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RESEARCH ARTICLE

Extraction rate of Valuable Compounds from Peanut Skin Waste by Ethanol-Assisted Supercritical Carbon Dioxide: Modelling and Optimization

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Abstract Response Surface Methodology (RSM) was employed to optimize the extraction rate of phenolic and flavonoid contents from peanut skin by supercritical carbon dioxide ($ScCO_2$) assisted by ethanol as entrainer. The studied extraction parameters were pressure (10 to 30 MPa), temperature (40 to 70 °C), and the ratio of ethanol (2.5 to 7.5%). Brunner's and Esquivel's models were applied to evaluate the extraction rate. The best-operating conditions, in the tested range, were 30 MPa, 40 °C, and 4.64% of ethanol ratio, with a maximum extraction rate of 0.22 mg/g.sec and 0.19 mg/g.sec of the phenolic and flavonoid content, respectively. The findings concluded that higher-pressure condition has a significant impact on the extraction rate of phenolic and flavonoid.

Keywords: Peanut skin; Phenolic; Flavonoid; Supercritical Carbon Dioxide/Ethanol; Extraction

Introduction

Peanut skin is a by-product of peanut-based industry and peeled out due to its astringent taste, as well as might reduce the quality of the final products [1] . Peanut (*Arachis Hypohea*) skin also comprises high-flavonoid and phenolic compounds, which could inhibit the oxidative degradation of lipids while improving the nutritional value [2]. Peanut skin extract also could act as anti-inflammatory, anti-viral, and anti-carcinogenic; which could prevent cancers [3].

Supercritical carbon dioxide ($ScCO_2$) extraction as green technology is preferable to extract peanut skin due to the application of high-purity and non-organic solvent. $ScCO_2$ offers a higher quantity of bioactive compounds extraction and high solubility of interest compounds (catechin, phenolic and flavonoid) compared to the conventional method (soxhlet extraction) [2, 4, 5]. Nevertheless, one of the drawbacks of $ScCO_2$ is the limited interest compounds, as it only applies to the non-polar. The addition of ethanol into $ScCO_2$ is one of the novel approaches to extract polar compounds, especially samples that contain flavonoid contents [6]. The advantage of using SC-CO2 is it provides high amounts of bioactive compounds at a low critical temperature, which is cost-effective and suitable for thermo-sensitive compounds.

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The application and optimization of $ScCO_2$ on phenolic and flavonoid extraction is widely studied. However, there are limited studies to determine the extraction rate of phenolic and flavonoid content in the $ScCO_2$ [7-10]. There are several ways to analyze the extraction rate data experimentally, Brunner's and Esquivel's models as empirical models are commonly used to correlate the experimental data to obtain the extraction rate [11, 12]. Response Surface methodology has been used to determine the parameter effects to enhance the extraction rate of phenolic and flavonoid. As a result, the optimum parameter could be obtained while predicting maximum responses [12].

Therefore, this study aimed to optimize the extraction parameters for high extraction rate of phenolic and flavonoid content from peanut skin by ethanol-assisted $ScCO_2$.

Materials and methods

Sample preparations

Peanut skin as a waste of peanut butter industry (G-Tech, Johor Bahru) was dried for 4 hours and sieved to 355-425 μm at 60 °C before kept in a refrigerator at -10 °C. The moisture content of the sample was reduced to less than 10% (Libherr EFL 3505, USA).

Chemical

CO_2 (99.99% purity) was purchased from Kras Instrument, Malaysia. Gallic acid and quercetin were bought from and Sigma-Aldrich (St. Louis, USA). Ethanol (99.86% purity), methanol (99.86% purity) HPLC grade, Folin-Ciocalteu, n-hexane (99.86% purity), Na_2CO_3, Al_2NO_3 and CH_3COOK were obtained from Fisher Scientific (Atlanta, USA).

$ScCO_2$ Extraction assisted by ethanol

The system of $ScCO_2$ consists of: 50 mL extraction vessel (dimensions: 1.4 cm internal diameter, 33 cm long), CO_2 pump (Supercritical 24, Japan), back pressure regulator (Jasco BP 2080, Japan), and oven (Memmert, Germany). The schematic diagram was shown in previous studies in Chiller's temperature was set to 6 °C. Next, about 5 ± 0.005 g of peanut skin was measured and put into an extraction vessel before kept in the chiller. Then, the heater of the back-pressure regulator (Jasco BP 2080, Japan) was set at 50 °C. Liquid CO_2 and ethanol (99.86% purity) were later controlled at a constant flow rate of 3 mL/min. The extraction time was 180 minutes and the extracted yield was recorded and collected every 30 minutes before stored in a refrigerator (Libherr EFL 3505, USA) at -10°C for further analysis.

