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Stirred maceration extraction of custard apple (*Annona squamosa* L.) peel

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Abstract. The custard apple, or *Annona squamosa* L., is one of the most popular fruits in tropical areas like Vietnam. The goal of this study was to use stirred maceration extraction to value-add the custard apple fruit's peel. On the total polyphenol content (TPC) and radical scavenging activity by DPPH and ABTS assays of the extracts, various values of extraction duration (30 - 60 min), ethanol concentration (40 - 70%), solvent to solid ratio (15 - 30 mL/g), and temperature (40 - 70°C) were studied. The best condition for the variables under investigation was determined using the Box-Behnken design and response surface methodology. Multiple linear regressions were used to determine the coefficients defining the effects of temperature, extraction duration, ethanol concentration, and solvent-to-solid ratio on the dependent responses. The ethanol concentration of 52%, the extraction duration of 51 min, the temperature of 60°C, and the solvent-solid ratio of 26 mL/g were determined to be the ideal values. The optimum TPC under these circumstances was 94.08 mg GAE/g, and the antioxidant activity was 536.64 µmol TE/g for the DPPH assay and 1310.83 µmol TE/g for the ABTS assay.

Keywords: *Annona squamosa* L., antioxidant activity, fruit by-product, maceration.

1. Introduction

Custard apple (*Annona squamosa* L.) is popular in tropical countries, including Vietnam. It is a commercially available edible fruit with a thin peel layer and a sweet whitish pulp. Most custard apple peel is discarded as organic waste, which can pollute the environment. Custard apple peel contains many biological phenolic compounds, including alkaloids, tannins, flavonoids, and saponins, which have antioxidant properties and can scavenge free radicals [1]. As a result, using the fruit's by-product antioxidants may benefit human health.

To extract the bioactive components from plant material, the traditional solid-liquid extraction method of maceration has been applied. It has been demonstrated that maceration extraction can be used to successfully extract antioxidant phenolic compounds from a variety of plant materials, including the kinnow (*Citrus reticulata* L.) peel [2], dried chokeberry [3], hazelnut (*Corylus avellana* L.) shell and skin [4], fresh olives [5], and the herb *Thymus serpyllum* [6]. Additionally, it was reported that stirring during maceration can improve extraction by enhancing diffusion and eliminating concentrated solution from the sample surface [7].

Many variables can influence the maceration process. Therefore, it is necessary to enhance extraction efficacy by minimizing the operation costs and obtaining the highest quality antioxidant extract. This paper reports the extraction of polyphenols from the peel of custard apple by stirred maceration at different extraction times, ethanol concentrations, solvent-to-solid ratios, and temperatures. The total polyphenol content and radical scavenging activity of the extracts were determined using DPPH and ABTS assays, and the maceration process was optimized using response surface methodology with the Box-Behnken design.



2. Materials and methods

2.1. Chemicals

Standard gallic acid (GA) ($\geq 98\%$) and the Folin-Ciocalteu reagent ($\geq 99.8\%$) were obtained from Merck (Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, $\geq 98\%$), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), and DPPH (2,2-diphenyl-1-picrylhydrazyl, $\geq 99.8\%$) were provided by Sigma-Aldrich (USA). The other substances were of analytical quality.

2.2. Materials

The ripe custard apple fruit was purchased from Tay Ninh province, Vietnam. The peel was separated, washed, and air-dried at 55 - 60°C until the moisture content is less than 12%. The dried peel powder was pulverized and sieved through a 0.5 mm sieve. Finally, until used, the peel powder was stored in PE bags.

2.3. Maceration extraction

1 g of powdered sample was blended with aqueous solvent at various extraction times (30, 40, 50, and 60 min), ethanol concentrations (40, 50, 60, and 70%), solvent-solid ratios (15:1, 20:1, 25:1, and 30:1 mL/g), and temperatures (40, 50, 60, and 70°C) using a magnetic hotplate stirrer at 200 rpm (MS-HS10, SCILOGEX).

