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Optimization and solubilization of interest compounds from roselle in subcritical ethanol extraction (SEE)

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Abstract A scientific investigation on the optimization and solubilization of anthocyanin from roselle by subcritical ethanol (SE) has not yet been conducted. Consequently, the purpose of this work is to evaluate the parameter influences on anthocyanin recovery by SE, followed by the identification of the solubilization behavior by semi-empirical modelling. The best conditions were 8.74 MPa, 383.51 K and 5.21 mL/min with responses of global yield of 0.765 g/g, anthocyanin of 921.43 mg/100 g, TPC of 40.57 mg/100 g and TFC of 559.14 μg/g. High pressure and temperature conditions are conducive to global yield, anthocyanin, flavonoid and phenolic recovery. The Del Valle Aguilera model fits the solubility of global yield and anthocyanin in SE effectively instead of Chrastil model since it has the lower average absolute relative deviation (AARD), which is 11.54 % and 7.15 %, respectively. The most influenced parameters were found to be the temperature, which gave a significant impact in enhancing the solubization power of global yield and anthocyanin.

Practical applications.

Roselle (*Hibiscus sabdariffa*) comprises several beneficial components, including anthocyanin, phenolic and flavonoid. It is used to treat pyrexia, liver damage, hypertension, and leukaemia, among other conditions. High cost production (supercritical fluid extraction), on the other hand, have often been utilised to recover these valuable compounds. To alleviate these disadvantages, the important chemicals in roselle are extracted using subcritical ethanol (SE) whereas this method offers short extraction time, low cost production cost and easily separation between solvent and solute. The solubilization behaviour of oil yield and anthocyanin were determined in this work using a semi-empirical model. The data indicate

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Nomenclature

ΔY(g)	the weight of yield extract (g)
W_S	the weight of roselle (g)
A	absorbance
MW	the molecular weight of cyanidin 3-glucoside
DF	a dilution factor
Ɛ	extinction coefficient of cyanidin 3-glucoside L is the cell path length
S	solubility of solute (g/L)
ρ	density of ethanol (g/L)
T	temperature (K)
k	the average number of solvent molecules in the solvato complex a and b adjustable parameter
n	the number of data points
C_{model}	the calculated data
C_{exp}	the the experimental data.
$\sum_i (y_i - f_i)^2$	the residual data of calculated and experimental data
$\sum_i (y_i - \bar{y})^2$	the variance of the data
$SCCO_2$	supercritical carbon dioxide
SFE	supercritical fluid extraction
SE	subcritical ethanol
SEE	subcritical ethanol extraction
TAC	total anthocyanin compounds
TFC	total flavonoid compounds
TPC	total phenolic compounds

that an endothermic process occurred to enhance solubility of yield extract and aexothermic process was suitable for anthocyanin, where high temperature condition was needed to enhance the solubility of global yield and low temperature is suitable for anthocyanin.

1. Introduction

Roselle (*Hibiscus sabdariffa L.*) has a rich history of use in a variety of geographies and medicinal areas. It is used to treat pyrexia, liver damage, hypertension, and leukaemia, among other conditions. Additionally, roselle has a variety of medical uses that have been explored globally (Elhefian, 2014). A possible use for roselle extract is in the creation of fruit juice, beverages, and jam (Yusoff and Leo, 2017). Calyces are often utilised to manufacture jam and fruit juices in a few sectors due to their distinctive vivid red colour and flavour. Additionally, roselle has a high concentration of phenolic, flavonoid, antioxidant compounds and anthocyanin to contribute for health and wealthness products [5,11]. The imbalance of antioxidant could result in different metabolic disorders and chronic diseases, such as cancer, hypertension and diabetes [11].

Numerous extraction methods for biochemical and biomass products from agricultural plants and herbs have been used; however, the most often used techniques include expeller or oil press, solvent extraction, supercritical fluid extraction (SFE), and ultrasound [3]. Current research indicates that supercritical carbon dioxide is often used to extract important chemicals, including athocyanin phenolic, flavonoid, and antioxidant molecules. Supercritical carbon dioxide ($SCCO_2$) is an innovative method for maximizing the extraction of interest compounds since it is a safe and environmentally friendly solvent. Mohd-Nasir et al. (2020) reported that the key advantages of this technique over conventional extraction processes include shortened extraction time, increased extract quality, lowered extraction agent costs, and an environmentally friendly operation. This method is quickly becoming a viable alternative to solid sample extraction [4]. However, the pure of supercritical of carbon dioxide offers low quantity of the anthocyanin from roselle due to different polarity between carbon dioxide and anthocyanin [9,10,12,15]; Lee, Durst, Wrolstad, Kupina, & JD, 2005; [19]; S. [24].Typically, roselle is also extracted using an organic solvent where energy used during the drying and solvent extraction procedures amounts for about two-thirds of the total energy consumed during the operation. The expense of various extraction technologies for commercial use may be too expensive. In light of economic and environmental concerns, it is important to conduct basic research on the utilisation of subcritical ethanol for the recovery of valuable components from anthocyanin [8].