Total Phenolic Content (TPC) Analysis

Analysis of total phenolic content was performed according by Rosli, Roslan [13]. 5 mL of Folin-Ciocalteu reagent was mixed with 50 mL of H_2O while 3 g of Na_2CO_3 was mixed with 40 mL of distilled water before sonicated for five minutes. Next, 1 mg of extract was diluted to 1 mL of ethanol and mixed by 5 mL of Folin-Ciocalteu mixture. The solution was then let to suspend by resting for ten minutes in room temperature. Later, 4 mL of Na_2CO_3 mixture was added into the solution and placed in room temperature for 30 minutes. A Spectrophotometer UV-Vis (Jasco, Japan) was used to measure the absorbance with a wavelength of 760 nm. Meanwhile, for a standard curve, gallic acid was prepared according to a method by Rosli, Roslan [13], and results were expressed as gallic acid equivalent (mg/g sample)

Total Flavonoid Content (TFC) Analysis

Analysis of total flavonoid content was performed according to Silva, Favaro-Trindade [14]. 1 mg of extract was diluted by 1 mL of ethanol. Next, in a different test tube, 1 mL ethanoic extract was mixed with 0.2 mL of Al_2NO_3 10%. Later, 0.2 mL of 1.0 M CH_3COOK was added to the solution. The solution was rested for 40 min in room temperature. The wavelength of a spectrophotometer UV-Vis (Jasco, Japan) was set at 415 nm. The results were expressed as quercetin equivalent (mg/g sample)

Empirical modeling

Brunner's and Esquivel's model were used to fitting the extraction rate data of phenolic and flavonoid

content. Esquivel model has two adjustable parameters (Y_2 and k_2) as shown in Eq. 1 [15]:

$$P_t = Y_2\left(\frac{t}{k_2+t}\right) \quad (1)$$

where P_t is the total phenolic or flavonoid content (mg/g), Y_2 is the predicted total phenolic or flavonoid content (mg/g), k_2 are the adjustable parameters (sec), and t is extraction time (sec).Therefore, the extraction rate can be obtained by Y_2/k_2 (mg/g.sec).
Brunner's model also has two adjustable parameters (Y_2 and k_2) that represents a specific solution of Fick's law, as shown in Eq.2 [16]:

$$P_t = Y_2(1 - e^{-k_2 t}) \quad (2)$$

where P_t is the total phenolic or flavonoid content (mg/g), Y_2 is the predicted total phenolic or flavonoid content (mg/g), $1/k_2$ is the adjustable parameter and t is extraction time (sec). Therefore, the extraction rate can be obtained by Y_2/k_2 (mg/g.sec).

Response Surface Methodology

In this study, pressure (X_1), temperature (X_2), and the ratio of ethanol (X_3) were chosen to optimize the extraction rate of phenolic and flavonoid content during supercritical CO_2 extraction. Table 1 shows the Factors and Levels of the extraction, and experiment were designed using Box-Behnken Design (BBD) with one central point data. Design Expert 6.0.4 study as statistical software was applied for this Finally, the response surfaces of the parameters were analyzed by Analysis of Variance (ANOVA). The general second-order coefficients as shown in Equation 3.

$$Y = \beta_0 + \sum_{i=1}^{k}\beta_i X_i + \sum_{i=1}^{k}\beta_{ii}{X_i}^2 + \sum\sum_{i<j}^{k}\beta_{ij}X_iX_j + \in \quad X_2X_k + \epsilon \quad (3)$$

Table 1 Range of parameters and responses applied in $ScCO_2$ extraction assisted by ethanol.

Parameters	Unit	Range	Increment	Responses
Pressure, X_1	MPa	10 - 30	$\Delta X_1 = 10$	Extraction rate of TPC, Y_1
Temperature, X_2	°C	40 - 70	$\Delta X_2 = 15$	Extraction rate of TFC, Y_2
Ratio of ethanol, X_3	%	2.5 - 7.5	$\Delta X_3 = 2.5$	

Statistical Analysis

The determination of the most suitable model was based on the average absolute relative deviation (AARD) and the coefficient of determination (R^2). A lower value of AARD and a high value of R^2 show that the model successfully fits the experimental data. The equations of AARD and R^2, are shown as in Eq 3 and 4, respectively.

$$\text{AARD (\%)} = \frac{100}{n}\sum_{i=1}^{n}\left|\frac{E_{model} - E_{exp}}{E_{exp}}\right| \quad (4)$$

Based on Eq 3, n is the number of data points, E_{exp} is the value of experimental extraction rate data, and E_{model} is the calculated extraction data by using Brunner's or Esquivel's model.

$$R^2 = 1 - \frac{\sum_i (EM_i - ED_i)^2}{\sum_i (x_i - \bar{x})^2} \quad (5)$$

Based on Eq 4, $\sum_i (EM_i - ED_i)^2$ is the residual data (i.e. an error between the model and the experimental data). Meanwhile, $\sum_i (x_i - \bar{x})^2$ is the variance of the data.

Table 2 Calculated parameters for the Applied Mathematical Models (modified Brunner's and Esquivel's Models) to fit the Total Phenolic Contents (TPC).

