing a range of health benefits (Kaderides and Goula, 2019). Chestnut (*Castanea sativa*) is one of the most economically important fruit crops (Aires *et al.*, 2016). The nut is processed into various products such as frozen fruit, chestnut purée, flour, and marron glacé (Fernández-Agulló *et al.*, 2014). After processing, significant amount of chestnut peels turns to waste and often becomes fuel (Vázquez *et al.*, 2012; Fernández-Agulló *et al.*, 2014).

Chestnut peel is a good source of phenolic compounds (Obiang-Obounou and Ryu, 2013; Lee *et al.*, 2016; Gullón *et al.*, 2018). Chestnut shell extracts have been reported to have antimicrobial (Fernández-Agulló *et al.*, 2014; Zhan *et al.*, 2014; Lee *et al.*, 2016), antioxidant (Tsujita *et al.*, 2011; Ham *et al.*, 2015), and anticancer (Jung *et al.*, 2016) activities, because polyphenols are natural antioxidants that have different biological activities and play important roles in human health (Ćujić

*et al.*, 2016; Džugan *et al.*, 2020; El Kantar *et al.*, 2020; Luca *et al.*, 2020; Yan *et al.*, 2020). Furthermore, they are able to prevent deterioration in food products (Babbar *et al.*, 2014), and hence have a positive influence on food quality by increasing shelf life (Fasolato *et al.*, 2016). Therefore, polyphenols obtained from agricultural and industrial residues are considered as remarkable sources for natural antioxidants to replace synthetic compounds (Zardo *et al.*, 2019; Jimenez *et al.*, 2020).

Developing an efficient and environmental friendly extraction process is very important to produce antioxidants from agrofood wastes or by-products (Franco *et al.*, 2018; Pérez-Armada *et al.*, 2019). Recently, different methods such as infrared radiation, pulsed electric field, microwave, and ultrasound as a pre-treatment and/or treatment have been used for the extraction of polyphenols from plants (Odabaş and Koca, 2016; Rajha *et al.*, 2019). For example, pre-treatment with OH, one of the novel electrotechnologies, has been shown to accelerate the extraction kinetics of total polyphenols from grape pomace (El Darra *et al.*, 2013). Pereira *et al.* (2016) reported that the existence of an electrical

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field generates electroporation of cellular tissues, thus causing an amplified extraction of bioactives. To the best of our knowledge, although the optimization of polyphenolic extraction from different natural sources has been reported by many studies, the literature on optimization of the extraction of polyphenols from pre-treated chestnut peels is very rare. Therefore, the purpose of the present work was to evaluate the effect of extraction conditions on TPC and AC of alcoholic extracts from chestnut peels pre-treated by OH using the response surface methodology. The optimized parameters were S/S ratio, extraction time, and solvent (methanol and ethanol) concentration, which influence the extraction efficiency (Ćujić *et al.*, 2016). These are also related to the reduction of extraction costs (Vázquez *et al.*, 2012; Soares and Ferreira, 2017).

## Materials and methods

### Materials

Chestnut peels were obtained from a chestnut processing plant (Özdem Şekerleme Company, Bursa, Turkey), filled in polyethylene bags, and refrigerated at 4°C until further analyses. All chemicals and reagents used were of analytical grade.

### Pre-treatment of chestnut peel by OH

Based on our preliminary trials, pre-treated chestnut peels showed higher TPC values than untreated ones. Therefore, the peels used in the present work were pre-heated by OH. It was carried out in an OH chamber which consisted of a rectangular Cast Polyamide/PA6G (15 × 6.6 × 8 cm) and two planar AISI 304 stainless steel electrodes (14.5 × 8 cm). The chamber had a capacity of 500 mL. Temperature was measured with type-K thermocouples coated with Teflon (to prevent interference from the electrical field) placed in the centre of the chamber. The electrodes of OH were connected to a variac (50 Hz, 0 - 600 V, 25 A) (Artsan Energy and Test Instruments, Turkey). The output data as voltage, current, and temperature were recorded at 1 s intervals on a data logger with special software, and monitored digitally using a computer. Peel samples were placed between two stainless steel electrodes inside the treatment chamber. The sample-to-liquid ratio in the treatment chamber was 1:20 (w/w). The distance between electrodes was adjustable, and adjusted to 10.8 cm. To assure a better contact between electrodes and sample, table salt solution was added to the chamber. The

pre-treatment conditions applied were optimized using response surface methodology. The following optimal conditions were applied: (1) concentration of salt solution was 0.32%, (2) the electrical field strength, E, was 20 V/cm, and (3) the treatment time was 100 s. The electric field strength within the treatment chamber was calculated using Eq. 1:

$$\text{Electrical field strength (E, V/cm)} = \frac{\text{output voltage (V)}}{\text{distance between the electrodes (cm)}} \quad (\text{Eq. 1})$$

