OPTIMIZATION OF POLYPHENOL EXTRACTION FROM CHESTNUT WASTE PRETREATED BY OHMIC HEATING USING BOX-BEHNKEN DESIGN

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***Abstract*— Chestnut processing produces a large amount of waste peels, which contain considerable polyphenols. The aim of this research was to optimize the extraction method for phenolic compounds from industrial chestnut peel pretreated by ohmic heating (OH) by using response surface methodology (RSM). Box-Behnken design (BBD) was used to investigate the effects of three independent variables, extraction time, solid to solvent (S/S) ratio, and temperature on total phenolic content (TPC) and antioxidant capacity (AC) of water extracts. All independent variables influenced the TPC and AC of the peel extracts. The optimum extraction conditions found were 22.02 min, S/S ratio of 1/39.70 (w/v), and 60 °C, resulting in the highest TPC of 34.83 mg gallic acid equivalent (GAE)/g dry matter (DM) and AC of 35.62 mol ascorbic acid equivalent (AAE)/100 g DM by DPPH. This study showed that water was almost effective for the extraction of polyphenols from the pretreated chestnut peel.**

***Keywords*— chestnut peel; extraction; ohmic heating; optimization; polyphenols.**

I. INTRODUCTION

Free radicals and reactive oxygen species generated in the body have potential risk factors regarding many diseases such as cancer, diabetes, heart disease and neurological disorder and accelerate ageing (Jung *et al*., 2016). Antioxidants are known to be able to prevent oxidation processes in the body and spoilage of food products (Babbar *et al*., 2014). It is well recognized that they can reduce the risk of human diseases (Amado *et al*., 2014), as a result of their bioavailability, and have a positive influence on food quality by increasing shelf life (Fasolato *et al*., 2016). The antioxidant compounds from agricultural waste/by-products can increase the stability of foods and also protect cell organelles from oxidative damage (Babbar *et al*., 2014). Recently, there has been increasing attention to naturally occurring antioxidants that can be introduced into our diet to replace artificial antioxidants the use of which is being limited owing to their carcinogenicity (Chen *et al*., 2013). Therefore, agricultural and industrial residues are thought to be remarkable sources of natural antioxidants such as polyphenols (Stevigny *et al*., 2007).

The chestnut (*Castanea sativa* Mill.) is one of the most economically important fruit crop. The outer shell and inner skins of the nut comprise 10% of the whole weight (Ham *et al*., 2015). The fruit has been processing mainly chestnut in syrup or marron-glacé, frozen chestnut and chestnut flour, as well as fresh consumption (Gullón *et al*., 2018; Ruiz *et al*., 2017; Vázquez *et al*., 2012). Chestnut processing generates a significant amount of lignocellulosic waste in the form of chestnut shells (Ruiz *et al*., 2017). The valorization of residues as value-added products not only reduces costs but also contributes to environmental pollution (Ham *et al*., 2015). Chestnut shells, which are used mainly as fuel (Fernández-Agulló *et al*., 2014; Vázquez *et al*., 2012), contain a high amount of polyphenols and hydrolysable tannins (Gullón *et al*., 2018). Chestnut shell extracts have been reported to have antimicrobial (Fernández-Agulló *et al*., 2014), antioxidant (Ham *et al*., 2015) and anticancer (Jung *et al*., 2016) activities.

Traditional solid-liquid extraction techniques are used to recover polyphenols from food processing by-products (Kumari *et al*., 2018) and based on using different solvents such as water, methanol, and ethanol or their mixtures (Rajha *et al*., 2019). Recently, novel electrotechnologies such as ohmic heating (OH) and pulsed electric field (PEF) have been tested to improve the efficiency of the conventional extraction of biomolecules from plant tissues causing membrane damage (El Darra *et al*., 2013; Rajha *et al*., 2019). In both methods, the existence of an electric field results in electroporation of cellular tissues enhancing the extraction of bioactive compounds (Pereira *et al*., 2016). OH is a technique in which an alternating current is passed through the food material and heat generation forms within the medium because of its inherent electric resistance (Nair *et al*., 2014). This process has some advantages such as rapid and uniform heating, high energy efficiency, and more environmentally friendly properties, which makes the minimal changes of structural, nutritional, or organoleptic properties in products (Saberian *et al*., 2018). Recently, the extraction of polyphenols from plants using the method assisted by OH has been tested. For example, pretreatment of pulsed OH increased the extraction kinetics of phenolic compounds from grape pomace, being 36% more than untreated samples (El Darra *et al*., 2013). However, this pretreatment, to best of our knowledge, was never applied for the extraction of polyphenols from

chestnut peels, although little research has been done on the effect of different extraction conditions on antioxidant capacity of chestnut peel (Vázquez *et al*., 2012).