Subcritical ethanol extraction (SEE) approach is more straightforward and capable of achieving large extraction rates in a relatively short period of time. SEE may have a variety of impacts on the product yield and quality depending on the operating circumstances (pressure, temperature, flowrate, residence time and particle size). The resulting bioactive products are an important source of resources for the chemical, pharmaceutical, food, and energy sectors. Additionally, hydrothermal processing of roselle biomass has been shown to be a promising approach for the production of anthocyanin. Subcritical ethanol is heated ethanol maintained in a liquid form at a pressure sufficient to maintain it at a temperature between 78.37 °C and 240.8 °C and a pressure between 1 and 6.13 MPa [17]. The viscosity, dielectric constant, and surface tension all drop as temperature increases, while diffusivity improves. An ideal pressure might be applied to maintain the ethanol liquid at a certain temperature.

Optimization through Box–Behnken response surface methodology (BBD) was used to construct models between the responses and the independent components, as well as to improve the extraction conditions for SEE [14]. BBD is a spherical, revolving response surface methodology (RSM) design composed of three interlocking 2^2 factorial designs with points, all lying on the surface of a sphere surrounding the center of the design. This design uses quantitative data from an appropriate experimental design to determine or simultaneously solve multivariate equations [7]. Furthermore,mathematical modeling is commonly applied to determine solubilization power of solvent to extract the solutes. semi empirical models, such as Chrastil and Del Valle Aguilera are preferred to determine the solubilization power of atnyocyanin in subcritical ethanol. This is because those models have a good agreement between the experimental and modeling data. Hence, the parameter-effect can be evaluated and determined [20]. This

models also have less adjustable parameters compared to semi-empirical and kinetic models. This justifies the models are easily correlated to the experimental data [2,25].

The subcritical ethanol extraction as a new extraction technique for extracting the anthocyanin, phenolic, and flavonoid compounds from roselle and the solubility model of these compounds in subcritical ethanol are thus two key novelties of this work. In this research, there are two objectives to achieve in order to establish this innovation. The first objective was to determine the extraction conditions for obtaining extracts from roselle using subcritical ethanol that were rich in global yield, anthocyanin, total phenolic compounds (TPC), and total flavonoid compounds (TFC). The second aim was to determine the optimal settings for enhancing the solubilization ability of global yield and anthocyanin in subcritical ethanol.

2. Material and methods

2.1. Preparation of raw material

Dried roselle was obtained from Ekomekar Resources, Terengganu. The dried roselle was grinded to the particle size of 355–425 μm using commercial blender (Panasonic, Japan) and sieved using Endecotts Octagon 2000 Digital Sieve Shaker. The moisture content of dried roselle is mainted below 8 %. Then, the dried roselle was placed in plastic bag and stored in a freezer (Libherr EFL 3505) at the temperatures −7 °C.

2.2. Chemicals

Sigma-Aldrich provided gallic acid and quercetin (St. Louis, USA). Fisher Scientific provided Ethanol (95 %) and ethanol (analysis grade), Folin-Ciocalteu, Al_2NO_3, CH_3COOK, and Na_2CO_3 (Atlanta, USA) and were of ACS grade or better.

2.3. Subcritical ethanol extraction (SEE)

The system of SEE consists of: 50 mL extraction vessel (dimensions: 1.4 cm internal diameter, 33 cm long), high pressure ethanol pump (Supercritical 24, Japan), back pressure regulator (Swagelok, USA), and oven (Memmert, Germany). Schematic diagram for experimental setup in this experiment was shown in shown in Fig. 1. Extraction was conducted under three independent variables; pressure (2 to 10 MPa), temperature (353 to 393 K) and flowrate (3 to 7 mL/min) as shown in Table 1. 1 ± 0.005 g of dried roselle was measured and put into an extraction vessel. ethanol (95 %) were pumped and controlled based on the variable of flow rate and pressure. Temperature of oven was also set based on the variable. The extracted yield was recorded and collected every 30 min.

