CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

MICROWAVE ASSISTED EXTRACTION OF CUSTARD APPLE (*ANNONA SQUAMOSAL* L.) PEEL

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https://doi.org/10.34302/crpjfst/2023.15.1.16

Article history:
Received:
10 January 2022
Accepted:
20 December 2022

Keywords:
Custard apple peel;
Microwave assisted extraction;
Polyphenols;
Antioxidants;
Response surface methodology.

ABSTRACT

In recent years, custard apple fruit has been applied in food processing with various products. The purpose of this study aimed to valorize the peel as organic food waste produced by fruit processing. Microwave assisted extraction (MAE) of bioactive polyphenols from custard apple peel was performed at different aqueous ethanol composition, extraction irradiation time, solvent to solid ratio, and microwave power. Total polyphenols content (TPC) and antioxidant activities of the extracts were investigated. Response surface methodology was applied to find the optimal condition according to the central composite design with ethanol concentration ranged from 50 to 70%, extraction time from 3 to 7 min, solvent to solid ratio from 20 to 30 mL/g, and microwave power from 154 to 274 W. A quadratic model was respectively developed to correlate the investigated variables to the TPC and radical scavenging activity by DPPH and ABTS of the extracts. Optimum condition was successfully selected at an ethanol concentration of 60%, extraction time of 5 min, solvent-solid ratio of 25 mL/g, and microwave power of 214 W. With a good correlation between predicted values and actual experimental results, the developed response surface model can be used to optimize the extraction of polyphenols from custard apple peel by MAE.

1.Introduction

Annona squamosal L., commonly known as custard apple, is cultivated mainly for its edible fruit. The plant is also attributed with antifertility, antitumor and abortifacient properties, and can be traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infestation, constipation, haemorrhage, antibacterial infection, dysuria, fever, and ulcer (Amudha & Varadharaj, 2017; Kaleem, Medha, Ahmed, Asif, & Bano, 2008). Custard apple is climacteric fruit, characterized by high respiration and rapid softerned after harvest, and are chilling sensitive (Pareek, Yahia, Pareek, & Kaushik, 2011).

Todays, fruit by-products are one of the main sources of organic waste that cause environmental pollution (Carciochi et al., 2017). Previous study showed that the custard apple peel contains polyphenols (Prajapti, Purohit, Sharma, & Kumar, 2006). Polyphenols have considerable significance as bioactive compounds with substantial health benefits because of their antioxidant potential to scavenge free radicals (Nag & Sit, 2018). It was reported that polyphenols from custard apple peel can be extracted by conventional solvent extraction methods and may have several medicinal properties (Sharma, Sharma, Chand, Khardiya, & Agarwal, 2013). However,

conventional solvent extraction method is associated with high solvent consumption and negative environmental impact, longer extraction times and generating relatively low yields (Jovanović et al., 2017). Recently, there have been many advanced techniques developed for extracting phenolic compounds from plant materials such as ultrasound assisted extraction, microwave assisted extraction (MAE), pressurized liquid extraction, enzymatic extraction and supercritical fluid extraction (Marchese et al., 2016). Among the techniques, MAE method has attracted significant interest because of its advantages in shorter extraction time, less energy and organic solvent consumption, reasonable cost and high efficiency (Kaderides, Papaoikonomou, Serafim, & Goula, 2019; Mellinas, Jiménez, & Garrigós, 2020; Sarfarazi, Jafari, Rajabzadeh, & Galanakis, 2020). There are several variables can influence the MAE. This work included a study of MAE of custard apple peel at different solvent composition, extraction irradiation time, solvent to solid ratio, and microwave power. Total polyphenols content, antioxidant activity according to DPPH and ABTS assays were investigated and the extraction process was optimized using response surface methodology. Up to now, there is no literature on optimization of the process for MAE of polyphenols from custard apple peel.

2. Materials and methods

2.1. Materials

2.1.1. Samples

The custard apples were from Tay Ninh Province, Vietnam. The peel was separated and rinsed with water to remove fleshy residues, then dried by hot-air at 60°C until the moisture content obtained ≤ 12%. The dried peel was powder and sieved through a 0.5 mm sieve before stored in PE bags with an average mass of 5 ± 0.03 g. PE samples were sealed and stored in the freezer at -20°C for subsequent experiments.

2.1.2. Chemicals and reagents

Folin-Ciocalteu reagent (≥ 99.8%) and standard gallic acid (GA) (≥ 99.9%) were supplied by Merck (Germany). DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (≥ 97%) reagent were purchased from Sigma-Aldrich (USA). All other chemicals were analytical grade.