Phenolic substances are usually extracted using organic solvents (methanol, ethanol, acetone, and ethyl acetate) with high extraction efficiency but high toxicity (Amado *et al*., 2014). Alternatively, the use of water has several advantages over commonly used organic solvents since it is an environmentally-friendly solvent and no toxicity for human health (Chen *et al*., 2013). The extraction process has importance because yield, composition, and purity of antioxidant compounds depend on the method used (Barizão *et al*., 2013). Extraction efficiency depends on extraction conditions such as solvent type, time, extraction method *etc*. (Fernández-Agulló *et al*., 2014; Jung *et al*., 2016). However, these variables have to be carefully selected to optimize the process (Fernández-Agulló *et al*., 2014). The response surface methodology (RSM) is an important multivariate method used in optimization procedures (Barizão *et al*., 2013).

To our knowledge, there has been no previous report on the optimization of the extraction conditions for phenolics and antioxidants from chestnut peel by RSM. So, the aim of this study was to optimize the extraction conditions (temperature, time, and solid to solvent (S/S) ratio) of phenolics from this agricultural by-product pretreated by OH using RSM.

II. METHODS

A. Plant material

Chestnut fruit (*C. sativa* Mill.) peels (both the outer brown peel and the inner skin) were supplied in December of 2019 by a chestnut processing plant, Kafkas Company (Bursa, Turkey). The peels were filled in the polyethylene bags and kept at 4 ºC before the experiments were conducted.

B. Chemicals

Folin-Ciocalteu's reagent, DPPH (2,2-diphenyl-1-picryhydrazyl), sodium carbonate and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (min. 99.9%) and ascorbic acid were from Merck (Darmstadt-Germany).

C. Pretreatment of chestnut peel by OH

According to preliminary trials, pretreated chestnut peels showed higher TPC values (56.29%) than untreated ones. Therefore, the peels used in this study were preheated by OH. The pretreatment of the peels was carried out in OH chamber, which consists of a rectangular plexiglass and two stainless steel electrodes (Fig. 1). The system of OH chamber was described previously by İncedayi (2020). Peel samples were placed between 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 fixed at 10.8 cm. Table salt solution was utilized to provide better contact between electrodes and material. Pretreatment conditions applied were optimized using RSM (data not shown). The following optimal conditions were applied: concentration of salt solution was 0.32%, elec- time rical field strength, E,

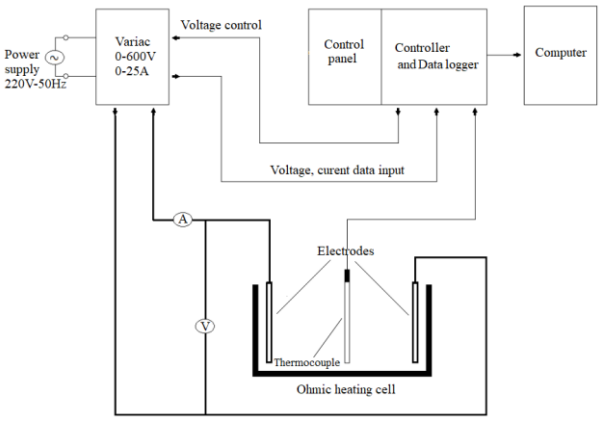

Figure 1: Schematic diagram of the OH system.

was 20 V/cm and treatment was 100 s. The electric field strength within the heating chamber was calculated as follows (Eq. 1):

$$Electrical\ field\ strength\ \left(E, \frac{V}{cm}\right) = \frac{Output\ voltage\ (V)}{Distance\ between\ the\ electrodes\ (cm)} \quad (1)$$

After OH, samples were drained off rapidly, rinsed, cooled in running water, and air-dried at room temperature until they reached constant moisture content. Peel samples were milled, passed through a 2 mm sieve, and stored at 4 °C before experiments.