Following OH, the peel samples were rapidly drained off, rinsed, cooled in running water, and air-dried at room temperature until they reached constant moisture content. The peel samples were milled, passed through a 2 mm sieve, and refrigerated at 4°C until further analyses.

### Experimental design

In the present work, the Box-Behnken Design (BBD) was employed to determine the extraction parameters for polyphenols from chestnut peels. The design comprised 30 experimental runs with three levels, -1 (lower limit), 0 (central point), and +1 (upper limit) for each factor. A, B, and C were chosen as the independent variables of which the selection and range were based on previous studies (Vázquez *et al.*, 2012; Zhou *et al.*, 2019), while response variables were TPC and AC. The experimental design (coded and actual values of the factors) for each run is shown in Table 1. The experiments were performed in duplicates, from which the averages were reported as the responses. The experimental data was fitted to the following second-order polynomial model (Eq. 2):

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_1^2 A^2 + \beta_2^2 B^2 + \beta_3^2 C^2 + \beta_1 \beta_2 AB + \beta_1 \beta_3 AC + \beta_2 \beta_3 BC \quad (\text{Eq. 2})$$

where, Y = predicted response;  $\beta_0$  = model intercept coefficient;  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  = regression coefficients for the linear effect terms;  $\beta_1^2$ ,  $\beta_2^2$  and  $\beta_3^2$  = square effect terms; and  $\beta_1 \beta_2$ ,  $\beta_1 \beta_3$  and  $\beta_2 \beta_3$  = interaction effect terms, respectively. Analysis of variance (ANOVA) was applied to determine the effect of each factor to analyse the predicted model on the response variable. Furthermore, to evaluate the fitness of the regression model, the regression coefficient ( $R^2$ ) and the *p*-value of the lack of fit (LOF) were employed. The relationship between the independent and response variables was presented by the response surface plots, and

the optimum conditions were determined. In order to find out the accuracy and suitability of the optimized conditions, an additional experiment was performed under optimal conditions. MINITAB 18 software (State College, PA) was used for data analysis.

#### Extraction of polyphenols

Ground chestnut peel samples were extracted in a Falcon® tube with ethanol, methanol, and their aqueous solutions at a known concentration (C, %) on a mechanical shaker. These solvents were chosen because they have been mostly used to extract polyphenols from plant materials (Vázquez *et al.*, 2012; Franco *et al.*, 2018; Riciputi *et al.*, 2018). Extractions were performed at a known S/S ratio (B, w/v) defined in Table 1. The Falcon® tube was wrapped in aluminium foil to prevent degradation during extraction. After extraction time period (A, min), the suspension was filtered through Whatman

No.1 filter paper, and the extract was frozen at -18°C until further analyses. The extraction conditions used are shown in Table 1.

#### Extract analyses

##### Total phenolic content (TPC)

The TPC of the peel extracts was determined using the Folin-Ciocalteu method (ISO, 2005). A calibration curve of gallic acid (5 - 50 µg/mL) was prepared, and the results determined from the regression equation of the calibration curve ( $y = 0.0157x$ ,  $R^2 = 0.99$ ) were expressed as mg gallic acid equivalents per gram of dry matter (mg GAE/g DM).

##### Antioxidant capacity (AC)

The AC was determined using the 2,2-diphenyl-2-picryl-hydrazyl (DPPH) method as described by Türkmen Erol *et al.* (2009), and calculated as the percentage inhibition of the DPPH radical using Eq. 3:

Table 1. Coded and uncoded independent variables in BBD design.