2.4. Determination of global yield

The percentage of the yield extract was calculated from the weight of the yield extract per unit weight of roselle at desired pressure, temperature and flow rate of CO_2 in extraction. Equation (1) shows the calculation of yield extract.

$$\text{Yield extract (g/g)} = \left(\frac{\Delta Y}{W_S}\right) \quad (1)$$

where ΔY(g) is the weight of yield extract (g), W_S is the weight of roselle (g).

2.5. Analysis of anthocyanin

A pH differential technique was used to assess the anthocyanin content of roselle calyces extract [16]. Two dilutions of the same material were prepared using potassium chloride (0.025 M) and sodium acetate trihydrate solutions, respectively (0.4 M). Both were adjusted to pH 1.0 and 4.5, respectively, using hydrochloric acid. The absorbance was determined using an ultraviolet–visible (UV–vis) spectrophotometer (Jasco, Japan) at 520 and 700 nm using Eq (1).

$$\textit{Absorbance},\ A: (A520 - A700)\ pH1.0 - - (A520 - A700)\ pH4.5 \quad (2)$$

The TAC was calculated as mg cyanidin-3-glucoside (cya 3-glu)/100 g of dry roselle as in Eq. (2);

$$\textit{Total anthocyanins content},\ TAC\ (mg/L) : A\ x\ MW\ x\ DF\ x\ 1000/\varepsilon\ x\ L \quad (3)$$

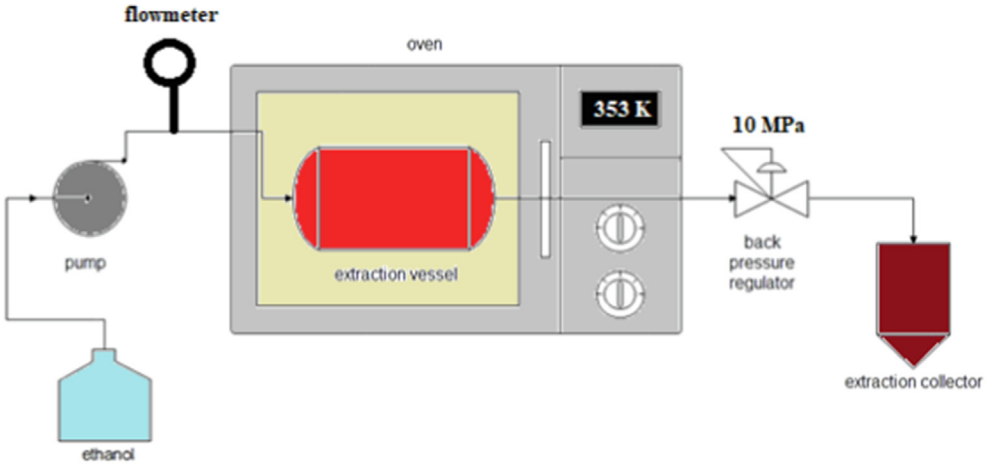

Fig. 1 The subcritical ethanol extraction (SEE) scheme.

Table 1 The parameters and responses of subcritical ethanol extraction.

Run	A:Pressure	B:Temperature	C:Flow rate	Global Yield	TAC	TPC	TFC
	MPa	C	mL/min	g/g	mg/100 g	mg/100 g	mg/100 g
1	2	353	5	0.573	676.505	17.457	300.141
2	6	393	7	0.642	998.593	15.026	593.846
3	6	373	5	0.689	828.265	34.961	663.776
4	2	373	3	0.573	783.244	29.768	147.133
5	6	373	5	0.688	876.625	40.539	593.217
6	10	353	5	0.478	952.104	18.428	221.818
7	6	353	3	0.472	750.381	14.437	472.867
8	6	373	5	0.676	839.486	34.714	590.28
9	6	393	3	0.558	756.126	23.559	449.79
10	6	373	5	0.669	835.612	34.268	587.273
11	6	373	5	0.664	836.815	34.406	591.469
12	10	373	7	0.5703	884.907	38.412	284.545
13	2	373	7	0.2491	727.671	21.818	516.713
14	10	373	3	0.538	884.507	38.366	516.643
15	10	393	5	0.764	890.251	40.570	516.713
16	2	393	5	0.484	859.792	28.597	299.79
17	6	353	7	0.381	633.756	14.067	300.14

whereby A is absorbance, MW is the molecular weight of cyanidin 3-glucoside (449.2 g/mol), DF is a dilution factor, Ɛ is an extinction coefficient of cyanidin 3-glucoside (26,900 L/cm/mol), and L is the cell path length (1 cm). Total anthocyanins content, TAC (mg/L) is converted to the mg extract of 100 g of dried roselle.