2.2. Methods

2.2.1. Extraction procedure

The polyphenols extraction was carried out by using a domestic microwave oven (Sanyo, Japan). For a typical experimental extraction procedure, 1.0 gram of custard apple peel powder was infused in aqueous ethanol and introduced to the oven equipped with reflux condenser in order to condense the vapors generated during MAE. The extraction variables evaluated were ethanol proportion (40-70%), solvent to solid material ratio (20:1- 35:1 v/w), extraction time (1-7 min) and microwave power (95 - 284 W). After MAE treatment, the extracts obtained were filtered through Whatman No. 4 filter paper then their total polyphenol content and antioxidant activity were determined.

2.2.2. Analysis of total phenolic content

Total polyphenol content (TPC) was determined according to the Folin Denis method as described in study of (Sripakdee, Sriwicha, Jansam, Mahachai, & Chanthai, 2015) with modification. Sample extract (0.1 mL) was reacted with 1.8 mL Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and incubated at room temperature for 5 min followed by the addition of 1.2 mL of sodium carbonate (15%, w/v). After 90 min absorbance was measured at 765 nm at room temperature. The results were expressed as mg gallic acid equivalent per g dry sample (mg GAE/g DW).

2.2.3. Determination of radical scavenging activity by DPPH

The free radical scavenging activity of custard apple peel extract on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was determined according to the method described by (Sripakdee et al., 2015) with modifications. Sample extract (0.1 ml) was added to 4 ml of

DPPH reagent and kept in darkness conditions at room temperature for 30 min. The absorbance was measured at 517 nm using a UV–visible spectrophotometer (Thermo, Genesys 10 UV). Trolox was used as standard and the results were expressed as μmol Trolox equivalents per gram dry sample (μmol TE/g DW).

2.2.4. Determination of radical scavenging activity by ABTS

The antioxidant capacity against 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was measured according to the method described by (Sripakdee et al., 2015) with modifications. 0.1 mL of extract was then added to 3 mL of ABTS solution and mixed at room temperature for 15 min in darkness conditions. The absorbance was determined at 734 nm using a UV–visible spectrophotometer (Thermo, Genesys 10 UV). Trolox was used as standard and the results were expressed as μmol Trolox equivalents per gram dry sample (μmol TE/g DW).

2.2.5. Statistical analysis

All results were subjected to statistical analyses. For screening individual process variables, experiments were carried out in triplicate and average values with standard deviations were computed by Statgraphics (Centurion XV). Significant difference was defined at $p<0.05$.

2.2.6. Experimental design and optimization

Response surface methodology (RSM) was used to determine optimal process parameters (variables) to extract polyphenols from custard apple peel. Equation (1) is the general form of a response surface of response variable Y as a function of n independent process variables from X_1 to X_n.

$$Y = B_o + \sum_{i=1}^{n} B_i X_i + \sum_{i=1}^{n} B_{ii} X_i^2 + \sum_{\substack{i,j=1 \\ i \neq j}}^{n} B_{ij} X_i X_j \quad (1)$$

where B_o is the constant; B_i is the linear coefficient; B_{ii} is the quadratic coefficient; and B_{ij} is the cross-product coefficient.

Table 1. Independent variables and selected levels used in the CCD for polyphenols extraction from custard apple peel.

Parameters/Variables	-1	0	+1
Ethanol concentration, X_1 (%)	50	60	70
Extraction time, X_2 (min)	3	5	7
Solvent-solid ratio, X_3 (mL/g)	20:1	25:1	30:1
Microwave power, X_4 (W)	154	214	274

A central composite design (CCD) was employed to the experimental data with different independent variables and selected levels (Table 1).

In this study, four independent process variables were ethanol concentration (% v/v; X_1), extraction time (min; X_2), solvent-solid ratio (mL/g; X_3), and microwave power (W; X_4). The selected response variables were TPC (mg GAE/g DW; Y_1), radical scavenging activity (RSA) by DPPH (μmol TE/g DW; Y_2), and RSA by ABTS (μmol TE/g DW; Y_3).

Different experimental combinations of levels were carried out in duplicate, and six experiments were performed at the center points of the design to allow the estimation of pure error. JMP version 10 was used to fit the quadratic response surface model (Equation 1 with n=4) to the experimental data.

3. Results and discussions

Influences of processing parameters including aqueous ethanol composition, extraction irradiation time, solvent to solid ratio, and microwave power on the TPC, antioxidant activity according to DPPH and ABTS assays of the extracts by MAE of custard apple peel are presented in Table 2.