2.4. Analysis of total phenolic content

With few adjustments, the total polyphenol content (TPC) assay was performed as described by ElShourbagy and El-Zahar [8]. After mixing 1.8 mL of Folin Ciocalteu's reagent (10%) with 0.1 mL of sample extract and incubating at room temperature for 5 minutes, 1.2 mL of Na_2CO_3 (15%) was added. The color creation was measured using a spectrophotometer to measure the absorbance at 765 nm after 90 minutes in complete darkness (Thermo, Genesys 10 UV). The results were expressed as mg of gallic acid equivalent per gram of dry sample (mg GAE/g DW) using gallic acid as the standard.

2.5. Antioxidant capacity assay (DPPH)

The DPPH assay was carried out with modifications as described by Sripakdee et al. [9]. In brief, 0.1 mL of sample extract was mixed with 4 mL of DPPH reagent and 0.9 mL of ethanol. For 30 minutes, the mixture was shaken briefly in the dark at room temperature. The absorbance of the color formation at 517 nm was measured using a spectrophotometer (Thermo, Genesys 10 UV). Trolox was used as a standard and water as a control. The findings were expressed in terms of mol Trolox equivalents per gram dry sample ($\mu\text{mol TE/g DW}$).

2.6. Antioxidant capacity assay (ABTS)

Similar to that, but with few adjustments, the ABTS assay was carried out as explained by Sripakdee et al. [9]. 3 mL of ABTS solution and 0.1 mL of extract were shaken and incubated for 15 minutes at room temperature and in complete darkness. The results were represented in terms of mol Trolox equivalents per gram of dry sample ($\mu\text{mol TE/g DW}$).

2.7. Experimental design

To extract polyphenols from the custard apple peel, optimal process parameters such as temperature, extraction time, ethanol concentration, and solvent-solid ratio were determined using a central composite design. The general form of a response surface of response variable Y as a function of n independent process variables ranging from X_1 to X_n is given by Equation (1).

$$Y = B_0 + \sum_{i=1}^n B_i X_i + \sum_{\substack{i,j=1 \\ i \neq j}}^n B_{ij} X_i X_j + \sum_{i=1}^n B_{ii} X_i^2 \quad (1)$$

where B_0 is the constant, B_i is the linear coefficient; B_{ij} is the cross-product coefficient, and B_{ii} is the quadratic coefficient.

The experimental data were analyzed using response surface methodology and a Box-Behnken design. Temperature (X_1), extraction time (X_2), ethanol concentration (X_3), and solvent-solid ratio (X_4) are four independent process variables in this study. Using JMP software (version 10), the quadratic response surface model was fitted to the experimental data. Table 1 shows the treatment levels and coded values for the independent variables (process parameters) used in the development of the experimental data. TPC (mg GAE/g DW; Y_1), radical scavenging activity (RSA) by DPPH ($\mu\text{mol TE/g DW}$; Y_2), and RSA by ABTS ($\mu\text{mol TE/g DW}$; Y_3) were the response variables chosen.

Table 1. Treatment levels and coded values of the independent process variables.

Independent variables	Symbols	Coded levels		
		-1	0	1
Temperature ($^{\circ}\text{C}$)	X_1	50	60	70
Extraction time (min)	X_2	40	50	60
Ethanol concentration (% v/v)	X_3	40	50	60
Solvent-solid ratio (mL/g)	X_4	20:1	25:1	30:1

3. Results and discussion

3.1. Effect of extraction time

Extraction time is an important variable that strongly influences the extraction of bioactive compounds. It can be observed from Table 2 that the total polyphenol content (TPC) increased from 84.19 to 91.15 mg GAE/g DW by increasing the extraction time from 30 to 50 minutes, while antioxidant activity by DPPH assay increased from 511.97 to 531.58 $\mu\text{mol TE/g DW}$ and antioxidant activity by ABTS assay increased from 903.80 to 989.51 $\mu\text{mol TE/g DW}$. However, as the extraction time was prolonged to 60 minutes, the TPC content decreased to 87.04 mg GAE/g DW, and the DPPH and ABTS radical scavenging activities decreased to 500.45 $\mu\text{mol TE/g DW}$ and 928.21 $\mu\text{mol TE/g DW}$, respectively.

Table 2. Effect of extraction time on total polyphenol content (TPC) and antioxidant activity.