2.6. Analysis of total phenolic compounds (TPC)

Each sample's of total phenolic compounds was measured and improved according to the recommendations of Putra et al. (2020) [20]. 5 mL of Folin-Ciocalteu reagent was sonicated for 5 min with 50 mL distilled water and 3 g of Na_2CO_3 with 40 mL of distilled water. 1 mg of the substance was dissolved in 1 mL ethanol and then suspended in 5 mL Folin-Ciocalteu mixture for ten minutes at room temperature. Following that, 4 mL of Na_2CO_3 solution was added and allowed to rest at room temperature for 30 min. The absorbance at 760 nm was determined using a UV–vis spectrophotometer (Jasco, Japan). The total phenolic content of each extract was evaluated using a gallic acid standard curve, and the findings are expressed as mg gallic acid equivalents per litre of extract (mg/100 g).

2.7. Analysis of total flavonoid compounds (TFC)

Analysis of total flavonoid compounds was performed according to the previous method by Silva et al. (2011) [23]. 1 mL of the material was mixed with 1 mL of ethanol. 1 mL of sample was combined with 0.2 mL of Al_2NO_3 10 % and 0.2 mL of 1.0 M CH_3COOK, rested for 40 min at room temperature. The absorbance of each sample was measured at 415 nm. A standard curve was prepared using quercetin, and results were represented as quercetin equivalent (mg/100 g).

2.8. Calculation of solubility of global yield and anthocyanin

Determination of the solubility is according to Eq. (4).

$$S(\frac{g}{L}) = \frac{\Delta Y(g)}{\Delta E(L)} S(\frac{g}{L}) = \frac{\Delta y(g)}{\Delta x(L)} = \frac{y_b - y_a}{x_b - x_a} \quad (4)$$

Where $\Delta Y(g)$ is total yield of global yield/anthocyanin (g) and $\Delta E(L)$ is the total volume of ethanol (L);

2.9. Semi -Empirical model

2.9.1. Chrastil model

The Chrastil model describes the equilibrium between a solute and solvent based on the assumption that temperature and density are the most influential parameters to obtain high solubility [2]. The Chrastil equation can be formed:

$$\ln S = k \ln \rho + \frac{a}{T} + b \quad (5)$$

Where S is solubility of solute (g/L), ρ is density of ethanol (g/L) and T is temperature (K). The coefficient value of k represents the average number of solvent molecules in the solvato complex related to the density and the coefficient value of a defined as $-\Delta H$ where ΔH is the sum of the of vaporization's enthalpies and solvation's enthalpies of the solute. The coefficient value of b depends on the molecular weights of the solute and solvent.

2.9.1.1. Del Valle Aguilera model. The DVA model was developed from the Chrastil model where the addition of one adjustable parameter maximizes the temperature dependence in the equation [25]. The Del Valle–Aguilera equation is shown in Eq. (6):

$$\ln S = k \ln \rho + \frac{a}{T} + \frac{b}{T^2} + c \quad (6)$$

Where S is solubility of solute (g/L), ρ is density of ethanol (g/L) and T is temperature (K). The coefficient value of k represents the average number of solvent molecules in the solvato complex related to the density and the coefficient value of a defined as $-\Delta H$ where ΔH is the sum of the of vaporization's enthalpies and solvation's enthalpies of the solute. The coefficient value of b depends on the quadratic effect of temperature and the coefficient value of c related to the molecular weights of the solute and solvent.

2.10. Experimental design for optimization

A Box-Behnken design was developed using the "Design Expert" software to analyse the effects and best of independent variables (Version 13.0.4, Stat-Ease Corporation, United States). For the SEE extraction, pressure (2 to 10 MPa), temperature (353 to 393 K) and flowrate (3 to 7 mL/min), and were studied, as well as global yield, TAC, TPC and TFC responses. The pareto chart was obtained using statistica version 10.0.