Table 2. Effect of processing parameters on polyphenol content and antioxidant activity.

Parameter	Level	TPC (mg GAE/g DW)	RSA by DPPH (μmol TE/g DW)	RSA by DPPH (μmol TE/g DW)
Ethanol concentration	40 (%)	$82.06^{a} \pm 2.07$	$489.90^{d} \pm 6.65$	$1119.52^{g} \pm 7.05$
	50 (%)	$91.39^{b} \pm 1.17$	$536.04^{e} \pm 20.25$	$1159.12^{g} \pm 24.84$
	60 (%)	$96.27^{c} \pm 0.74$	$589.46^{f} \pm 17.39$	$1242.98^{h} \pm 21.79$
	70 (%)	$89.06^{b} \pm 0.57$	$582.21^{f} \pm 15.65$	$1122.48^{g} \pm 42.14$
Solvent-solid ratio	20:1 (mL/g)	$92.09^{b} \pm 1.92$	$550.22^{d} \pm 25.57$	$1267.39^{f} \pm 21.69$
	25:1 (mL/g)	$96.12^{c} \pm 0.21$	$617.35^{e} \pm 15.54$	$1361.38^{g} \pm 19.91$
	30:1 (mL/g)	$91.16^{ab} \pm 2.13$	$587.55^{e} \pm 13.57$	$1298.38^{f} \pm 9.07$
	35:1 (mL/g)	$88.94^{a} \pm 0.61$	$546.99^{d} \pm 4.37$	$1261.69^{f} \pm 30.99$
Extraction time	1 (min)	$92.13^{a} \pm 0.56$	$549.03^{c} \pm 4.38$	$1159.24^{e} \pm 13.72$
	3 (min)	$95.02^{b} \pm 0.74$	$561.38^{c} \pm 9.00$	$1236.51^{f} \pm 8.29$
	5 (min)	$98.63^{c} \pm 1.05$	$603.02^{d} \pm 18.73$	$1283.58^{g} \pm 11.52$
	7 (min)	$95.37^{b} \pm 0.84$	$587.59^{d} \pm 6.91$	$1180.64^{e} \pm 15.47$
Microwave power	95 (W)	$90.07^{a} \pm 1.06$	$518.29^{d} \pm 6.94$	$1009.40^{g} \pm 5.44$
	166 (W)	$91.40^{a} \pm 1.67$	$573.94^{e} \pm 11.41$	$1198.78^{i} \pm 11.32$
	214 (W)	$97.62^{b} \pm 1.72$	$613.15^{f} \pm 13.89$	$1279.37^{j} \pm 60.61$
	284 (W)	$91.76^{a} \pm 1.89$	$463.51^{c} \pm 2.21$	$1121.40^{h} \pm 11.75$

Within one parameter group, different superscripts within the same column indicate significant differences between values ($p < 0.05$).

3.1. Effect of ethanol concentration

Ethanol and water are among the most commonly used solvents to extract polyphenols from plant because of their effectiveness and environmental friendliness (Chan et al., 2009; Mustafa & Turner, 2011). It was reported that by creating a more polar medium and breaking hydrogen bonding, addition of a quantity of water to ethanol facilitated the extraction of polyphenols from both high and low polarity ends (Jovanović et al., 2017). Therefore, aqueous ethanol composition is an importance factor to be optimized in order to obtain a good extraction yield with economic advantage.

As shown in Table 2, the effect of ethanol concentration with different polarities on TPC is presented. The result shows that increasing the ethanol concentration from 40% to 60%, the total polyphenol content (TPC) increases gradually from 82.06 mg GAE/g DW to 96.27 mg GAE/g DW. Meantime, radical scavenging activity by DPPH increased from 489.90 to 589.46 μmol TE/g DW and activity by ABTS was from 1119.52 to 1242.98 μmol TE/g DW. This can be explained by the difference in dielectric properties of the solvents towards microwave heating. The dielectric constant of water is higher than ethanol; therefore, increase in ethanol concentration led to a slower microwave energy absorption and reduced heating of the sample with a limited thermal degradation of the extracted phenolic compounds (Dahmoune et al., 2014). A similar effect was reported for the extraction of polyphenols from other plant sources (Dahmoune, Nayak, Moussi, Remini, & Madani, 2015; Li et al., 2012).