Exp.	A*	B**	C***	Time (min)	S/S ratio (g/mL)	Solvent concentration (%)
1	0	0	0	90	1:25	80
2	1	1	0	120	1:40	80
3	0	-1	-1	90	1:10	60
4	1	0	-1	120	1:25	60
5	1	-1	0	120	1:10	80
6	-1	0	1	60	1:25	100
7	-1	1	0	60	1:40	80
8	-1	1	0	60	1:40	80
9	1	0	1	120	1:25	100
10	0	-1	1	90	1:10	100
11	1	1	0	120	1:40	80
12	-1	0	-1	60	1:25	60
13	0	-1	-1	90	1:10	60
14	-1	0	1	60	1:25	100
15	0	0	0	90	1:25	80
16	0	1	-1	90	1:40	60
17	0	1	1	90	1:40	100
18	-1	-1	0	60	1:10	80
19	1	0	1	120	1:25	100
20	-1	0	-1	60	1:25	60
21	1	0	-1	120	1:25	60
22	0	0	0	90	1:25	80
23	0	0	0	90	1:25	80

\*time; \*\*solid/solvent ratio; and \*\*\*solvent concentration.

$$AC(\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \quad (\text{Eq. 3})$$

The AC of the samples was converted to ascorbic acid equivalent (AAE) defined as mmol of AAE per 100 g of DM.

## Results and discussion

### Analysis of the model for chestnut peel extracts

The responses of TPC and AC of the chestnut peel extracts are shown in Table 2. The lowest values were obtained with pure ethanol and methanol, which is in agreement with previous studies (Ashraf *et al.*, 2016; Riciputi *et al.*, 2018; DiNardo *et al.*, 2019; Lasano *et al.*, 2019). Results showed that the highest value of TPC (41.92 mg GAE/g DM) was achieved with ethanol, which is in concordance with the result of Stevigny *et al.* (2007), while the highest value of AC (42015.95 mmol AAE/100 g DM) was obtained with methanol. Ethanol is thought to be superior to methanol because it is a cheap, reusable, non-toxic, and environmentally friendly organic solvent (Amado *et al.*, 2014; Gunathilake *et al.*, 2019; Sablania *et al.*,

2019).

The ANOVA analysis for TPC and AC of ethanolic and methanolic peel extracts showed that the model was highly significant ( $p < 0.05$ ) with high values of determination coefficients ( $R^2$ ) (Table 3). This result represented a good correlation between the experimental and predicted data for TPC and AC of ethanolic and methanolic peel extracts (Figure 1). For the two model responses, the lack of fit was not significant ( $p > 0.05$ ), thus indicating that the model could be used accurately to predict the responses.

### Effect of extraction parameters on TPC and AC of chestnut peel extracts

The  $p$ -values determine the statistical significance of each term (Table 3);  $p < 0.05$  represent that the model is significant, while  $p > 0.05$  is insignificant (Rai *et al.*, 2019). Based on these criteria, for ethanolic extraction, S/S ratio and ethanol concentration had significant linear effects on two responses ( $p < 0.05$ ), which indicated that TPC and AC were strongly influenced by these factors. The square term of extraction time had a significant effect on both TPC and AC while ethanol concentration had a significant square effect on only AC ( $p < 0.05$ ). All of the interaction terms had no