Extraction time (min)	TPC (mg GAE/g DW)	Antioxidant activity ($\mu\text{mol TE/g DW}$)	
		DPPH	ABTS
30	$84.19^a \pm 1.10$	$511.97^d \pm 12.55$	$903.80^f \pm 29.45$
40	$86.44^b \pm 1.11$	$511.70^d \pm 9.32$	$968.22^{gh} \pm 24.42$
50	$91.15^c \pm 1.45$	$531.58^e \pm 3.74$	$989.51^h \pm 25.46$
60	$87.04^b \pm 0.75$	$500.45^d \pm 5.98$	$928.21^{fg} \pm 34.34$

Different superscripts within the same column indicate significant differences between values ($P < 0.05$).

According to Fick's second law of diffusion, the solute concentration in the extracting material and the bulk solvent will eventually reach equilibrium, leading to the greatest possible amount of extracts to be produced [10]. On the other hand, a prolonged extraction period facilitates the condition of exposure to light and oxygen, which further degrades the polyphenols by oxidation [11]. Other research in the extraction of phenolic compounds from plant material has also shown that the TPC and

antioxidant activity are linearly correlated [12, 13]. So, when TPC increases, antioxidant activity also increases, and vice versa. In all cases, the antioxidant capacity by ABTS assay is higher than that obtained by the DPPH assay. This is due to different radical scavenging action mechanisms [14, 15].

Therefore, with the best extraction efficiency obtained, the extraction time of 50 minutes had been chosen for the next optimization experiments.

3.2. Effect of ethanol concentration

Polyphenols are a mixture of many substances with high biological activity and different polarities. Therefore, it is not possible to extract all polyphenols with a single type of solvent. Water and ethanol are commonly used solvents for extracting plant polyphenols because they are both environmentally friendly and cost-effective [16, 17]. Besides, a combination of water and ethanol may increase the swelling of plant materials as well as the contact surface area between the solvent and the plant material extracted [18]. Therefore, the composition of the aqueous ethanol mixture is an important factor affecting extraction efficiency.

Table 3. Effect of ethanol concentration on total polyphenol content (TPC) and antioxidant activity.

Ethanol concentration (%)	TPC (mg GAE/g DW)	Antioxidant activity ($\mu\text{mol TE/g DW}$)	
		DPPH	ABTS
40	85.88 ^a $\pm$ 2.12	486.16 ^c $\pm$ 14.0	815.10 ^e $\pm$ 15.46
50	93.71 ^b $\pm$ 2.07	529.03 ^d $\pm$ 13.52	968.67 ^f $\pm$ 10.63
60	88.09 ^a $\pm$ 2.34	466.84 ^c $\pm$ 13.57	793.06 ^e $\pm$ 18.83
70	86.99 ^a $\pm$ 1.77	476.26 ^c $\pm$ 10.34	778.98 ^e $\pm$ 17.54

Different superscripts within the same column indicate significant differences between values ($P < 0.05$).

The content of TPC and antioxidant activity increased significantly when the ethanol concentration was raised from 40% to 50%, as indicated in Table 3. The amount of TPC specifically increased from 85.88 mg GAE/g DW to 93.71 mg GAE/g DW. Antioxidant activity according to DPPH from 486.16 $\mu\text{mol TE/g DW}$ increased to 529.03 $\mu\text{mol TE/g DW}$ and according to ABTS from 815.10 to 968.67 $\mu\text{mol TE/g DW}$. It was reported that a tiny amount of water can be added to the organic solvent to make the mixture more polar and break hydrogen bonds, which would enhance the extraction of polyphenols [6]. In a binary solvent system, the solubility of the polyphenols may be improved by one solvent, while the other solvent could enhance the desorption [17]. However, as the ethanol concentration increased from 50% to 60%, the TPC decreased to 88.09 mg GAE/g DW and continued to decrease to 86.99 mg GAE/g DW when the ethanol concentration reached 70%. This is consistent with the maceration of *Thymus serpyllum* L. herb reported by Jovanović et al., in which 50% ethanol was also used to achieve the highest amount of total polyphenols [6]. The antioxidant capacity measured by the DPPH assay decreased to 466.84 $\mu\text{mol TE/g DW}$, while antioxidant activity measured by the ABTS assay decreased to 793.06 $\mu\text{mol TE/g DW}$ and then to 778.98 $\mu\text{mol TE/g DW}$. Because water has a higher polarity than ethanol, increasing the ethanol concentration resulted in a decrease in the polarity of the binary solvent system. To achieve the best extraction efficiency during the maceration extraction, the ethanol concentration of 50% was selected for the response surface optimization process.