2.11. Average absolute relative deviation (AARD), coefficient of determination (R^2) and root mean square error (RMSE)

The equation of AARD between model and experimental is presented below:

$$AARD(\%) = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{C_{model} - C_{exp}}{C_{exp}}\right| \quad (7)$$

From Eq. (7), n is the number of data points, C_{model} is the calculated data, and C_{exp} is the the experimental data. Meanwhile, Eq. (8) shows the equation of R^2.

$$R^2 = 1 - \frac{\sum_i (y_i - f_i)^2}{\sum_i (y_i - \bar{y})^2} \quad (8)$$

From the above equation, $\sum_i (y_i - f_i)^2$ represents the residual data of calculated and experimental data where It is a measure of the discrepancy between the data and an estimation model. $\sum_i (y_i - \bar{y})^2$ is the variance of the data.

RMSE represents the standard deviation of residual values (prediction errors). Residuals are a measure of how distant data points are from the regression line; RMSE is a measure of how dispersed these residuals are. In other words, it indicates the degree of data concentration around the line of best fit. In regression analysis, root mean square error is widely used to validate experimental findings. Eq. (9) shows the equation of RMSE.

$$RMSE = \sqrt{\frac{(C_{model} - C_{exp})^2}{n}} \quad (9)$$

From Eq. (9), n is the number of data points, C_{model} is the calculated data, and C_{exp} is the the experimental data.

3. Results and discussion

A extraction technique of SEE was utilized to recover anthocyanin, a naturally occurring red pigment, from the calyces of roselle. RSM was applied to maximize the global yield, anthocyanin phenolic and flavonoid recovery as a red pigment. Table 1 presents the results of optimizing the extraction variables and the responses of SEE. The reason of choosing the parameters of pressure 2 to 10 MPa is based on limitation operations of SEE apparatus in our lab. The pressure condition was constraint to 10 MPa. Furthermore, the minimum temperature condition was set 353 K due to the subcritical region of ethanol is above 353 K. The maximum temperature condition was set 393 K due to prevention of degradation of anthocyanin. The maximum flow rate was set to 7 mL/min due to high flow rate will reduce the residence time of subcritical ethanol to extract the interest compounds.

Table 2 to 5 further demonstrate that the ANOVA table for all responses and parameters for SEE. The results of multiple optimization for the extraction conditions of SWE are presented in Table 6. To validate the model, the ideal predicted value was then compared to the experimental result. Fig. 2 depicts the predicted and actual yield, TAC, TPC and TFC responses for SEE extraction. Fig. 3 shows that the pareto chart of variables to obtain the significance effect to the responses of yield, TAC, TPC and TFC.

The parameter values (pressure, temperature, and flow rate) in the optimization section were set within the range and the responses (yield extract, TAC, TPC and TFC) were set to maximum. The best conditions were 8.74 MPa, 383.51 K and 5.21 mL/min with responses of global yield of 0.765 g/g, anthocyanin of 921.43 mg/100 g, TPC of 40.57 mg/100 g and TFC of 559.14 μg/g. ANOVA was done to analyze the quadratic effect, interactions, and coefficients of the treatment factors on the response variables, as shown in Tables 2 to 5. P-value of subcritical ethanol extraction was set 0.05.

3.1. Statistical data analysis and process effects on global yield

Pressure, temperature, and flowrate variables were investigated using a second order polynomial model to determine the response of global yield recovery. Analysis of variance was used to determine the quadratic effect of the treatments factors, their interactions, and coefficients on the response variables. Additionally, a quadratic model was chosen since the coefficient (R^2) was greater than 0.8 and the p-value was less than 0.05, as shown in Table 2. Fig. 2 similarly depicts the predicted vs actual data. The Model F-value of 13.26 indicates that the model is statistically significant. Due to noise, there is a 2.06 % probability that an F-value this significant will occur based on the lack of fit. Significant terms are those with p-values less than 0.05, as indicated in Table 2.

The coefficients of A and B are critical model terms in this instance. As a result, pressure and temperature are more important than flowrate in maximising global yield recovery. At a flowrate of 5 mL/min and a pressure of 10 MPa, Fig. 4 demonstrates that a substantial increase in temperature increased the global yield recovery. Due to the predominance of the vapor/sublimation pressure state throughout the extraction process, the global yield is easily diluted in the solvent. Additionally, when the temperature rises, the diffusivity of the solvents increases. Increase the solvating capacity of solvents by raising their diffusivity to carry out the target chemicals [1,18]. Increased pressure at a flow rate of 5 mL/min while maintaining a temperature of 393 K improves global yield recovery, as illustrated in Fig. 4. This is because ethanol penetration into roselle increased with increasing pressure. As

Table 2 ANOVA table for the response of global yield.