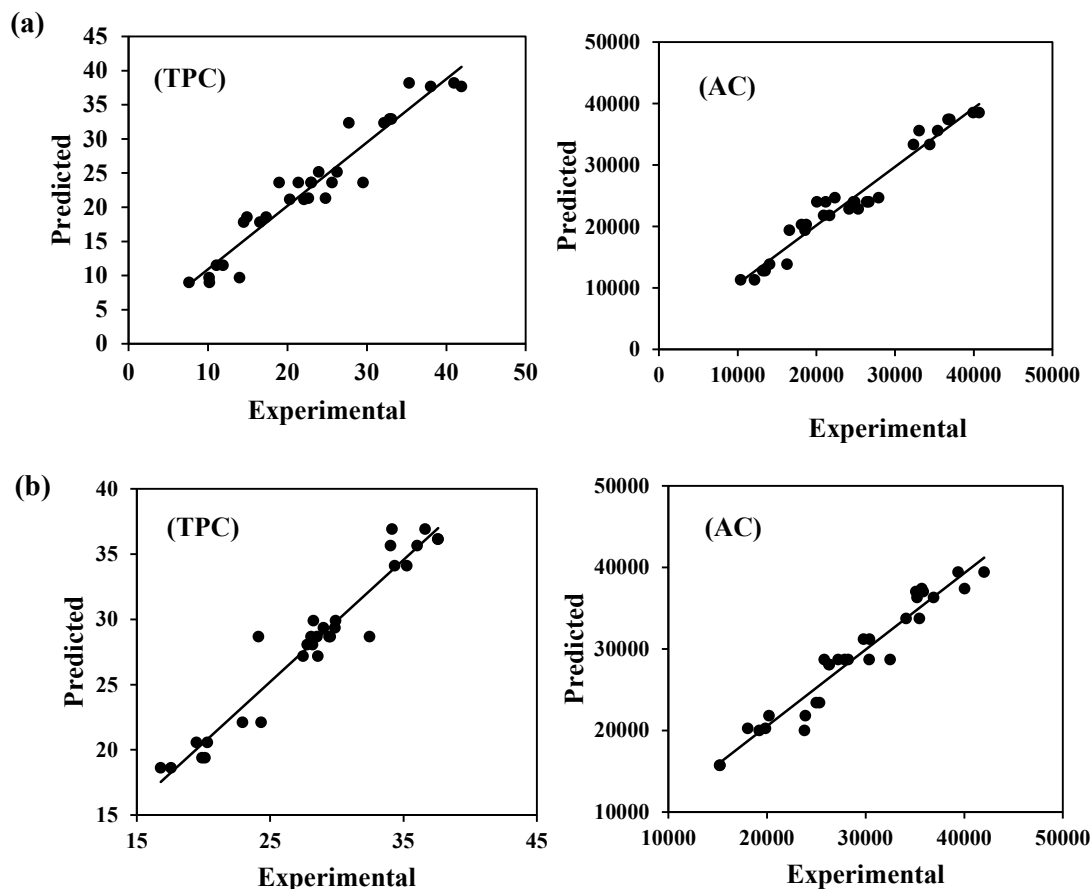


Figure 1. Experimental values *versus* predicted values for TPC and AC of chestnut peel ethanolic (a) and methanolic (b) extracts.

Table 2. Experimental results and predicted values for TPC (in mg GAE/g DM) and AC (in mmol AAE/100 g DM) of chestnut peel ethanolic and methanolic extracts.

Exp.	Ethanolic extraction				Methanolic extraction			
	TPC		AC		TPC		AC	
	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	23.02	23.58	24724.49	23995.90	29.50	28.68	25827.27	28672.50
2	22.07	21.11	20932.03	21794.20	29.91	29.91	26293.31	28071.52
3	35.32	38.16	36738.09	37397.36	34.15	36.91	36894.24	36293.49
4	32.16	32.33	35441.75	35568.15	37.57	36.14	39377.92	39397.95
5	24.81	21.32	25316.14	22877.02	29.87	29.35	30405.34	31170.38
6	10.15	9.68	16278.99	13857.45	16.79	18.62	18058.72	20255.63
7	17.33	18.53	18124.24	20291.95	27.78	28.07	35449.02	33717.47
8	14.92	18.53	18723.32	20291.95	28.19	28.07	34118.44	33717.47
9	10.18	8.97	12134.83	11316.63	19.47	20.58	19224.40	19981.96
10	14.48	17.82	18589.81	19395.08	19.91	19.40	15247.99	15701.89
11	20.30	21.11	21694.47	21794.20	28.25	29.91	26315.13	28071.52
12	32.86	32.88	34414.19	33318.33	34.34	34.10	35843.67	37001.47
13	40.97	38.16	36967.82	37397.36	36.63	36.91	35227.36	36293.49
14	13.98	9.68	14083.46	13857.45	17.57	18.62	19854.74	20255.63