Run	X_1 (MPa)	X_2 (C)	X_3 (%)	Yield TPC (mg/g)	Brunner's Model					Esquivel's Model				
					k_2 (sec)	Y_2 (g)	Y_2/k_2 (mg/g.sec)	AARD (%)	R^2	k_2 (sec)	Y_2 (g)	Y_2/k_2 (mg/g.sec)	AARD (%)	R^2
1	30	55	2,5	279.87 ± 2.4	5452	438	0,080	6,497	0,986	286	0,000340	0,097	4,124	0,995
2	20	55	5	183.26 ± 3.1	1996	218	0,109	0,849	1,000	182	0,000447	0,081	2,064	0,999
3	10	55	7,5	112.22 ± 2.2	585492	7183	0,012	22,376	0,950	144	0,000157	0,023	12,357	0,983
4	20	70	2.5	166.26 ± 2.6	8079	309	0,038	3,164	0,996	203	0,000180	0,037	2,772	0,997
5	20	40	2,5	165.94 ± 2.7	4032	234	0,058	6,525	0,990	176	0,000278	0,049	4,955	0,995
6	20	70	7,5	88.57 ± 1.4	1252	99	0,079	1,784	0,998	88	0,000614	0,054	1,958	0,996
7	10	70	5	236.71 ± 1.3	5160	348	0,067	1,950	0,999	253	0,000230	0,058	3,467	0,998
8	10	40	5	122.19 ± 1.8	8600	229	0,027	14,048	0,989	152	0,000163	0,025	8,721	0,991
9	30	70	5	202.72 ± 2.6	4243	293	0,069	7,137	0,980	206	0,000409	0,084	4,877	0,993
10	20	40	7.5	102.52 ± 2.1	728	110	0,150	0,487	1,000	100	0,000823	0,083	1,653	0,998
11	10	55	2,5	127.77 ± 3.4	7076	211	0,030	1,326	0,999	100	0,000823	0,083	21,759	0,878
12	30	55	7,5	220.85 ± 1.4	904	243	0,269	1,133	0,999	217	0,000762	0,165	0,879	1,000
13	30	40	5	287.33 ± 3.4	1412	328	0,233	2,405	0,997	286	0,000575	0,164	0,680	1,000
Average							0,098	4,299	0,993			0,078	4,619	0,989

Table 3 Calculated parameters for the Applied Mathematical Models (modified Brunner's and Esquivel's Models) to fit the Total Flavonoid Contents (TFC).

Run	X_1 (MPa)	X_2 (C)	X_3 (%)	Yield TFC (mg/g)	Brunner's Model					Esquivel's Model				
					k_2 (sec)	Y_2 (g)	Y_2/k_2 (mg/g.sec)	AARD (%)	R^2	k_2 (sec)	Y_2 (g)	Y_2/k_2 (mg/g.sec)	AARD (%)	R^2
1	30	55	2,5	477.91 ± 5.2	4150	683	0,165	6,510	0,990	491	0,000339	0,167	4,125	0,995
2	20	55	5	208.51 ± 2.1	4985	329	0,066	9,569	0,980	205	0,000440	0,090	2,070	0,999
3	10	55	7,5	42.79 ± 1.4	218806	1177	0,005	22,414	0,952	62	0,000156	0,010	12,376	0,983
4	20	70	2.5	49.40 ± 1.1	218806	1177	0,005	22,016	0,955	62	0,000156	0,010	6,410	0,996
5	20	40	2,5	353.26 ± 4.5	4881	534	0,109	6,472	0,988	363	0,000339	0,123	4,125	0,995
6	20	70	7,5	338.90 ± 3.4	1255	405	0,323	1,773	0,998	357	0,000613	0,219	1,959	0,996
7	10	70	5	35.32 ± 2.4	3554	44	0,012	3,729	0,996	38	0,000224	0,009	3,381	0,998
8	10	40	5	66.06 ± 0.9	17645	188	0,011	13,284	0,983	82	0,000163	0,013	8,722	0,991
9	30	70	5	189.84 ± 2.2	4008	270	0,067	7,156	0,981	203	0,000316	0,064	5,706	0,990
10	20	40	7.5	159.11 ±1.1	713	170	0,238	0,567	1,000	158	0,000623	0,098	2,653	0,995
11	10	55	2,5	12.06 ± 0.3	7073	20	0,003	1,327	0,999	16	0,000623	0,010	40,782	0,917
12	30	55	7,5	91.62 ± 1.5	901	101	0,112	1,134	0,999	90	0,000768	0,069	0,981	1,000
13	30	40	5	353.83 ± 3.5	1421	405	0,285	2,404	0,997	351	0,000574	0,202	0,680	1,000
Average							0,098	8,037	0,985			0,085	6,015	0,991

Results and Discussions

Modelling

Brunner's and Esquivel's models were preferable for the extraction rate analysis as they require less adjustable parameters, and the models are easily fitted to experimental data [11]. Table 2 shows that the results of Brunner's and Esquivel's model fitted the recovery TPC and TFC from peanut skin by $ScCO_2$ assisted by ethanol. Meanwhile, Figure 1 and 2 show the example of Brunner's and Esquivel's model fitted the obtained data at a pressure of 10 to 30 MPa, temperature of 40 to 70 ^oC, and ethanol ratio of 2.5 to 7.5 %.

Based on the average coefficients of determination (R^2) and average absolute relative deviation (AARD), the most suitable model was determined and presented in Table 2 and Table 3, respectively. The highest average coefficients of determination (i.e. $R^2 \geq 1$) and the lowest average absolute relative deviation (AARD ≤ 10%) presented the most suitable mathematical model. Table 2 indicates that Brunner's model gave better fitting for TPC recovery; thus, proved that it could provide more accurate data on the extraction rate of TPC compared to Esquivel's model. However, Table 3 shows the Esquivel's model suggested the best fitting for the extraction rate of TFC. Therefore, in this study, the calculated TPC and TFC extraction rate using Brunner's and Esquivel's model, respectively, were then applied in RSM for optimization. The maximum calculated extraction rate of TPC and TFC were later set to obtain the optimum parameters. Furthermore, the Brunner and Esquivel model is suitable to fit the experimental data of high-pressure instead of low-pressure condition as shown in Table 2 and 3. This is similar studies to the recovery of peanut skin oil that the models gives the low percentage of error to correlate the experimental data at high-pressure condition (30 MPa) [12]. The models also gives the similar results to give low percentage of error to correlate the waste of palm-fiber to obtain the extraction rate of carotenoid and tocopherol at high-pressure condition (30 MPa) [11].