D. Experimental design

In this study, Box–Behnken design (BBD) was applied to determine the parameters for polyphenol extraction from waste of chestnut peel. The current design comprised 30 experimental runs with three levels, −1 (lower limit), 0 (central point), and +1 (upper limit) for each factor. A (time), B (S/S ratio), and C (temperature) were chosen as the independent variables whose selection and range were based on previous studies, while response variables were TPC and AC. Table 1 shows the experimental design (coded and actual values of the factors) for each run. The experiments were performed in duplicate, and the average of the duplicate has been taken as a response. The experimental data were fitted to the following second-order polynomial model (Eq. 2):

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

where Y is the predicted responses; β_0 is the model intercept coefficient; β_1, β_2 and β_3 are the regression coefficients for the linear effect terms; β_1^2, β_2^2 and β_3^2 are the square effect terms; and $\beta_1\beta_2$, $\beta_1\beta_3$ and $\beta_2\beta_3$ are the interaction effect terms, respectively. A, B and C are the independent variables (Table 1). The adequacy of the model was predicted through the regression analysis (R^2) and the analysis of variance (ANOVA). Optimal conditions were chosen considering the response surface plots. MINITAB 18 software (State College, PA) was used for data analysis.

E. Extraction of polyphenols

In this study, water was used as an extraction solvent because preliminary trials showed that water was found to

Table 1. BBD including independent variables and the respective responses of dependent variables i.e. TPC (mg GAE/g DM) and AC (mol AAE/100 g DM) of chestnut peel water extracts.

Exp.	Independent variables			Dependent variables			
				TPC		AC	
	A	B	C	Experimental	Predicted	Experimental	Predicted
1	-1	1	0	6.12	7.08	7.44	7.41
2	0	1	1	14.84	13.11	20.27	19.29
3	1	-1	0	21.11	21.45	14.83	15.85
4	0	1	-1	4.70	4.12	3.08	3.63
5	1	0	-1	23.38	22.11	21.50	19.14
6	0	0	0	25.64	27.41	26.54	28.13
7	0	0	0	26.13	27.41	28.64	28.13
8	-1	0	1	35.40	33.30	33.51	33.64
9	-1	0	-1	7.49	7.16	5.55	5.22
10	1	-1	0	22.09	21.45	15.81	15.85
11	0	-1	1	27.41	29.69	27.31	26.57
12	0	0	0	29.52	27.41	30.80	28.13
13	0	0	0	25.05	27.41	26.12	28.13
14	0	1	-1	3.77	4.12	2.98	3.63
15	0	-1	1	31.74	29.69	27.04	26.57
16	0	0	0	31.04	27.41	33.59	28.13
17	0	-1	-1	10.13	11.37	9.51	10.83
18	-1	-1	0	21.17	18.89	19.36	18.35
19	0	1	1	15.05	13.11	21.78	19.29
20	1	0	-1	24.24	22.11	21.31	19.14
21	1	0	1	23.74	23.27	22.04	22.12
22	1	1	0	8.02	9.43	11.07	12.32
23	0	-1	-1	8.93	11.37	8.69	10.83
24	-1	0	1	27.82	33.30	29.26	33.64
25	1	0	1	22.73	23.27	22.06	22.12
26	-1	-1	0	20.21	18.89	20.64	18.35
27	0	0	0	27.09	27.41	23.12	28.13
28	1	1	0	7.23	9.43	10.27	12.32
29	-1	0	-1	6.91	7.16	5.04	5.22
30	-1	1	0	7.75	7.08	8.44	7.41

Independent variables	Levels		
	-1	0	+1
A: Time (min)	20	30	40
B: S/S ratio (w/v)	1/25	1/50	1/75
C: Temperature (ºC)	40	50	60

be almost effective. Ground peel sample was extracted with distilled water in a controlled water bath. Extractions were performed at a known S/S ratios (w/v), temperatures (ºC) and times (min) defined in Table 1. After extraction time period, the suspension was filtered through Whatman No. 1 filter paper and the extract was rapidly cooled under tap water.