3.3. Effect of the solvent to solid ratio

Another important variable is the ratio of the volume of extraction solvent to the amount of plant materials which is related to the economic aspects of solvent consumption. Experimental results in Table 4 showed that with an increasing solvent-solid ratio from 15:1 to 25:1, the polyphenol content increased gradually from 87.54 to 95.60 (mg GAE/g DW). Meantime, radical scavenging activity by

DPPH increased from 450.70 to 541.01 ($\mu\text{mol TE/g DW}$) and activity by ABTS was from 642.35 to 896.92 ($\mu\text{mol TE/g DW}$). The more solvents used for extraction, the greater the difference in concentration gradient between the outside and inside of the plant cell. This makes it easier for the solvent to penetrate the center of the plant cells and dissolve the components to be extracted. The diffusivity of polyphenol molecules into the solvent is thus also enhanced and antioxidant activity also increased [19, 20].

However, continuing to increase the solvent-solid ratio to 30:1, the polyphenol content, DPPH, and ABTS radical scavenging activities decreased to 89.18 (mg GAE/g DW), 453.77 ($\mu\text{mol TE/g DW}$), and 799.21 ($\mu\text{mol TE/g DW}$), respectively. This may explain that the extraction reached equilibrium. Besides, the dissolved oxygen level increase with the amount of solvent used, facilitating the condition to reduce the TPC and antioxidant capacity of the extracts [16, 21]. Since both observed TPC and antioxidant activity obtained maximal values at the solvent to solid ratio of 25:1, this ratio was chosen to be included in the Box-Behnken design.

Table 4. Effect of solvent-solid ratio on total polyphenol content (TPC) and antioxidant activity.

Solvent-solid ratio (mL/g)	TPC (mg GAE/g DW)	Antioxidant activity ($\mu\text{mol TE/g DW}$)	
		DPPH	ABTS
15:1	87.54 ^a $\pm$ 2.12	450.70 ^d $\pm$ 14.53	642.35 ^f $\pm$ 14.32
20:1	91.56 ^b $\pm$ 0.99	451.59 ^d $\pm$ 6.90	740.96 ^g $\pm$ 18.31
25:1	95.60 ^c $\pm$ 0.51	541.01 ^e $\pm$ 9.73	896.92 ^h $\pm$ 17.92
30:1	89.18 ^{ab} $\pm$ 2.91	453.77 ^d $\pm$ 9.19	799.21 ^g $\pm$ 11.03

Different superscripts within the same column indicate significant differences between values ($P < 0.05$).

3.4. Effect of temperature

The temperature has a significant impact on both the quality of the target chemicals and the extraction efficiency of biological molecules. The results in Table 5 indicated that polyphenol content increased gradually from 40 to 60°C. However, the polyphenol concentration declined from 95.47 to 93.66 (mg GAE/g DW) as the extraction temperature increased from 60 to 70°C. On the other hand, the same trend was observed with antioxidant capacity. The antioxidant activity according to DPPH and ABTS also increased significantly from 40 to 60°C followed by a decrease afterward. For both observed assays, a temperature of 60°C showed significantly higher antioxidant activity than it was achieved at other temperatures.

Table 5. Effect of temperature on total polyphenol content (TPC) and antioxidant activity.

Temperature (°C)	TPC (mg GAE/g DW)	Antioxidant activity ($\mu\text{mol TE/g DW}$)	
		DPPH	ABTS
40	85.80 ^a $\pm$ 1.41	479.51 ^e $\pm$ 11.42	814.17 ^g $\pm$ 12.47
50	90.19 ^b $\pm$ 1.54	479.85 ^e $\pm$ 9.41	899.94 ^h $\pm$ 12.89
60	95.47 ^c $\pm$ 2.19	527.82 ^f $\pm$ 14.40	1080.91 ⁱ $\pm$ 24.53
70	93.66 ^{bc} $\pm$ 2.93	509.63 ^f $\pm$ 4.83	970.65 ⁱ $\pm$ 13.09

Different superscripts within the same column indicate significant differences between values ($p < 0.05$).