Source	Sum of Squares	df	Mean Square	F-value	p-value	Coefficient
Model	0.2511	9	0.0279	13.26	0.0013	0.6772
A-Pressure	0.0279	1	0.0279	13.28	0.0082	0.0591
B-Temperature	0.0371	1	0.0371	17.62	0.0040	0.0681
C-Flowrate	0.0112	1	0.0112	5.32	0.0545	−0.0374
AB	0.0350	1	0.0350	16.64	0.0047	0.0936
AC	0.0316	1	0.0316	15.03	0.0061	0.0889
BC	0.0076	1	0.0076	3.62	0.0987	0.0437
$\mathbf{A^2}$	0.0186	1	0.0186	8.83	0.0207	−0.0664
$\mathbf{B^2}$	0.0053	1	0.0053	2.51	0.1569	−0.0354
$\boldsymbol{C^2}$	0.0690	1	0.0690	32.81	0.0007	−0.1280
Residual	0.0147	7	0.0021			
Lack of Fit	0.0142	3	0.0047	38.03	0.0021	
Std. Dev.	0.0459		$\mathbf{R^2}$	0.9446		
Mean	0.5690					
C.V. %	8.06					

Table 3 ANOVA table for the response of anthocyanin.

Source	Sum of Squares	df	Mean Square	F-value	p-value	Coefficient
Model	1.286E + 05	9	14286.95	7.36	0.0077	843.36
A-Pressure	39840.42	1	39840.42	20.52	0.0027	70.57
B-Temperature	30259.96	1	30259.96	15.59	0.0055	61.50
C-Flowrate	624.28	1	624.28	0.3216	0.5884	8.83
AB	15023.36	1	15023.36	7.74	0.0272	−61.28
AC	783.29	1	783.29	0.4035	0.5455	13.99
BC	32236.91	1	32236.91	16.60	0.0047	89.77
$\mathbf{A^2}$	1415.52	1	1415.52	0.7291	0.4214	18.34
$\mathbf{B^2}$	1221.55	1	1221.55	0.6292	0.4537	−17.03
$\boldsymbol{C^2}$	7291.34	1	7291.34	3.76	0.0938	−41.61
Residual	13590.15	7	1941.45			
Lack of Fit	12137.87	3	4045.96	11.14	0.0206	
Std. Dev.	44.06		$\mathbf{R^2}$	0.9044		
Mean	824.39					
C.V. %	5.34					

a result of the enhanced contact between the solvent molecules, the solute dissolves more readily. As a result, increasing the penetration strength of ethanol improves the solubility of the extraction [13].

3.2. Statistical data analysis and process effects on anthocyanin recovery

Analysis of variance was used to determine the quadratic effect of the treatment factors, their interactions, and coefficients on the anthocyanin recovery as response variables. Instead of linear and 2FI models, a quadratic model was chosen since the coefficient (R^2) was greater than 0.8 and the p-value was less than 0.05, as shown in Table 3. With an F-value of 7.36, the model is statistically significant. Fig. 2 also depicts the predicted vs actual data for the anthocyanin recovery. Due to noise, an F-value of this significance has a 10.64 % probability of occuring. The coefficients *A* and *B* are critical model terms for optimising anthocyanin recovery. This is because the *p*-value for pressure is less than 0.05 in the quadratic model, as seen in Table 3.

As illustrated in Fig. 5, increasing the pressure parameter at 353 K and a flow rate of 5 mL/min increased anthocyanin recovery. However, increasing the pressure at the maximum temperature (393 K) had no effect on the anthocyanin recovery (mg/100 g). This due to the temperature effect is more dominant compared to pressure effect to enhance the antocyanin for this condition. the augmentation of solubility; and mass transfer effects caused by changes in the characteristics of ethanol at elevated temperatures and pressures, which improve the capacity of analytes to be dissolved with more disturbance of surface equilibria [3]. Subcritical ethanol has a lower viscosity and a higher diffusivity, which enables it to penetrate more deeply through matrix particles [6]. Elevated pressure and temperature upset surface equilibria as a result of the weakening of hydrogen bonding and van der Waals forces between the ana-

Table 4 ANOVA table for the response of TPC.