However, continuing increase concentration of ethanol to 70%, the TPC decreased to 89.06 mg GAE/g DW, DPPH and ABTS radical scavenging activities decreased to 582.21 (μmol TE/g DW) and 1122.48 (μmol TE/g DW), respectively. A high ethanol concentration will reduce the polarity of the solvent and molecular movement resulting in light dissolution of polyphenol compounds and decrease of

solubility (Jovanović et al., 2017). Therefore, to achieve the optimal polyphenol extraction efficiency and antioxidant activity, the 60% ethanol concentration was finally selected as center point for the next RSM trials.

3.2. Effect of solvent-solid ratio

In the MAE extraction method, the ratio of solid material to solvent is an important factor affecting the extraction efficiency. Solvent can diffuse through the porous matrix of dried peel material and extract the interested compounds. The solvent-solid ratio influences the mass transfer. Using a high ratio also provides an increase in the gradient concentration of the polyphenols between surface and interior part of the dried custard apple peel. The influence of ratio of solvent to solid on the polyphenol content and antioxidant activities of the extract is shown in Table 2.

TPC content increased from 92.09 mg GAE/g DW at solvent-solid ratio of 20:1 to a maximum of 96.12 mg GAE/g DW at the solvent-solid ratio of 25:1. With the same trend, DPPH scavenging activity increased from 550.22 μmol TE/g DW to 617.35 μmol TE/g DW and ABTS scavenging activity increased from 1267.39 μmol TE/g DW to 1361.38 μmol TE/g DW. However, when the solvent-solid ratio continued to rise, the TPC and antioxidant activity gradually decreased. The total polyphenol content decreased from 91.16 mg GAE/g DW at solvent-solid ratio of 30:1 and continued to decrease to 88.94 mg GAE/g DW at the ratio of 35:1. The radical scavenging activity by DPPH decreased from 587.55 μmol TE/g DW to 546.99 μmol TE/g DW and the activity by ABTS was also reduced from 1298.38 μmol TE/g DW to 1261.69 μmol TE /g DW. At a certain amount of solvent, the bioactive substances will not continue to increase when the extraction reaches equilibrium. The more solvent used, the greater the amount of dissolved oxygen in it. As a result, the presence of oxygen not only reduces the TPC but also weakens the antioxidant activity of polyphenols (Chan et al., 2009; Thoo, Ho, Liang, Ho, & Tan, 2010). Therefore, when increasing the solvent-solid ratio to 30:1 and 35:1, the total polyphenol content and antioxidant activity decrease. The solvent-solid ratio of 25:1 was statistically different from the remaining ratios and this is the highest for TPC and antioxidant activity. Therefore, to achieve the best extraction efficiency, this will be selected for the next optimization experiments.

3.3. Effect of extraction irradiation time

Effect of extraction irradiation time on polyphenols content and antioxidant activity is also shown in Table 2. Increased extraction time from 1 minute to 5 minutes, the total polyphenol content (TPC) increased from 92.13 mg GAE/g DW to a maximum of 98.63 mg GAE/g DW. With the same trend, the antioxidant activity according to DPPH and ABTS assays increases from 549.03 μmol TE/g DW to 603.02 μmol TE/g DW and from 1159.24 μmol TE/g DW to 1283.58 μmol TE/g DW, respectively. In contrast, when extraction time continued to increase to 7 minutes, TPC decreased to 95.37 mg GAE/g DW and antioxidant activity also reduced to 587.59 μmol TE/g DW (DPPH assay) and to 1180.64 μmol TE/g DW (ABTS assay). Thus, extraction time is an important factor and affects the microwave extraction of custard apple peels. The TPC content and antioxidant activities increase as the extraction time increases, but too long extraction time may lead to degradation of the polyphenols resulting in a decrease of these important values. This is consistent with the study of (Zhao, Zhang, Li, Meng, & Li, 2018) showing that in microwave-assisted extraction of phenolic compounds from *Melastoma sanguineum* fruit the TPC value increased with duration increasing from 15 to 45 min; while the extraction efficacy decreased with extended duration to 60 min (Zhao et al., 2018). Therefore, to achieve the best extraction efficiency during polyphenol extraction, the irradiation duration of 5 minutes was selected for the optimization process.