Commonly, solubility and diffusion of the extracted solute within the material into the solvent typically increase with increasing temperature during solid-liquid extraction, making saturation of solvent reach more rapidly. It was reported that the surface tensions and viscosity of the solvent would decrease as extraction temperature increased which supports the solvent in penetrating the sample matrices [6]. However, increasing the extraction temperature increases energy costs and may cause

degradation in thermolabile polyphenols. The same phenomena have been also reported with the extraction of milled black alder wood and bark [22]. With the maximum TPC and antioxidant activity obtained at 60°C, this temperature was considered proper for further experiments.

3.5. Optimization of polyphenol extraction

The combined effects of processing parameters including the temperature, extraction time, ethanol concentration, and solvent-solid ratio on the TPC and antioxidant activity according to DPPH and ABTS of the extracts were studied using the Box-Behnken design. Different combinations of process parameters and their corresponding response variables are listed in Table 6.

Table 6. Experimental Box-Behnken matrix design and results obtained of different response variables.

Exp.	Coded values				Experimental values			Exp.	Coded values				Experimental values		
	X ₁	X ₂	X ₃	X ₄	Y ₁	Y ₂	Y ₃		X ₁	X ₂	X ₃	X ₄	Y ₁	Y ₂	Y ₃
1	-1	-1	-1	-1	84.23	440.25	1203.83	28	-1	-1	-1	-1	84.80	444.48	1204.55
2	1	-1	-1	-1	86.13	433.84	1213.02	29	1	-1	-1	-1	86.64	429.46	1217.01
3	-1	1	-1	-1	86.18	432.96	1179.61	30	-1	1	-1	-1	86.40	437.79	1184.09
4	1	1	-1	-1	89.17	409.96	1160.52	31	1	1	-1	-1	88.55	405.65	1177.49
5	-1	-1	1	-1	88.28	444.30	1208.31	32	-1	-1	1	-1	89.93	452.76	1212.54
6	1	-1	1	-1	87.61	438.75	1214.72	33	1	-1	1	-1	87.15	429.94	1209.87
7	-1	1	1	-1	89.35	428.38	1246.53	34	-1	1	1	-1	90.08	437.39	1225.36
8	1	1	1	-1	87.97	489.01	1189.95	35	1	1	1	-1	87.02	489.01	1177.02
9	-1	-1	-1	1	87.90	456.63	1203.11	36	-1	-1	-1	1	87.69	444.12	1203.59
10	1	-1	-1	1	89.98	443.00	1223.08	37	1	-1	-1	1	90.73	455.90	1223.44
11	-1	1	-1	1	91.13	452.15	1247.27	38	-1	1	-1	1	91.03	456.98	1235.43
12	1	1	-1	1	90.67	453.34	1221.74	39	1	1	-1	1	89.33	473.24	1221.74
13	-1	-1	1	1	92.26	468.94	1240.19	40	-1	-1	1	1	91.68	468.94	1250.51
14	1	-1	1	1	89.83	459.55	1218.23	41	1	-1	1	1	89.21	450.97	1255.25
15	-1	1	1	1	90.56	442.22	1297.33	42	-1	1	1	1	90.36	432.57	1257.38
16	1	1	1	1	88.19	508.56	1248.02	43	1	1	1	1	89.76	508.66	1239.79
17	-1	0	0	0	94.46	516.13	1309.64	44	-1	0	0	0	94.56	503.44	1313.87
18	1	0	0	0	93.67	501.19	1288.99	45	1	0	0	0	94.57	505.51	1297.46
19	0	-1	0	0	94.14	524.10	1318.63	46	0	-1	0	0	95.22	528.78	1314.40
20	0	1	0	0	94.34	521.79	1297.20	47	0	1	0	0	94.27	525.76	1309.90
21	0	0	-1	0	94.62	518.89	1335.83	48	0	0	-1	0	94.93	524.41	1324.45
22	0	0	1	0	93.57	521.42	1318.37	49	0	0	1	0	93.65	521.42	1326.83
23	0	0	0	-1	94.08	528.20	1312.96	50	0	0	0	-1	94.29	523.21	1336.77
24	0	0	0	1	90.80	521.63	1323.13	51	0	0	0	1	90.18	534.66	1318.89
25	0	0	0	0	96.89	583.89	1327.36	52	0	0	0	0	96.87	590.99	1323.39
26	0	0	0	0	95.36	584.01	1319.16	53	0	0	0	0	96.54	574.08	1335.30
27	0	0	0	0	97.58	588.66	1323.39	54	0	0	0	0	95.81	590.99	1310.16