Source	Sum of Squares	df	Mean Square	F-value	p-value	Coefficient
Model	1400.09	9	155.57	9.49	0.0036	35.78
A-Pressure	181.79	1	181.79	11.08	0.0126	4.77
B-Temperature	235.03	1	235.03	14.33	0.0068	5.42
C-Flowrate	35.31	1	35.31	2.15	0.1857	−2.10
AB	30.26	1	30.26	1.85	0.2165	2.75
AC	15.99	1	15.99	0.97	0.3564	2.00
BC	16.66	1	16.66	1.02	0.3471	−2.04
$\mathbf{A^2}$	35.46	1	35.46	2.16	0.1849	2.90
$\mathbf{B^2}$	649.17	1	649.17	39.58	0.0004	−12.42
$\boldsymbol{C^2}$	182.78	1	182.78	11.15	0.0124	−6.59
Residual	114.80	7	16.40			
Lack of Fit	86.17	3	28.72	4.01	0.1064	
Std. Dev.	4.05		$\mathbf{R^2}$	0.9242		
Mean	28.20					
C.V. %	14.36					

Table 5 ANOVA table for the response of TFC.

Source	Sum of Squares	df	Mean Square	F-value	p-value	Coefficient
Model	3.901E + 05	9	43347.02	38.75	less than 0.0001	605.20
A-Pressure	9518.14	1	9518.14	8.51	0.0224	34.49
B-Temperature	39927.82	1	39927.82	35.70	0.0006	70.65
C-Flowrate	1479.98	1	1479.98	1.32	0.2878	13.60
AB	21792.37	1	21792.37	19.48	0.0031	73.81
AC	90504.20	1	90504.20	80.91	less than 0.0001	−150.42
BC	25087.90	1	25087.90	22.43	0.0021	79.20
$\mathbf{A^2}$	1.353E + 05	1	1.353E + 05	120.94	less than 0.0001	−179.24
$\mathbf{B^2}$	35130.45	1	35130.45	31.41	0.0008	−91.34
$\boldsymbol{C^2}$	15006.34	1	15006.34	13.42	0.0080	−59.70
Residual	7829.91	7	1118.56			
Lack of Fit	3522.58	3	1174.19	1.09	0.4492	
Std. Dev.	33.44		$\mathbf{R^2}$	0.9803		
Mean	449.77					
C.V. %	7.44					

Table 6 The best value of each parameter and response for SEE.

Parameters	Set goal	Values	Responses	Predicted	Observed	Error (%)
Pressure, MPa	In range	8.74	Yield, g/g	0.765	0.133	2.43
Temperature, K	In range	383.51	TAC, mg/100 g	921.43	944.21	2.39
Flow rate, mL/min	In range	5.21	TPC, mg/100 g	40.57	45.27	
			TFC, mg/100 g	559.14	578.82	

lyte and matrix, as well as the softening of the dipole attraction between matrix active sites and analyte molecules. Continuous fluid flow through the extraction cell avoids solid–fluid equilibrium, resulting in higher extraction rates for the dynamic (continuous) mode of SEE over the static mode.

3.3. Statistical data analysis and process effects on TPC

Analysis of variance was used to determine the quadratic effect of the treatments factors, their interactions, and coefficients on the response variables. Additionally, a quadratic model was chosen since the coefficient (R^2) was greater than 0.8 and the p-value was less than 0.05, as shown in Table 4. Fig. 2 also depicts the predicted vs actual data for the TPC recovery. The Model F-value of 9.49 is higher than F tabulated indicates that the model is statistically significant. Due to noise, there is a 10.64 % confidence that an F-value this significant will occur. Significant words are those with *p*-values less than 0.05, as indicated in Table 4. the coefficient of *B* is the significant model term in this situation. As a result, temperature parameters, rather than pressure and flowrate, are critical in enhancing TPC.