3.4. Effect of microwave power

Microwave power is also an important factor in the extraction of polyphenols by MAE

method. Results (Table 2) showed that microwave power significantly influenced the TPC and antioxidant activities in the tested condition. There were correlations among TPC, antioxidant activities and microwave power. As microwave power increased from 95 W to 214 W, the total phenol and antioxidant capacity also increased. A maximum extraction of 97.62 (mg GAE/g DW) total poly phenol content, together with scavenging against DPPH and ABTS (613.15 μmol TE/g and 1279.37 μmol TE/g DW) was obtained at 214 W. It was reported that the main effect of microwaves in many cases of MAE is the heating effect (Dahmoune et al., 2014). Therefore, this can be explained that as temperature increases at high microwave power, the solubility and diffusion of the compounds from the material matrix into the solvent will be enhanced. In addition, increasing temperature will decreases viscosity of the solvent, facilitate solvent to penetrate deeply into the material matrix and increase the contacting surface area.

However, when continue to increase the microwave power to 284 W, the total polyphenol content and antioxidant activity according to DPPH and ABTS assays decreased to 91.76 mg GAE/g DW, 463.51 μmol TE /g DW and 1121.4 μmol TE /g DW, respectively. The results obtained were according with (Alara, Abdurahman, Ukaegbu, & Azhari, 2018), who studied microwave-assisted extraction of *Vernonia cinerea* leaves and observed a reduction in the TPC and antioxidant activity at a very high microwave power because of thermal degradation of phenolic compounds in the plant sample. Therefore, with the maximum TPC and antioxidant activity obtained at 214 W, this microwave power was considered proper for further experiments.

3.5. Optimization of polyphenol extraction

A total of 54 runs were used to optimize the four individual parameters in the CCD applied to total extracted polyphenols and antioxidant activities. The response values at different experimental combination were listed in Table 3.

Table 3. Experimental matrix design for response surface and results obtained of different response variables.

No.	X_1	X_2	X_3	X_4	Y_1	Y_2	Y_3
1	50	3	20	154	90.26	404.53	1228.76
2	70	3	20	154	92.87	570.90	1208.06
3	50	7	20	154	93.04	419.36	1255.71
4	70	7	20	154	91.43	453.05	1240.78
5	50	3	30	154	91.70	400.21	1223.41
6	70	3	30	154	92.59	557.90	1206.49
7	50	7	30	154	94.98	452.00	1236.03
8	70	7	30	154	91.52	426.06	1241.90
9	50	3	20	274	91.40	453.41	1275.61
10	70	3	20	274	92.63	487.62	1231.49
11	50	7	20	274	92.37	439.58	1279.87
12	70	7	20	274	91.65	504.33	1280.31
13	50	3	30	274	90.69	417.16	1280.38
14	70	3	30	274	91.04	436.57	1245.83
15	50	7	30	274	92.21	444.32	1313.37
16	70	7	30	274	90.56	427.88	1231.12
17	50	5	25	214	96.37	593.92	1341.40
18	70	5	25	214	97.92	595.01	1316.25
19	60	3	25	214	96.75	646.84	1344.58

20	60	7	25	214	96.76	586.46	1317.84
21	60	5	20	214	96.98	559.53	1348.46
22	60	5	30	214	97.47	547.12	1336.50
23	60	5	25	154	98.81	589.88	1310.44
24	60	5	25	274	97.28	593.98	1351.33
25	60	5	25	214	100.5	680.26	1415.12
26	60	5	25	214	100.24	697.03	1410.65
27	60	5	25	214	100.42	692.75	1409.95
28	50	3	20	154	90.68	409.21	1241.65
29	70	3	20	154	92.27	575.45	1225.38
30	50	7	20	154	92.73	419.15	1231.11
31	70	7	20	154	91.61	457.47	1240.78
32	50	3	30	154	90.45	404.89	1236.31
33	70	3	30	154	91.55	553.34	1207.21
34	50	7	30	154	95.40	451.50	1229.89
35	70	7	30	154	91.10	430.63	1262.75
36	50	3	20	274	91.40	476.23	1281.34
37	70	3	20	274	92.75	462.18	1231.49
38	50	7	20	274	92.76	444.46	1265.79
39	70	7	20	274	91.44	538.06	1285.26
40	50	3	30	274	90.69	421.68	1281.15
41	70	3	30	274	90.01	436.44	1255.34
42	50	7	30	274	92.52	457.89	1308.67
43	70	7	30	274	91.70	419.41	1225.44
44	50	5	25	214	96.98	581.61	1341.40
45	70	5	25	214	98.23	594.95	1322.79
46	60	3	25	214	96.13	638.54	1349.27
47	60	7	25	214	96.76	591.86	1319.41
48	60	5	20	214	96.87	563.67	1339.89
49	60	5	30	214	97.57	547.61	1336.50
50	60	5	25	154	98.91	585.03	1304.88
51	60	5	25	274	97.18	580.41	1356.03
52	60	5	25	214	99.35	650.43	1396.44
53	60	5	25	214	99.35	649.98	1419.88
54	60	5	25	214	101.05	667.55	1405.48

[X_1: ethanol concentration (% v/v); X_2: extraction time (min); X_3: solvent-solid ratio (mL/g); X_4: microwave power (W) and Y_1: TPC (mg GAE/g DW); Y_2: radical scavenging activity by DPPH (μmol TE/g DW); Y_3: radical scavenging activity by ABTS (μmol TE/g DW)].