[X₁: temperature; X₂: extraction time; X₃: ethanol concentration; X₄: solvent-solid ratio; and Y₁: TPC (mg GAE/g DW); Y₂: radical scavenging activity by DPPH (μmol TE/g DW); Y₃: radical scavenging activity by ABTS (μmol TE/g DW)].

Second-order regression fitting was utilized on the experimental results to develop the response function. Effects of linear, cross-product and quadratic coefficients of second-order models on the response variables are shown in Table 7. For the TPC (Y₁), radical scavenging activity (RSA) by DPPH (Y₂), and RSA by ABTS (Y₃), the unaffected factors ($P > 0.05$) are removed from the quadratic

regression equations. As a result, the final form in terms of coded factors for the developed models may be written as follows:

$$Y_1 = 95.93 + 0.45 X_3 - 0.86 X_1 X_3 - 0.53 X_2 X_3 - 1.32 X_{12} - 1.15 X_{22} - 1.45 X_{32} - 3.30 X_{42} \quad (2)$$

$$Y_2 = 560.39 + 7.77 X_3 + 9.35 X_4 - 9.52 X_1 X_2 + 8.12 X_1 X_3 - 41.30 X_1^2 - 22.76 X_2^2 - 26.33 X_3^2 - 20.94 X_4^2 \quad (3)$$

$$Y_3 = 1337.62 - 6.27 X_1 + 9.9 X_3 + 15.39 X_4 - 8.90 X_1 X_2 - 5.68 X_1 X_3 + 9.21 X_2 X_4 - 41.38 X_1^2 - 34.83 X_{22} - 18.50 X_{32} - 21.93 X_{42} \quad (4)$$

The R^2 values of the TPC and antioxidant capacity by DPPH and ABTS models were found to be 0.901, 0.909, and 0.946 respectively, whereas the adjusted R^2_{Adj} values were 0.865, 0.877, and 0.927 respectively. It has been recommended that the value of R^2 should be at least 0.75 to get a satisfactory correlation between experimental and estimated values [23]. In this study, the adjusted determination coefficients R^2_{Adj} and the determination coefficients R^2 were comparable, further supporting that the model well fitted the experimental results [24].

Table 7. Estimated regression coefficients of second-order models of response variables.

Term	TPC, Y_1 (mg GAE/g DW)		RSA by DPPH, Y_2 ($\mu\text{mol TE/g DW}$)		RSA by ABTS, Y_3 ($\mu\text{mol TE/g DW}$)	
	Regression coefficient	Pvalue	Regression coefficient	P-value	Regression coefficient	P-value
Constant B_0	95.92932	<.0001	560.3916	<.0001	1337.62	<.0001
Linear						
X_1	-0.13056	0.5383	3.475278	0.2497	-6.27222	0.0139
X_2	0.304167	0.1561	2.519722	0.4021	-0.4975	0.8392
X_3	0.454167	0.037	7.770556	0.0127	9.9	0.0002
X_4	0.928611	<.0001	9.353333	0.0032	15.38806	<.0001
Cross-product						
B_{12}	-0.15438	0.493	9.25	0.0056	-8.8975	0.0014
B_{13}	-0.8625	0.0004	8.1225	0.0139	-5.68313	0.0338
B_{23}	-0.53188	0.0221	4.601875	0.1527	4.234375	0.1093
B_{14}	-0.18438	0.4135	3.855	0.2291	0.678125	0.7943
B_{24}	-0.25625	0.2576	1.978125	0.5343	9.205625	0.001
B_{34}	-0.37188	0.1035	-2.19063	0.4916	2.5975	0.3209
Quadratic						
B_{11}	-1.32481	0.0222	-41.3016	<.0001	-42.3763	<.0001
B_{22}	-1.14731	0.0459	-22.7616	0.0062	-34.8338	<.0001
B_{33}	-1.44731	0.0131	-26.3341	0.0018	-18.4963	0.0066
B_{44}	-3.30231	<.0001	-20.9441	0.0112	-21.9288	0.0016
R^2	0.901016		0.909528		0.946229	
R^2_{Adj}	0.865483		0.877051		0.926927	