Statistical Analysis

The fixing the parameters studies were based on the preliminary studies, reviewing previous studies and the capacity of the $ScCO_2$ system. The limitation of parameters is that the maximum operating pressure has been restricted to 30 MPa due to the capacity of our $ScCO_2$ extraction system. Furthermore, the maximum temperature condition was restricted to 70 ^OC due to the prevention of degradation of flavonoid content [2]. Furthermore, the ratio of modifier was restricted to 7.5% due to the prevention the changing supercritical phase to subcritical phase [17].

The quadratic models have successfully to describe the experimental analysis due to the satisfactory coefficient (R^2) of the quadratic model was over than 0.8, and the *p*-value was below than 0.05, as shown in Table 2 and Table 3, respectively. Furthermore, the final model is shown in Equations 6 and 7 and Table 2 and 3. Effects of the treatments variables, their interactions, and coefficients on the response variables were obtained by analysis of variance (ANOVA). This is the optimum conditions were 30 MPa, 40 °C and ratio of entrainer (ethanol) of 4.64% with a maximum extraction rate of the phenolic and flavonoid content of 0.22 mg/g.sec and 0.19 mg/g.sec, respectively. Furthermore, the pressure, X_1 effect is the most significant effect on the extraction rate of phenolic due to the lowest value of p-value (0.0001). The parameter of $X_1^2, X_2^2\ X_2^2$ and $X_2\ X_3$ are not significant parameters on recovery of TPC due to high p-value (p-value >0.05). In the recovery of TFC, pressure and quadratic pressure are only the significant factor based on p value (p-value < 0.05).

$$Y_1 = 0.11 + 0.064\,X_1 - 0.027\ X_2 + 0.038\ X_3 + 0.00288\,X_1^2 - 0.013\,X_2^2 - 0.015\,X_3^2 - 0.051\ X_1\,X_2 + 0.051\,X_1\,X_3 - 0.013\,X_2\,X_3 \quad (6)$$

$$Y_2 = 0.09 + 0.058\,X_1 - 0.023\ X_2 + 0.00458\ X_3 - 0.04\,X_1^2 + 0.022\,X_2^2 + 0.013\,X_3^2 - 0.033\ X_1\,X_2 + 0.024\,X_1\,X_3 - 0.071\,X_2\,X_3 \quad (7)$$