Effects of linear, quadratic and cross-product coefficients of second-order models on TPC, antioxidant activity according to DPPH and ABTS assays are shown in Table 4. The R^2 values of the TPC, antioxidant activity according to DPPH and ABTS assays were found to be 0.968, 0.928 and 0.916 respectively, whereas the adjusted R^2_{Adj} values were 0.957, 0.902 and 0.885 respectively. The adjusted determination coefficients R^2_{Adj} were comparable to determination coefficients R^2 indicating that the models were highly significant (Myers & Montgomery, 2002).

Table 4. Estimated regression coefficients of second-order models of different response variables.

Term	TPC, Y_1 (mg GAE/g DW)		RSA by DPPH, Y_2 (μmol TE/g DW)		RSA by ABTS, Y_3 (μmol TE/g DW)	
	Regression coefficient	***P*-value**	**Regression coefficient**	***P*-value**	**Regression coefficient**	***P*-value**
Constant B_o	99.54179	<.0001*	647.75611	<.0001*	1381.1099	<.0001*
Linear						
B_1	-0.104444	0.3735	23.226111	<.0001*	-10.92167	0.0029*
B_2	0.4077778	0.0011*	-10.82306	0.0263*	5.8966667	0.0947
B_3	-0.038611	0.7411	-11.26611	0.0211*	-0.929167	0.7887
B_4	-0.322778	0.0083*	-3.304167	0.4850	18.007778	<.0001*
Quadratic						
B_{11}	-1.861852	<.0001*	-43.76167	0.0011*	-36.41148	0.0003*
B_{22}	-2.636852	<.0001*	-19.20917	0.1295	-34.09648	0.0006*
B_{33}	-2.014352	<.0001*	-80.65167	<.0001*	-26.53398	0.0059*
B_{44}	-1.191852	0.0004*	-47.80917	0.0004*	-36.20148	0.0003*
Cross-product						
B_{12}	-0.7325	<.0001*	-17.63906	0.0010*	3.913125	0.2905
B_{13}	-0.330625	0.0105*	-10.76719	0.0365*	-3.65125	0.3235
B_{23}	0.265625	0.0371*	1.4240625	0.7761	-1.336875	0.7163
B_{14}	0.06375	0.6074	-15.81844	0.0029*	-7.824375	0.0384*
B_{24}	-0.15125	0.2264	14.120312	0.0071*	-1.7025	0.6436
B_{34}	-0.355625	0.0063*	-9.747812	0.0571	1.199375	0.7443
R^2	0.968259		0.92794		0.915536	
R^2_{Adj}	0.956865		0.902073		0.885215	