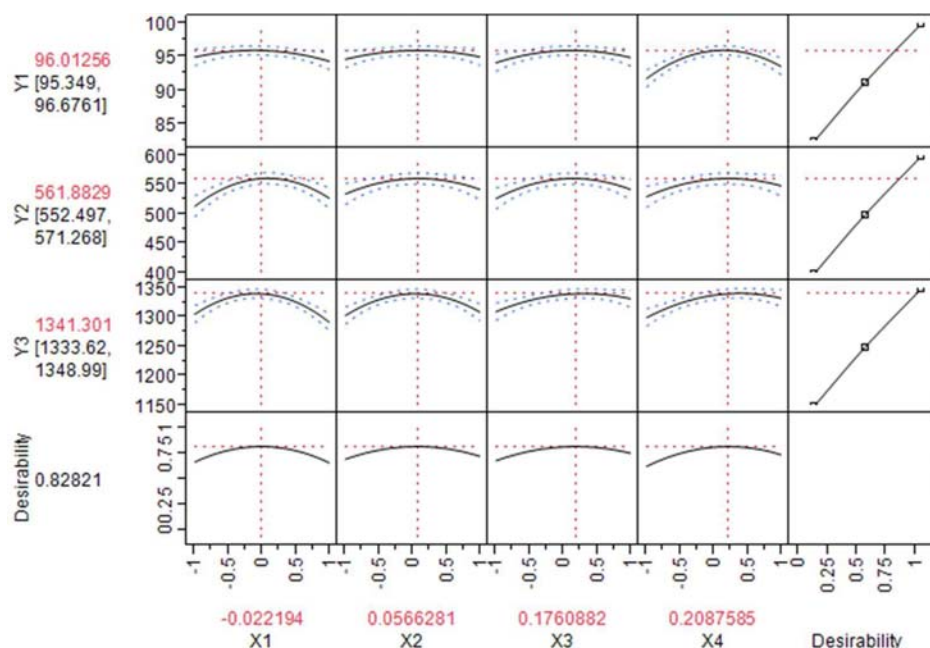


Figure 1. Prediction profiler of the optimization of AS peel extraction. [X₁: Temperature (°C); X₂: extraction time (min); X₃: ethanol concentration (% v/v); X₄: solvent to solid ratio (mL/g)].

The desirability function for maximizing the TPC, and radical scavenging activity by DPPH and ABTS assays was applied. The solution having the highest desirability of 0.8282 was finally chosen (Figure 1). The optimal conditions are predicted at 60°C, 51 min extraction time, 52% ethanol concentration, and the solvent-solid ratio of 26 mL/g. To be confirmed, the extraction of polyphenols from the custard apple peel was performed under the selected optimal conditions. The confirmation experiment resulted in the TPC of 94.08 mg GAE/g, radical scavenging activity by DPPH of 536.64 $\mu\text{mol TE/g}$, and activity by ABTS of 1310.83 $\mu\text{mol TE/g}$, compared to 96.01 mg GAE/g DW, 561.88 $\mu\text{mol TE/g}$ and 1341.30 $\mu\text{mol TE/g}$ of the predicted values, respectively. No significant difference between predicted and experimental data indicated that the developed Box-Behnken model was effective in reflecting the predicted optimization.

4. Conclusions

Stirred maceration technique was successfully performed to extract the polyphenols from custard apple peel. Extraction time, ethanol concentration, solvent-solid ratio, and temperature showed significant effects on the total extracted polyphenols and antioxidant activities in the DPPH and ABTS assays. Response surface methodology with Box-Behnken design can be used as a good tool to optimize conditions for the extraction of custard apple peel. The optimum conditions are finally chosen at the temperature of 60°C, the extraction duration of 51 min, 52% ethanol concentration, and the solvent to solid ratio of 26 mL/g. The developed model was validated by a good correlation between the actual and predicted values of TPC and antioxidant activities.

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