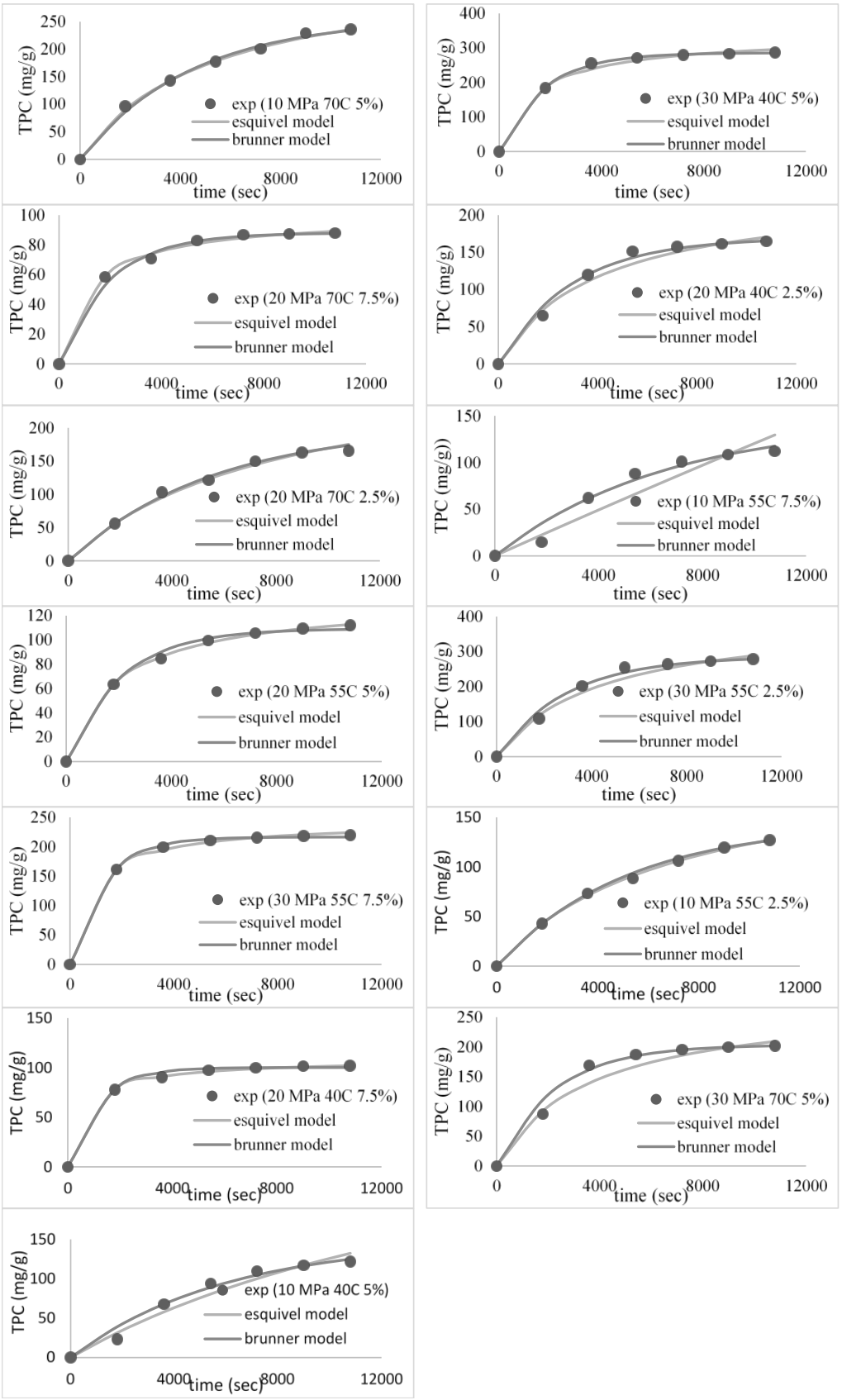

Figure 1 Brunner's and Esquivel's model fitted the experimental data of total phenolic content

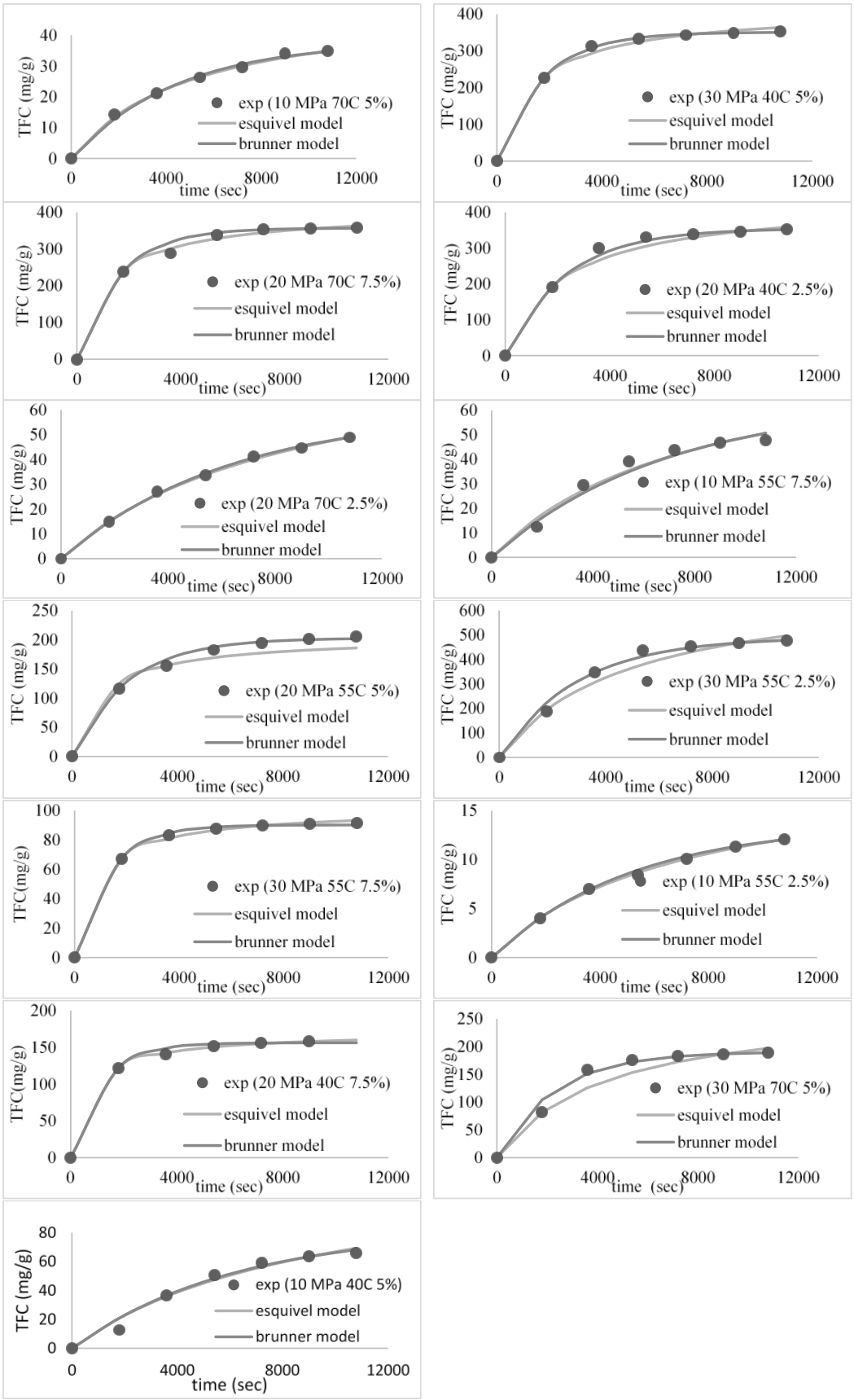

Figure 2 Brunner's and Esquivel's model fitted the experimental data of total flavonoid content

Table 4. ANOVA table for the responses.

Source	Sum of square	df	Mean Square	F	p-value
Extraction rate of TPC (mg/g.sec)					
Regression	0.074	9	0.0081	37.57	<0.0001
Residual	0.0015	7	0.00021		
Total	0.75	16			
Extraction rate of TFC (mg/g.sec)					
Regression	0.067	9	0.0073	6.44	0.011
Residual	0.0081	17	0.0012		
Total	0.75	26			

Table 5. Coefficient of polynomial extraction rate of TPC and TFC.