15	25.63	23.58	21188.62	23995.90	28.52	28.68	28252.25	28672.50
16	41.92	37.67	40703.47	38520.72	36.02	35.64	40033.71	37391.31
17	11.06	11.46	13192.08	12810.60	22.93	22.11	20215.17	21821.07
18	26.26	25.17	27920.56	24670.26	27.47	27.20	25330.48	23401.61
19	7.59	8.97	10371.54	11316.63	20.29	20.58	23808.80	19981.96
20	33.08	32.88	32349.22	33318.33	35.26	34.10	35090.16	37001.47
21	27.72	32.33	33046.87	35568.15	37.59	36.14	42015.95	39397.95
22	22.99	23.58	26684.43	23995.90	28.06	28.68	27871.17	28672.50
23	29.52	23.58	24882.46	23995.90	32.46	28.68	32490.59	28672.50
24	16.52	17.82	16590.87	19395.08	20.13	19.40	15219.09	15701.89
25	23.94	25.17	22381.76	24670.26	28.57	27.20	25007.50	23401.61
26	18.96	23.58	20066.95	23995.90	29.42	28.68	30367.87	28672.50
27	22.63	21.32	24174.13	22877.02	28.99	29.35	29803.10	31170.38
28	11.90	11.46	13517.77	12810.60	24.33	22.11	23892.50	21821.07
29	38.05	37.67	39947.30	38520.72	34.03	35.64	35685.80	37391.31
30	21.37	23.58	26428.26	23995.90	24.12	28.68	27226.10	28672.50

TPC = total phenolic content; AC = antioxidant capacity.



Table 3. Analysis of variance (ANOVA) of RSM modelling for TPC and AC of chestnut peel ethanolic and methanolic extracts.

Source	SD <sup>a</sup>	TPC						AC							
		Ethanolic extraction			Methanolic extraction			Ethanolic extraction			Methanolic extraction				
		SS <sup>b</sup>	MS <sup>c</sup>	F-value	SS	MS	F-value	SS	MS	F-value	SS	MS	F-value		
Model	9	2391.55	265.73	28.02*	998.958	110.995	31.70*	2080996660	231221851	45.47*	1479772090	164419121	33.77*		
Linear	3	2215.54	738.51	77.88*	981.865	327.288	93.48*	1940677017	646892339	127.23*	1364270610	454756870	93.41*		
Time	1	1.60	1.60	0.17	15.952	15.952	4.56*	84679	84679	0.02	4506319	4506319	0.93		
S/S ratio	1	46.88	46.88	4.94*	2.051	2.051	0.59	29823886	29823886	5.87*	52085050	52085050	10.70*		
Solvent conc.	1	2167.07	2167.07	228.54*	963.862	963.862	275.29*	1910768451	1910768451	375.79*	1307679242	1307679242	268.60*		
Square	3	138.09	46.03	4.85*	9.157	3.052	0.87	93708906	31236302	6.14*	9352074	3117358	0.64		
Time*Time	1	99.91	99.91	10.54*	2.688	2.688	0.77	48081206	48081206	9.46*	5816935	5816935	1.19		
S/S ratio*S/S ratio	1	19.66	19.66	2.07	2.255	2.255	0.64	6864324	6864324	1.35	1629759	1629759	0.33		
Solvent conc.*Solvent conc.	1	8.38	8.38	0.88	3.801	3.801	1.09	31670024	31670024	6.23*	1186131	1186131	0.24		
Interaction	3	37.91	12.64	1.33	7.936	2.645	0.76	46610737	15536912	3.06	106149405	35383135	7.27*		
Time*S/S ratio	1	20.72	20.72	2.19	0.049	0.049	0.01	5430148	5430148	1.07	89977613	89977613	18.48*		
Time*Solvent conc.	1	0.01	0.01	0.00	0.003	0.003	0.00	11475132	11475132	2.26	3564851	3564851	0.73		
S/S ratio*Solvent conc.	1	17.18	17.18	1.81	7.883	7.883	2.25	29705458	29705458	5.84*	12606941	12606941	2.59		
Residual error	20	189.64	9.48		70.024	3.501		101692162	5084608		97369512	4868476			
Lack of fit	3	67.34	22.45	3.12	23.818	7.939	2.92	36124163	12041388	3.12	34304389	11434796	3.08		
Pure error	17	122.30	7.19		46.206	2.718		65567999	3856941		63065123	3709713			
Total	29	2581.19			1068.98			218268822			1577141602				
		$R^2 = 92.65$	Adj- $R^2 = 89.35$		$R^2 = 93.45$	Adj- $R^2 = 90.50$	Pred- $R^2 = 87.07$		$R^2 = 95.34$	Adj- $R^2 = 93.24$	Pred- $R^2 = 90.01$		$R^2 = 93.83$	Adj- $R^2 = 91.05$	Pred- $R^2 = 86.22$