F. Extract analyses

Total polyphenol content (TPC): TPC of the peel was analyzed using the Folin-Ciocalteu method (ISO 14502-1, 2005). In this method, 0.5 mL of extract or pure water (as a blank) was mixed with 2.5 mL of Folin-Ciocalteu reagent (10%, v/v). 2 mL of 7.5% sodium carbonate solution is added to the mixture after 5 min and shaken thoroughly. The mixture was allowed to stand for 60 min and blue color formed was measured at 765 nm against blank using a spectrophotometer (Shimadzu UV-VIS 1208). A calibration curve of gallic acid (5-50 µg/mL) was prepared, and the results calculated from the regression equation of the calibration curve [y (absorbance)=0.0157x (gallic acid concentration), R^2=0.99] were defined as mg gallic acid equivalents (GAE) per gram of dry matter.

Antioxidant capacity (AC): AC was determined by using DPPH method of Turkmen Erol *et al*. (2009). AC was calculated as the percentage inhibition of the DPPH radical by the following equation 3:

$$AC(\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \qquad (3)$$

$\boldsymbol{Abs_{control}}$ is the absorbance of the DPPH solution without sample and $\boldsymbol{Abs_{sample}}$ is the absorbance of the test sample.

AC of samples was converted to ascorbic acid equivalent (AAE) defined as mol of ascorbic acid equivalents per 100 g of DM.

III. RESULTS

A. Analysis of the model

The responses of AC determined by DPPH assay and TPC of the chestnut peel water extracts are shown in Table 1. According to the results, the values of TPC and AC of the extracts varied from 3.77 to 35.40 mg GAE/g DM and from 29.78 to 33.59 mol AAE/100 g DM, respectively.

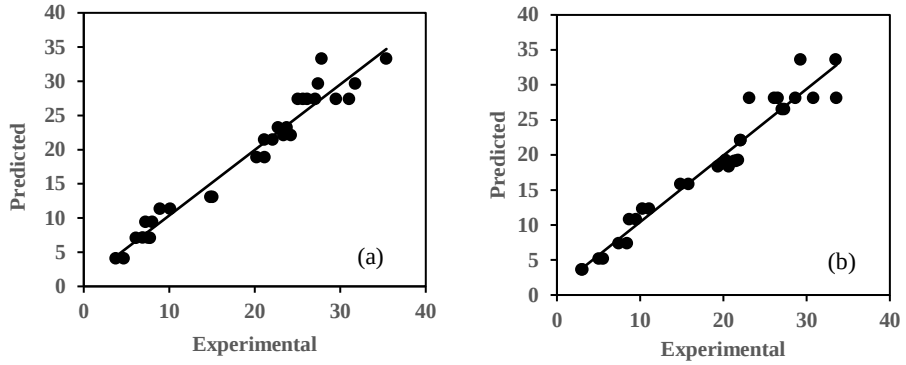

Figure 2: Experimental *versus* predicted values for TPC (a) and AC (b) of the peel water extracts.