Extraction rate, (mg/g.s)	Coefficient, (β)	*p*-value	Extraction rate, (mg/g.s)	Coefficient, (β)	*p*-value
TPC, Y_1			TFC, Y_2		
Intercept	0.11		Intercept	0.090	
X_1	0.064	< 0.0001	X_1	0.058	0.0020
X_2	-0.027	0.0014	X_2	-0.023	0.0947
X_3	0.038	0.0002	X_3	0.00458	0.7165
X_1^2	0.00288	0.7009	X_1^2	-0.040	0.0472
X_2^2	-0.013	0.1073	X_2^2	0.022	0.2349
X_3^2	-0.015	0.0830	X_3^2	0.013	0.4501
X_1X_2	-0.051	0.0002	X_1X_2	-0.033	0.0917
X_1X_3	0.051	0.0002	X_1X_3	-0.024	0.1940
X_2X_3	-0.013	0.1267	X_2X_3	0.071	0.0041
R^2= 0.972			R^2=0.8923		

Parameter Effects

Figure 3 shows that increased in pressure from 10 to 30 MPa had significantly enhanced the extraction rate of TPC at constant temperatures of 40 °C and 70 °C.This is because the density, solubility, and diffusivity effect are dominant in the extraction process [4]. The high-pressure condition will enhance the density of solvents; thus, high interaction of the solvent molecules will then increase the dissolution of the solute [17] [18]. As a result, the increase in density enhances the solubility and diffusivity [19]. Furthermore, Figure 3 shows the increase in TPC with pressure is more evidenced at 40 C than at 70 C due to the vapor pressure and density. At pressure of 10 MPa and temperature 70 °C, the vapor pressure effect was occurred in this condition, thus the recovery of TPC was high. Contractively in pressure of 30 MPa and temperature of 70 °C, the effect of reducing of solubility was occurred instead of vapour pressure in this condition, therefore the extraction rate of TPC was not significantly increased.

Increase in the rate of ethanol from 2.5 to 7.5% also could enhance the extraction rate of TFC at a constant temperature of 55 °C and pressure of 10 MPa, as shown in Figure 4(b). The addition of ethanol increases the polarity of carbon dioxide; thus, the flavonoid – a polar compound, is easily soluble. However, an increase in the rate of ethanol will slightly decrease the extraction rate of TFC at constant pressure and temperature of 30 MPa and 55 °C, respectively (Figure 3(b)). This is due to ethanol could prevent the $ScCO_2$ from diffusing into the peanut skin and increasing the rate of ethanol would reduce the mixture density. The compactness of material inside of the vessel was also occurred due to in the high pressure condition, thus the yield of flavonoid content will decrease. Maran, Manikandan [32] also found that extraction pressure and entrainer flow rate give significant effect to obtain high flavonoid contents from tea (*Camellia sinensis L.*) leaves due to high solubility and polarity of solvent.