TPC = total phenolic content; AC = antioxidant capacity; <sup>a</sup>degree of freedom; <sup>b</sup>sum of square; and cmean square.

significant effect on TPC ( $p > 0.05$ ), while the interaction between S/S ratio and ethanol concentration was significant for AC ( $p < 0.05$ ). The predicted models for TPC and AC of ethanolic extracts are as Eq. 4:

$$\begin{aligned} \text{TPC} &= 23.58 - 0.32A - 1.71B - 11.64C - 3.68A^2 + 1.63B^2 + 1.07C^2 + 1.61AB - 0.04AC - 1.47BC \\ \text{AC} &= 23995.9 - 72.75A - 1365.28B - 10928.1C - 2551.676A^2 + 964.13B^2 + 2070.91C^2 + 823.87AB - 1197.66AC - 1926.96BC \end{aligned} \quad (\text{Eq. 4})$$

The negative values indicate that an increase in the factors tends to decrease the responses; on the other hand, positive values in the models show that an increase in them tends to increase the response values (Barizão *et al.*, 2013). As seen in Eq. 4, TPC and AC increased with decreasing extraction time, S/S ratio, and ethanol concentration due to the  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  terms. For the two responses, square and interaction terms of these independent variables also had positive and negative values.

In the case of methanolic extraction, extraction time and methanol concentration had significant linear effects on TPC ( $p < 0.05$ ), while linear terms of S/S ratio and ethanol concentration had significant effects on AC ( $p < 0.05$ ). Also, the interaction between S/S ratio and extraction time was significant ( $p < 0.05$ ) for AC. The predicted models for TPC and AC of methanolic extracts are as Eq. 5:

$$\begin{aligned} \text{TPC} &= 28.68 + 0.10A + 0.36B - 7.76C - 0.60A^2 + 0.55B^2 - 0.72C^2 - 0.078AB - 0.024AC + 0.99147BC \\ \text{AC} &= 28672.5 + 530.70A + 1804.25B - 9040.46C + 887.53A^2 - 469.78B^2 - 400.78C^2 - 3353.68AB - 667.54AC + 1255.34BC \end{aligned} \quad (\text{Eq. 5})$$

As shown in Eq. 5, TPC and AC increased with increasing extraction time and S/S ratio due to the  $\beta_1$  and  $\beta_2$  terms; but increased with decreasing methanol concentration due to  $\beta_3$  term. For the two responses, square and interaction terms of these independent variables also had positive and negative values.

Three dimensional response surface plots were used to represent the relationships between the independent and dependent variables (Figures 2 and 3), in which it is clear that increments of TPC and AC depended mostly on ethanol and methanol concentration. These plots were generated by maintaining one factor at a constant level, whereas the other two factors were varied within their range. As seen in Figures 2 and 3, independently on the solvent used, solvent concentration from 100% (code = +1) to 60% (code = -1) yielded high TPC and AC. This could be due to the increase in the polarity of the solvent. The extraction of polyphenols from agricultural materials depends mostly on the polarity of the extraction solvent (Gunathilake *et al.*, 2019; Haya *et al.*, 2019). Our results are in accordance with previous studies (Türkmen *et al.*, 2006; Vázquez *et al.*, 2012; Čujić