Table 2. ANOVA of RSM modeling for TPC and AC

	Source	DF[a]	SS[b]	MS[c]	F value	p-value
TPC	Model	9	2505,57	278,397	51.00	0.000
	Linear	3	1337.01	445.669	81.64	0.000
	Time	1	24.170	24.170	4.43	0.048
	S/S ratio	1	567.735	567.735	104.00	0.000
	Temperature	1	745.102	745.102	136.49	0.000
	Square	3	812.91	270.971	49.64	0.000
	Time * Time	1	73.447	73.447	13.45	0.002
	S/S ratio * S/S ratio	1	745.188	745.188	136.51	0.000
	Temperature * Temperature	1	57.681	57.681	10.57	0.004
	Interaction	3	355.65	118.551	21.72	0.000
	Time * S/S ratio	1	0.024	0.024	0.00	0.948
	Time * Temperature	1	312.063	312.063	57.17	0.000
	S/S ratio * Temperature	1	43.565	43.565	7.98	0.010
	Residual Error	20	109.18	5.459		
	Lack-of-Fit	3	38.19	12.732	3.05	0.057
	Pure error	17	70.98	4.175		
	Total	29	2614.75			
	R^2 = 95.82 Adj-R^2 = 93.95 Pred-R^2 = 90.51					
AC	Model	9	2422.82	269.202	42.21	0.000
	Linear	3	1201.24	400.412	62.78	0.000
	Time	1	5.81	5.81	0.91	0.351
	S/S ratio	1	209.33	209.327	32.82	0.000
	Temperature	1	986.10	986.101	154.60	0.000
	Square	3	870.35	290.118	45.49	0.000
	Time * Time	1	173.62	173.616	27.22	0.000
	S/S ratio * S/S ratio	1	709.54	709.537	111.24	0.000
	Temperature * Temperature	1	78.01	78.014	12.23	0.002
	Interaction	3	351.23	117.076	18.36	0.000
	Time * S/S ratio	1	27.49	27.494	4.31	0.051
	Time * Temperature	1	323.73	323.730	50.76	0.000
	S/S ratio * Temperature	1	0.00	0.003	0.00	0.984
	Residual Error	20	127.56	6.378		
	Lack-of-Fit	3	45.89	15.296	3.18	0.051
	Pure error	17	81.68	4.804		
	Total	29	2550.38			
	R^2 = 95.00 Adj-R^2 = 92.75 Pred-R^2 = 90.22					

a: Degree of freedom b: Sum of square c: Mean square

The ANOVA of the second-order polynomial model for TPC and AC from chestnut peels indicated that the model was significant ($p < 0.05$) with calculated and adjusted coefficients of determination (R^2) > 0.9 (Table 2). These values represent a reliable agreement between predicted and experimental data (Fig. 2). For two model responses, lack of fit (LOF) was not significant ($p > 0.05$), and this showed that the model could be used to predict TPC and AC.

All linear and square terms were significant ($p < 0.05$) except the linear term of time for AC, which indicated TPC and AC were strongly influenced by these factors. The interaction between time and temperature was significant ($p < 0.05$) for both responses. Also, the interaction between S/S ratio and the temperature was significant ($p<0.05$) for only TPC response. The predicted model equations (Eqs. 4 and 5) for TPC and AC were given below, respectively:

$$TPC = 27.41 + 1.23A - 5.96B + 6.82C - 3.15A^2 - 10.05B^2 - 2.79C^2 - 0.06AB - 6.25AC - 2.33BC \quad (4)$$

$$AC = 28.13 + 0.60A - 3.62B + 7.85C - 4.85A^2 - 9.80B^2 - 3.25C^2 + 1.85AB - 6.36AC - 0.02BC \quad (5)$$

The positive values in the models show that an increase of factors tends to increase the response values; otherwise, negative values indicate that an increase tends to reduce the responses (Barizão *et al*. 2013). As seen in equations above, TPC and AC increased with increasing extraction time and temperature due to the β_1 and β_3 terms but decreased with increasing S/S ratio due to β_2 term. Square and interaction terms of these independent variables had mostly negative values.

B. Effect of extraction parameters on TPC and AC

The results illustrated that S/S ratio, extraction time and temperature greatly affected TPC and AC from chestnut peel (Table 2). The response surface plots were used to demonstrate the effects of S/S ratio, extraction time and the temperature on the responses (Fig. 3). These plots were generated by maintaining one factor at a constant level, whereas the other two factors were varied in their range.

A significant effect was produced on the maximization of TPC by the two-factor interaction between extraction temperature and S/S ratio ($p<0.05$). It has been shown in Fig. 3a that temperature from 50 °C (code = 0) to 60 °C (code = +1) and S/S ratio from 1/50 (code = 0)