* *Terms with P-value < 0.05 are significant at α = 0.05.*

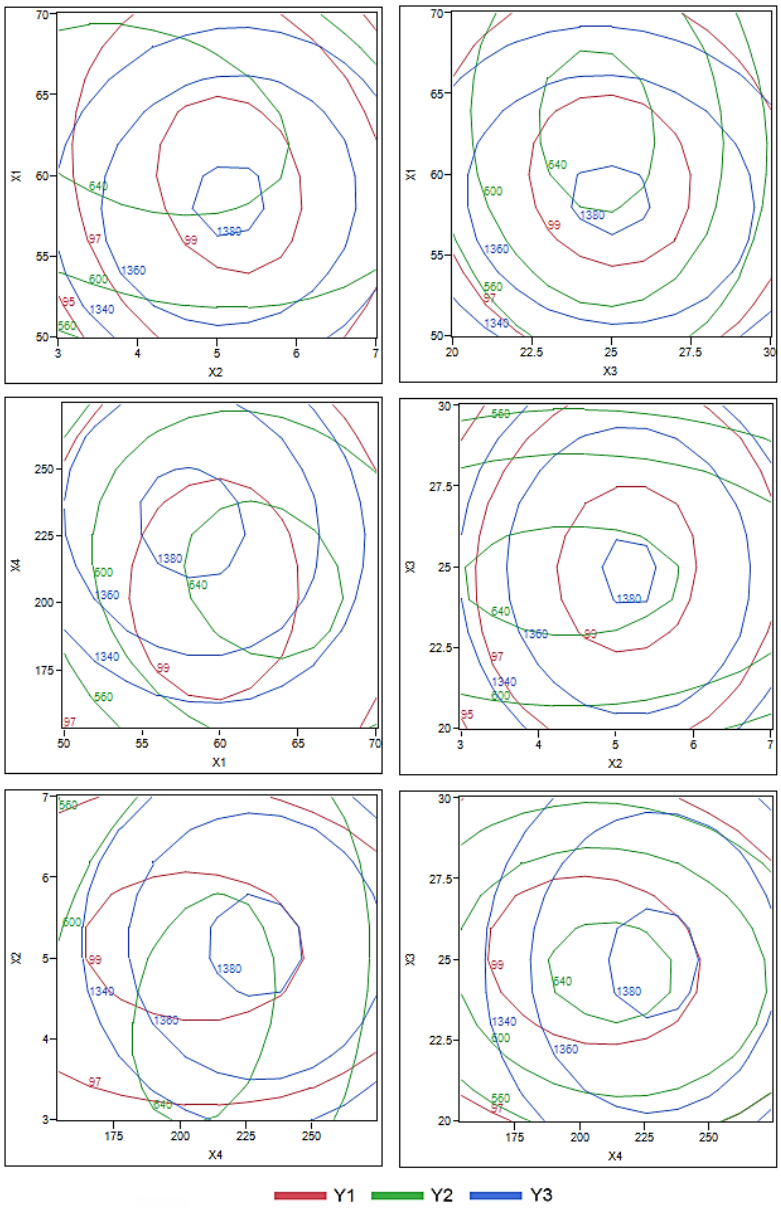

Figure 1. Effects of four investigated factors on three chosen response values. [X_1: ethanol concentration (% v/v); X_2: extraction time (min); X_3: solvent-solid ratio (mL/g); X_4: microwave power (W) and Y_1: TPC (mg GAE/g DW); Y_2: radical scavenging activity by DPPH (μmol TE/g DW); Y_3: radical scavenging activity by ABTS (μmol TE/g DW)].