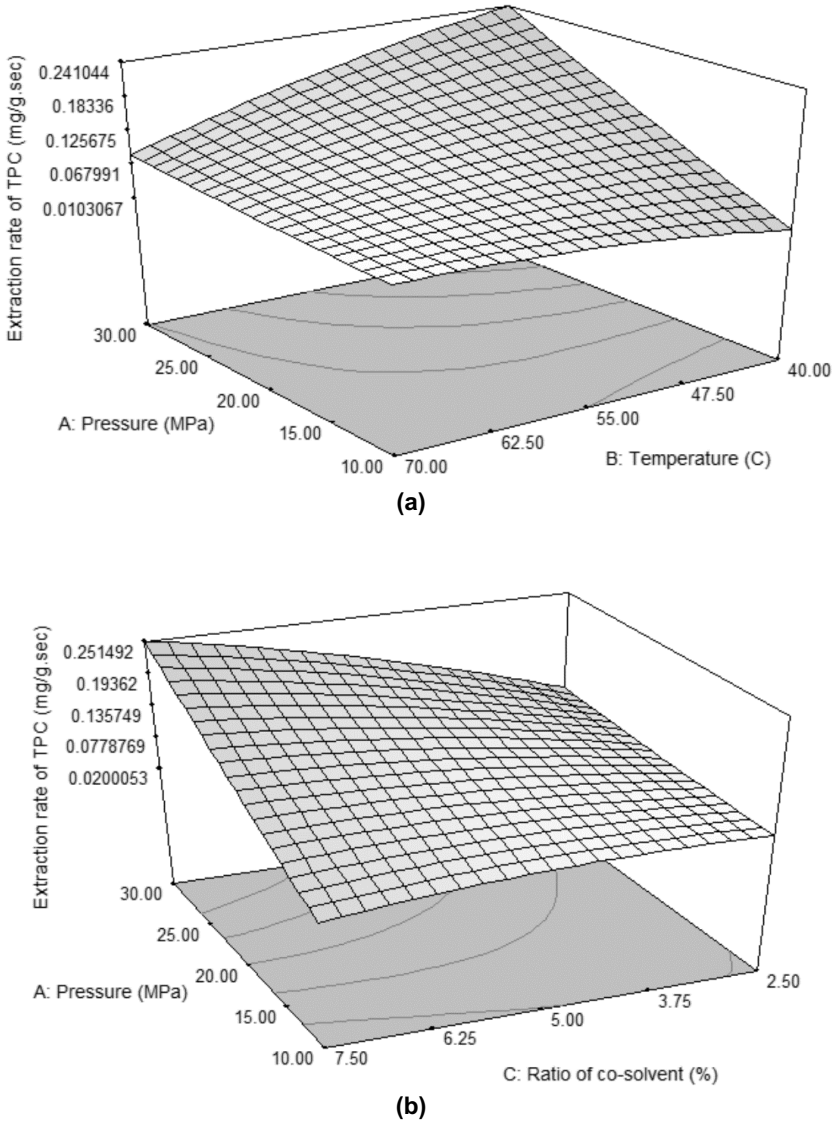

Figure 3 Response of the effect of variables on extraction rate of TPC (mg/g.sec) at (a) constant ratio of ethanol 5% and (b) constant temperature 55 °C.

The recovery of TPC from fermented orange pomace by supercritical carbon dioxide also has similar results, whereby pressure was found to be the most significant factor to enhance the TPC recovery [20]. Adil, Cetin [21] also found that pressure could give a significant effect compared to other parameters for enhancing the recovery of phenolic contents from apple and peach pomaces . Guo-qing, Hao-ping [22] found that high pressure encourages the bulk density of the fluid, which increased the solubility. However, as pressure increases further, the fluid is less compressible, thus has an insignificant impact in bulk density. However, Duba and Fiori [23] revealed contradictive results by which the pressure over 35 MPa has no significant effect on the recovery of flavanol/phenolic compounds. Machmudah, Zakaria [24] also found that the selectivity of $ScCO_2$ is also weakened by high-pressure condition (i.e. 40 MPa), as all the studied compounds will be extracted.

An increase in temperature (40 to 70 °C) at a constant pressure of 10 MPa slightly enhanced the extraction rate of TPC at constant rate of ethanol 5% from 0.027 to 0.067 mg/g.sec. The sublimation pressure is dominant during the low-pressure condition; thus, the TPC is easily diluted in the mixture

solvent [18]. High temperature condition (70 °C) also escalates the diffusivity, thus, the solvating power of solvents will be enhanced to extract phenolic content. However, at constant pressure of 30 MPa, enhancement of temperature decreased the extraction rate of TPC from 0.233 mg/g.sec to 0.069 mg/g at constant rate of ethanol 5%. as shown in Figure 3(a). This is due to a high-temperature could decrease the density of the solvent; thus, the solubility of solutes and diffusivity effect will be alleviated [25, 26]. Therefore, the solvation power of solvent will reduce to carry out the phenolic contents. Meanwhile, Figure 3(b) shows that increase in the ratio of ethanol (2.5 to 7.5%) does not give a significant impact on the enhancement of the TPC extraction rate at constant pressures of 10 and 30 MPa. This is contradictive that phenolic compounds are easily diluted/dissolved in a polar solvent compared to the nonpolar solvents. Hasmida, Liza [27] also found that the effect of entrainer does not gives the significant effect to enhance the recovery of phenolic content in the extraction of *Quercus infectoria* galls. Molino et al. [29] also found the contradictive results that effectiveness of ethanol as an entrainer for astaxanthin extraction was not significant due to the selectivity of solvents. This is contradictive result with the recovery of tocopherol and carotenoids from wasted palm-fiber that increase of entrainer ratio (5 to 7.5 %) enhance the yield [11]. This is due to the swelling matrix of raw material by ethanol; thus, the supercritical carbon dioxide is easily to penetrate the raw material. On the other hand, the presence of ethanol was found to be effective for the extraction of lutein, as an enhancement was observed both in terms of recovery and purity. Thus, the phenolic was unsuccessful extracted due to the ethanol is not enough to swell peanut skin.

A similar trend of TPC extraction rate is shown as in Figure 4(a). Increase in pressure (10 to 30 MPa) encouraged TFC rate at a constant ethanol ratio of 5% and temperatures of 40 and 70 °C. Molino, Mehariya [28] found that the density of solvents could play a significant role in enhancing flavonoid recovery. Putra, Rizkiyah [29] also reported that the increase in pressure could be resulting in the enhanced extraction of polar and high-molecular-mass compounds such as polyphenols (i.e. the major antioxidants in plant extracts) suggesting the significance of operating at lower temperature and higher pressure. Ouédraogo, Dicko [30] also found that to enhance the recovery of flavonoids from *Odontonema strictum* leaves with high antioxidant activity by increasing of pressure (200 Bar).

Besides that, increase of temperature from 40 to 70 °C also could decrease the extraction rate of TFC from 0.185 to 0.041 mg/g.sec, at a constant pressure of 30 MPa, 5% ratio of ethanol and temperature 40 to 70 °C, as shown in Figure 4(a). Due to the low density of $ScCO_2$, the alleviation of solubility relies on the increase in temperature. On the other hand, temperature affects the volatility of the solute; therefore, it is difficult to predict the outcome. The similar results with the recovery of flavonoid from *Odontonema strictum* leaves that increase temperature condition (55 to 65 °C) decrease the flavonoid extract [30]. A higher temperature (65 °C) accelerate mass transfer to improve the extraction yield by enhancement of the solubility of the solute. In contrast, increase in temperature (40 to 60 °C) increased the solvating power when applying $ScCO_2$ assisted by ethanol in recovery of flavonoid content from brewer's spent grain. The predominance of the vapor pressure effect depended on the high-temperature condition to enhance the extraction rate of flavonoid content [31].