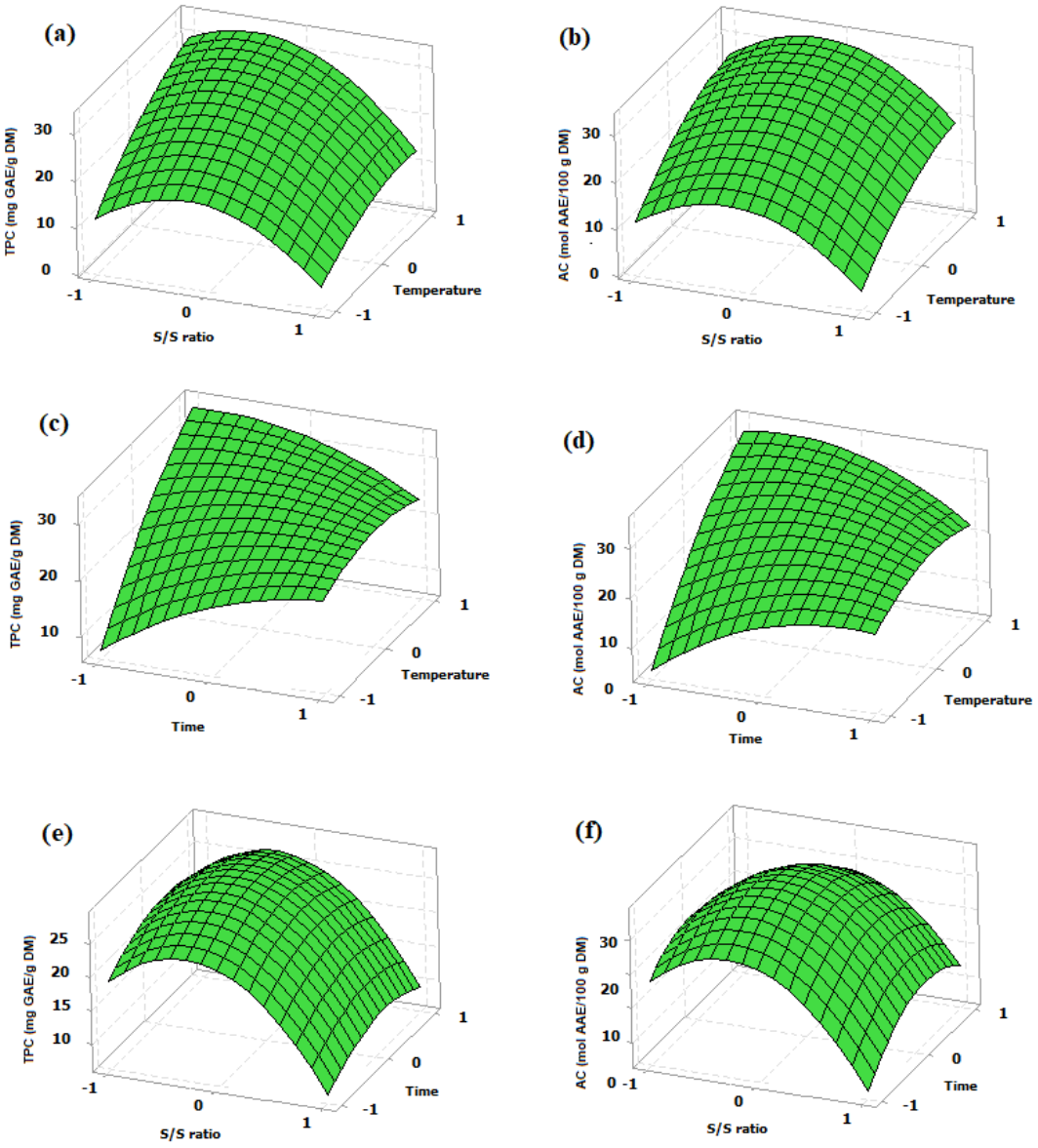

Figure 3: Response surface plots of TPC (a, c and e) and AC (b, d and f) from chestnut peel as a function of temperature, time and S/S ratio. Extraction time was kept at 30 min (a and b); S/S ratio was kept at 1/50 (c and d) and temperature was kept at 50 ºC (e and f).