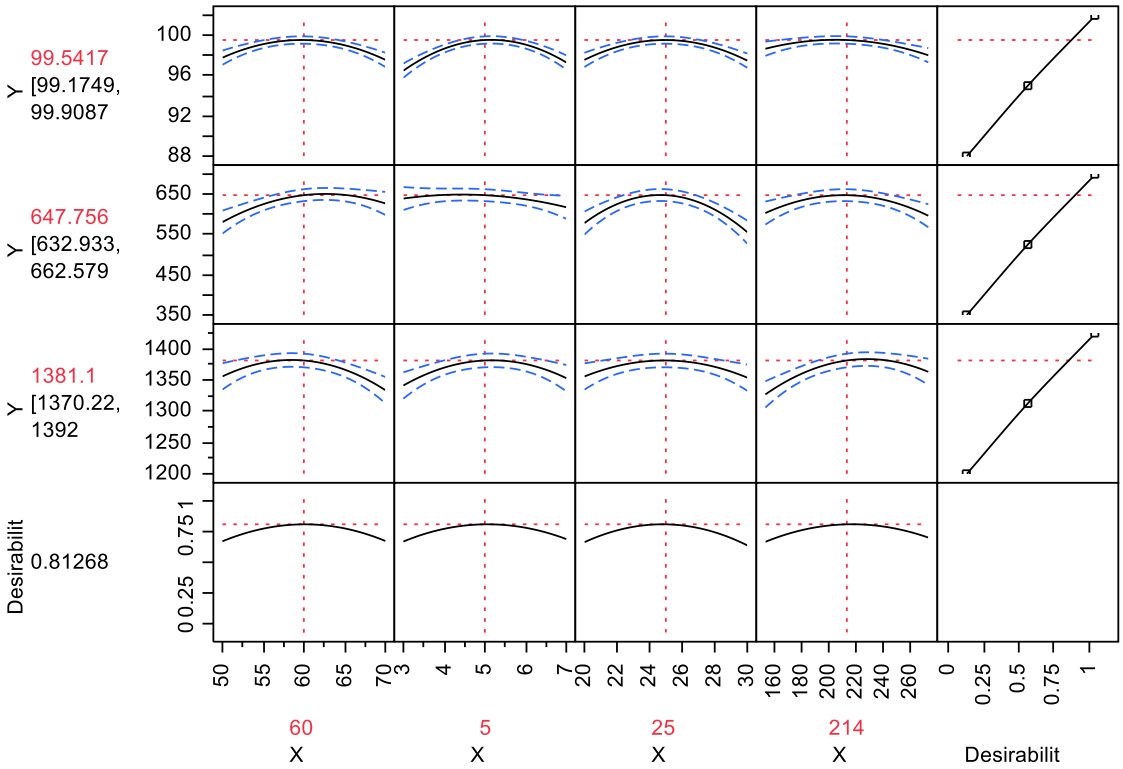

Figure 2. Prediction profiler of the optimization of polyphenol extraction. [X_1: ethanol concentration (% v/v); X_2: extraction time (min); X_3: solvent-solid ratio (mL/g); X_4: microwave power (W) and Y_1: TPC (mg GAE/g DW); Y_2: radical scavenging activity by DPPH (µmol TE/g DW); Y_3: radical scavenging activity by ABTS (µmol TE/g DW)].

Omitted terms which are insignificant at $p \geq 0.05$, the linear regression equations in terms of coded factors for the three models developed are as follows:

$Y_1 = 99.54 + 0.41X_2 - 0.32X_4 - 1.86X_1^2 - 2.64X_2^2 - 2.01X_3^2 - 1.19X_4^2 - 0.73X_1X_2 - 0.33X_1X_3 + 0.27X_2X_3 - 0.36X_3X_4$

$Y_2 = 647.76 + 23.23X_1 - 10.82X_2 - 11.27X_3 - 43.76X_1^2 - 80.65X_3^2 - 47.81X_4^2 - 17.64X_1X_2 - 10.77X_1X_3 - 15.82X_1X_4 + 14.12X_2X_4$

$Y_3 = 1381.11 - 10.92X_1 + 18.01X_4 - 36.41X_1^2 - 34.1X_2^2 - 26.53X_3^2 - 36.2X_4^2 - 7.82X_1X_4$

The model equations allowed the prediction of the effects of the four investigated factors on the TPC, antioxidative activity according to DPPH and ABTS assays. Six independent contour plots showing the effects of four investigated factors on three chosen response values are presented in Figure 1. These contour plots of response surfaces can be used to explore the dependence of chosen response values on the changes of process parameters around the center values developed in the CCD design.

As shown in Figure 1, an increase in ethanol concentration (X_1), extraction time (X_2), solvent-solid ratio (X_3), microwave power (X_4) up to a threshold level led to increased total polyphenol content (Y_1), radical scavenging activity by DPPH and ABTS assays (Y_2 and Y_3). Beyond this level, all the response values slightly decreased, which indicated that a greater TPC and antioxidant activity could be obtained when the moderate variables were selected.

Optimization of polyphenol extraction from custard apple peel was done to obtain maximum chosen response values. As observed, the developed contours allow the optimum combination of process variables to be ascertained. The desirability function was used as a tool for optimization in order to find the combinations of process parameters, which would achieve maximum TPC, antioxidative activity according to DPPH and ABTS assays.

The solution having highest desirability of 0.81 was finally selected (Figure 2).

An ethanol concentration of 60%, extraction time of 5 minutes, solvent-solid ratio of 25:1 (mL/g), and microwave power 214 W are predicted and selected as conditions for the polyphenol extraction from custard apple peel. Under the selected optimal conditions, experimental results showed an optimum TPC of 100.15 ± 0.68 mg GAE/g DW, 673.0 ± 20.44 μmol TE/g DW (DPPH assay) and 1409.6 ± 8.11 μmol TE/g DW (ABTS assay), compared to the predicted values of TPC of 99.54 mg GAE/g DW, 647.76 μmol TE/g DW (DPPH assay) and 1381.11 μmol TE/g DW (ABTS assay). The very good correlation between these results confirmed that the developed quadratic response surface model was adequate for reflecting the predicted optimization.

4. Conclusions

MAE can be successfully used to extract polyphenols from the custard apple peel. Ethanol composition, extraction irradiation time, solvent to solid ratio and microwave power show a strong effect on the TPC and antioxidant activities of the extracts. The response surface method can be used as a good tool to describe the dependency of investigated factors on the response values. Optimum conditions are selected at an aqueous ethanol concentration of 60%, extraction time of 5 minutes, solvent-solid ratio of 25:1 mL/g, and microwave power of 214 W. The model was successfully validated as the results of actual and predicted values of TPC, RSA by DPPH and ABTS showed a very good correlation.

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