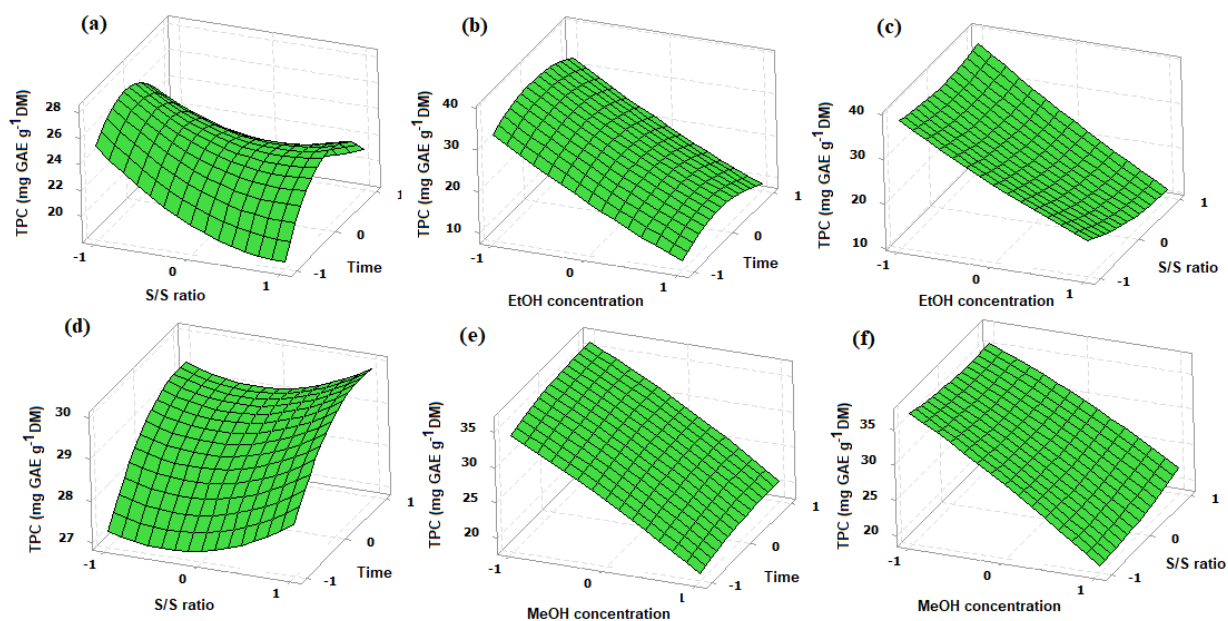


Figure 2. Response surface plots of TPC of (a) ethanolic (a, b, c) and (b) methanolic (d, e, f) extracts from chestnut peel as a function of time, S/S ratio and solvent concentration. Extraction time was kept at constant at 90 min (c and f); S/S ratio was kept at constant at 1/25 of ratio (b and e) and solvent concentration was kept at 80 (a and d).