Increase in the rate of ethanol from 2.5 to 7.5% also could enhance the extraction rate of TFC at a constant temperature of 55 °C and pressure of 10 MPa, as shown in Figure 4(b). The addition of ethanol increases the polarity of carbon dioxide; thus, the flavonoid – a polar compound, is easily soluble. However, an increase in the rate of ethanol will slightly decrease the extraction rate of TFC at constant pressure and temperature of 30 MPa and 55 °C, respectively (Figure 3(b)). This is due to ethanol could prevent the $ScCO_2$ from diffusing into the peanut skin and increasing the rate of ethanol would reduce the mixture density. The compactness of material inside of the vessel was also occurred due to in the high pressure condition, thus the yield of flavonoid content will decrease. Maran, Manikandan [32] also found that extraction pressure and entrainer flow rate give significant effect to obtain high flavonoid contents from tea (*Camellia sinensis L.*) leaves due to high solubility and polarity of solvent.

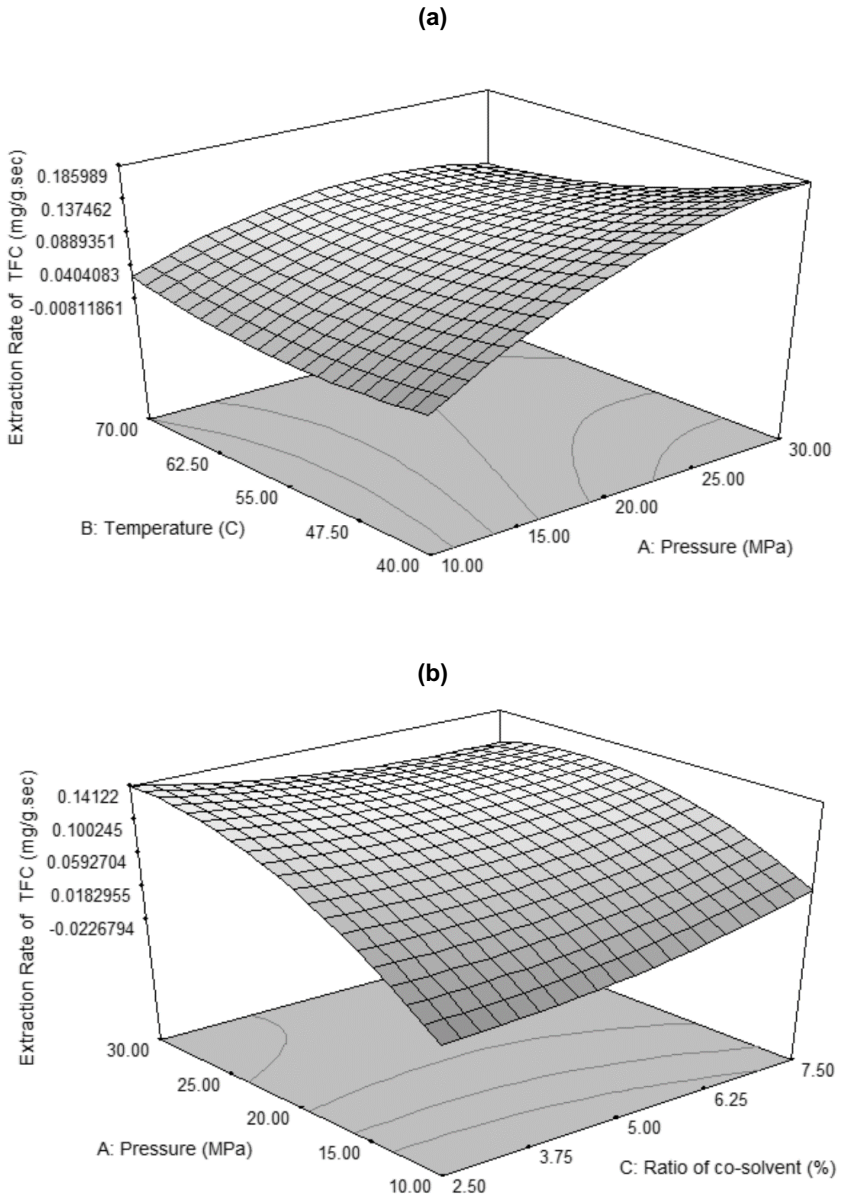

Figure 4 Response of the effect of variables on extraction rate of TFC (mg/g.sec) at (a) constant ratio of ethanol 5% and (b) constant temperature 55 ºC.

Conclusion

In this research, Brunner's model gives better fitting for TPC recovery to obtain its extraction rate, as compared to Esquivel's. In contrast, Esquivel's model gives better fitting for TFC recovery. Therefore, the calculated TPC extraction rate in Brunner's model and TFC extraction rate in Esquivel's model were used for optimization. The quadratic polynomial showed that the model was successfully fitted to the extraction rate of TPC and TFC. Based on the optimization study using RSM, the best conditions were reported to be at 30 MPa, 40 °C and rate of ethanol of 4.64%, with a maximum phenolic and flavonoid extraction rate content of 0.22 mg/g.sec and 0.19 mg/g.sec, respectively. Nevertheless, this study found that increasing pressure to a certain level would enhance the extraction rate of phenolic and flavonoid contents.

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