to 1/37.5 (code = -0.5) yielded high TPC. As indicated in the study of Gunathilake *et al*. (2019), increasing the extraction temperature increase in the solubility of polyphenols, diffusion rate, mass transfer rate, and extraction rate. Additionally, plant tissues might soften more as the temperature increased. Thus more polyphenols would diffuse into the solvent (Vázquez *et al*., 2012). The interaction between extraction time and the temperature had a significant effect ($p < 0.05$) on TPC, which had the highest coefficient in all interaction effects. But, this is different from the previous study on *Centella asiatica* (Gunathilake *et al*., 2019), which might be because they used alcohol instead of water as the extraction solvent. Temperature within the range 50-60 °C and extraction time from 25 min (code = -0.5) to 20 min (code = -1) resulted in higher TPC (Fig. 3c). On the other hand, the combined effect of time and S/S ratio on TPC was not significant according to the results of ANOVA (Table 2) (Fig. 3e), which also have been reported in another work on apple (Barizão *et al*. 2013).

Similarly, the interaction effect between extraction time and temperature on AC was found to be significant, as observed in TPC, which is in consistent with the study of Franco *et al*. (2018) for peanut skin. Moreover, it had the highest coefficient among all interaction effects. Increasing the temperature from 50 to 60 °C and decreasing extraction time from 25 min (code = -0.5) to 20 min (code = -1) resulted in higher AC (Fig. 3d). However, other two interaction effects (S/S ratio x temperature and S/S ratio x time) on AC were not significant (Figs. 3b and 3f) (Table 2), which is in agreement with the study reported by Barizão *et al*. (2013) for apple flesh.

C. Optimization procedure

The optimal conditions predicted by the models were developed for maximizing both responses, TPC and AC, and tested to evaluate the extraction method. The predicted optimal temperature of the bath, S/S ratio and extraction time were 60 °C, 1/39.70, and 22.02 min, respectively. For these optimum extraction conditions, the corresponding predicted response values for TPC and AC were 35.13 mg GAE/g DM and 35.00 mol AAE/100 g DM, respectively. Experimental values of TPC and AC at optimal conditions were 34.83 mg GAE/g DM and 35.62 mol AAE/100 g DM, respectively, near to the predicted values (TPC and AC values for untreated peel were 16.37 mg GAE/g DM and 15.63 mol AAE/100 g DM, respectively). According to this, predicted results matched well with the experimental results obtained at optimum extraction conditions. However, the optimum extraction conditions from this study were different from those reported by some researchers who also investigated the optimization of polyphenol extraction from different natural sources (Barizão *et al*., 2013; Franco *et al*., 2018). This disparity may be attributed to differences in solvents, extraction times, and materials used, which also makes difficult the comparison of the results from one study to another. On the other hand, TPC obtained at our optimal conditions was higher than that reported in the study of Vella *et al*. (2018). The researchers found 17.68 mg GAE/g DM from chestnut shell by boiling water at 1/40 (w/v) solid/liquid ratio, indicating that our procedure was effective. Additionally, water was less effective for the extraction of polyphenols from chestnut peel compared to organic solvents such as ethanol and methanol from our preliminary experiment (data not shown). Despite this fact, TPC obtained from chestnut peel using water (34.83 mg GAE/g DM) was found to be higher than TPC obtained with organic solvents from some other materials such as tomato peel (21.0 mg GAE/g dry weight-dw), pea pod (13.6 mg GAE/g dw); cauliflower waste (9.2 mg GAE/g dw) (Babbar *et al*., 2014) and hazelnut shell (9.18 GAE mg/g of shell) (Stevigny *et al*., 2007). On the other hand, TPC obtained at our optimal conditions was higher than that reported in the study of Vella *et al*. (2018). The researchers found 17.68 mg GAE/g DM from chestnut shell by boiling water at 1/40 (w/v) solid/liquid ratio, indicating that our procedure was effective.

IV. CONCLUSIONS

Box-Behnken design was a remarkable tool to determine the best extraction conditions of polyphenols from the chestnut peel. Results showed that both the value of R^2 and LOF validate the suitability of the predicted model. RSM study showed that the highest values of TPC and AC were obtained when the following conditions were used: extraction temperature of 60 °C, 22.02 min, and a S/S ratio of 1/39.70 (w/v). From this study, it could be concluded that water is almost effective when applied at optimal conditions for extraction of polyphenols from the chestnut peel, which is more meaningful due to its economic, environmentally-friendly and non-toxic solvent.

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