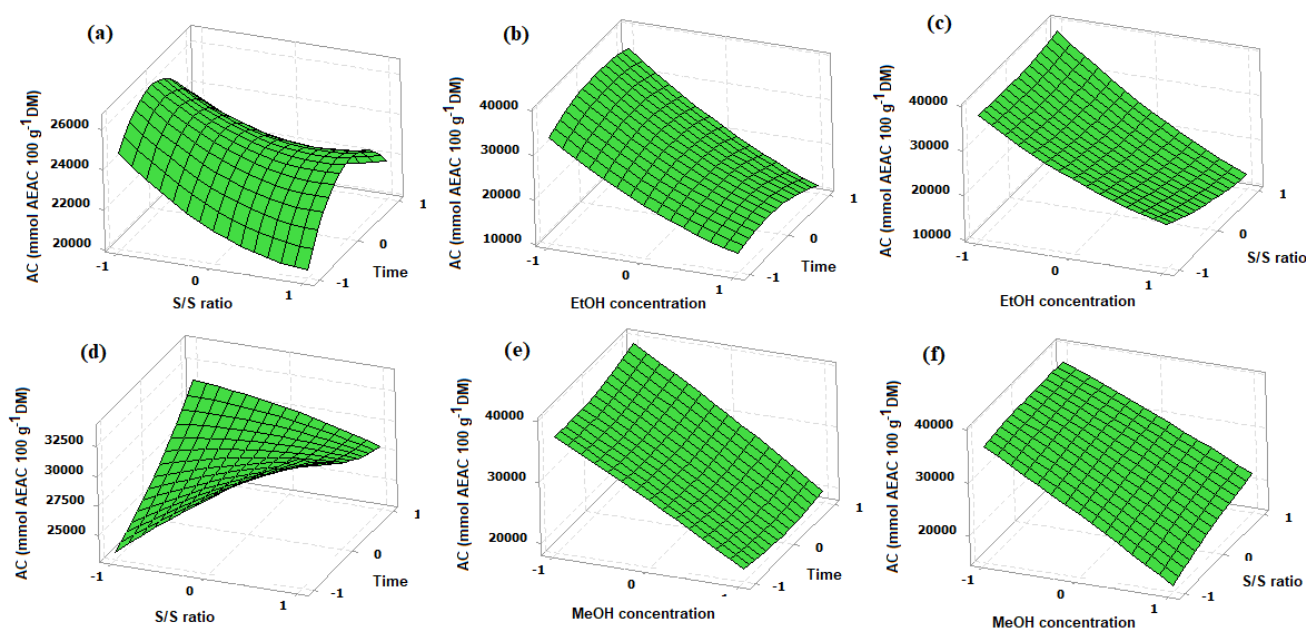


Figure 3. Response surface plots of AC of (a) ethanolic (a, b, c) and (b) methanolic (d, e, f) extracts from chestnut peel as a function of time, S/S ratio and solvent concentration. Extraction time was kept at constant at 90 min (c and f); S/S ratio was kept at constant at 1/25 of ratio (b and e) and solvent concentration was kept at 80 (a and d).

*et al.*, 2016; Liu *et al.*, 2018; Strati *et al.*, 2018).

Regarding the S/S ratio, for ethanol extractions, a significant effect ( $p < 0.05$ ) was produced on maximisation of AC by the interaction between S/S ratio and ethanol concentration (Figure 3c). This result is in accordance with the study of Zhou *et al.* (2019) for antioxidant activity of seed coat extracts from red bean. Decreasing S/S ratio from 1/40 (code = 1) to 1/10 (code = -1) and ethanol concentration from 100% (code = +1) to 60% (code = -1) resulted in the highest AC of peel extract, which is consistent with the study reported by Kumar *et al.* (2019). In the case of methanolic extractions, the interaction between S/S ratio and extraction time had a significant effect ( $p < 0.05$ ) on AC (Figure 3d), which was also reported in the study of Barizão *et al.* (2013). The highest AC of the extracts was obtained when extraction time was at its minimum value and S/S ratio at its maximum value.

#### Optimization procedure

Taking into consideration the results from response surface analysis for polyphenolic extraction, a S/S ratio of 1/10 and a solvent concentration of 60% were concluded as the optimum conditions for both solvents. As the optimum extraction time 82.41 and 116.97 min were selected for ethanolic and methanolic extractions, respectively. Under these optimum extraction conditions, the predicted values of TPC

were 38.40 and 37.41 mg GAE/g DM for ethanolic and methanolic extractions, respectively. Afterwards, the validity of the model was analysed; and the experimentally observed values were 39.02 and 38.79 mg GAE/g DM for ethanolic and methanolic extracts, respectively. This indicated that the predicted results matched well with the experimental results obtained at optimal extraction conditions (TPC for unpretreated and ethanol extracted peel (control) was 29.68 mg GAE/g DM).

In the literature, there have been a lot of studies about the extraction optimization of polyphenols from different sources. However, it is very difficult to compare the optimum extraction conditions from the present work with those from previous studies that reported the same independent variables to optimize extraction method using ethanol and/or methanol as the extraction solvent. The main reasons for that are differences in extraction conditions applied and materials used. Vázquez *et al.* (2012) reported that aqueous solutions of methanol and ethanol (50%) were selected as the optimum solvent concentration and optimum extraction time for methanolic extraction (75 min) was longer than that for ethanolic one (30 min) for chestnut, which is partly consistent with our results. Furthermore, ethanol was observed to be at 60.2% by Zhou *et al.* (2019) for red bean seed coat extracts, methanol at 60% by Strati *et al.* (2018) for leek extracts, and a S/S ratio of 1/10 by Riciputi

et al. (2018) for potato by-product extracts as optimum extraction conditions, which are in accordance with our results.

## Conclusion

In the present work, the extraction conditions for polyphenols from pre-treated chestnut peel were optimized using RSM, and a second-order polynomial model provided a satisfactory fit to the experimental data. The results showed that both the values of  $R^2$  and LOF validated the convenience of the predicted model. BBD was a suitable model for optimization of the extraction process, and a good agreement between predicted and experimental values was found. Solvent concentration and S/S ratio were found to be the most significant parameters affecting TPC and AC of peel extracts. Under optimal conditions selected for polyphenol extraction, TPC values of ethanol and methanol extracts were 39.02 and 38.79 mg GAE/g DM, respectively, that were close to the values of predicted responses (38.40 and 37.41 mg GAE/g DM, respectively). Our results suggest that chestnut shell could be a potentially a good source of polyphenols, and ethanol is a more appropriate solvent for the extraction of polyphenols due to shorter extraction time and being environmentally friendly.

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