As illustrated in Fig. 6, increasing the pressure parameter at 353 K and a flow rate of 5 mL/min increased anthocyanin

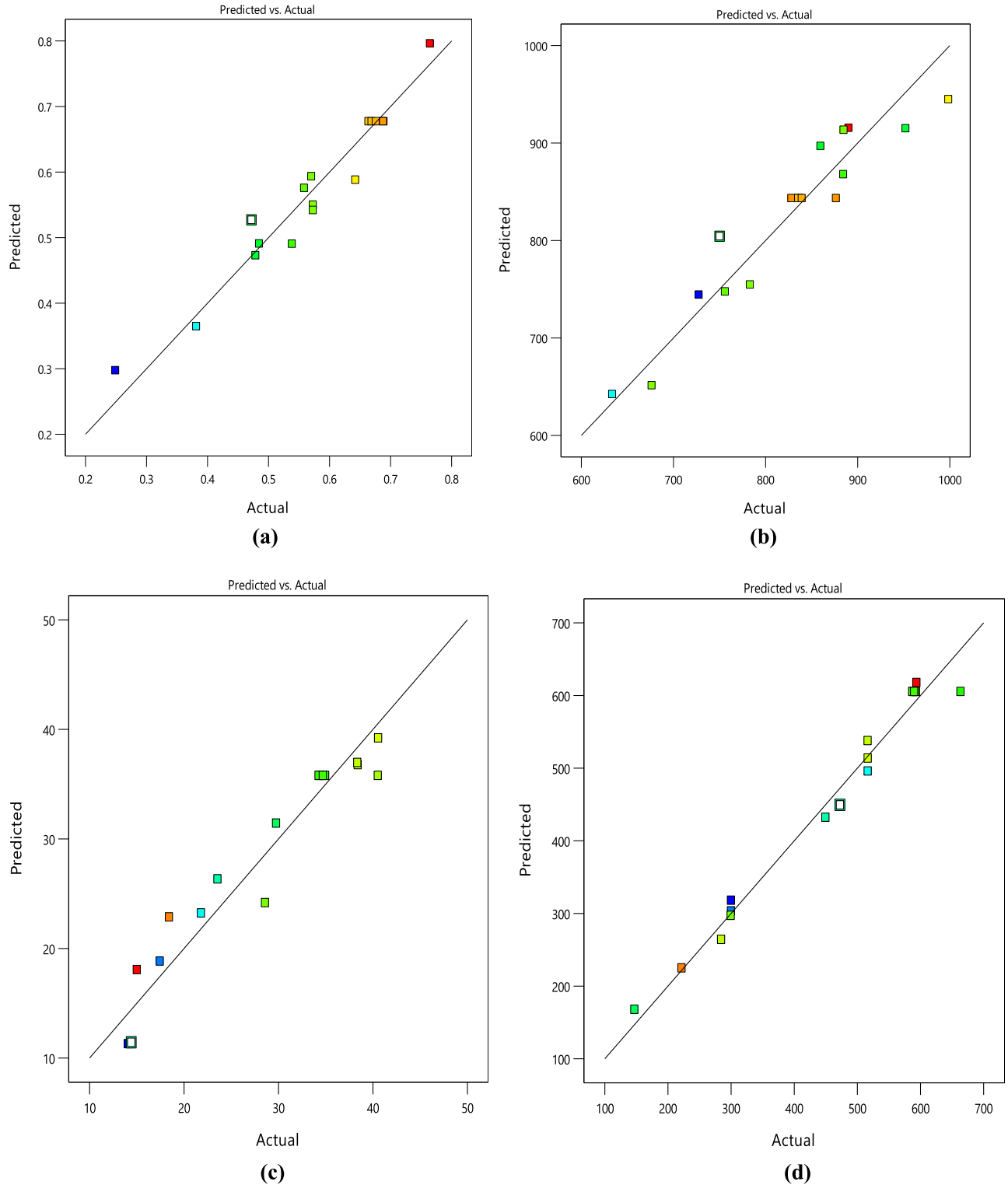

Fig. 2 Predicted vs Actual responses: (a) global yield (b) TAC (c) TPC and (d) TFC on SEE.

recovery. However, increasing the pressure at the maximum temperature (393 K) had no effect on the anthocyanin recovery (g/g). This due to the temperature effect is more dominant compared to pressure effect to enhance the antocyanin for this condition. the augmentation of solubility; and mass transfer effects caused by changes in the characteristics of ethanol at elevated temperatures and pressures, which improve the capacity of analytes to be dissolved with more disturbance of surface equilibria [3]. Subcritical ethanol has a lower viscosity and a higher diffusivity, which enables it to penetrate more deeply through matrix particles [6]. Elevated pressure and temperature upset surface equilibria as a result of the weakening of

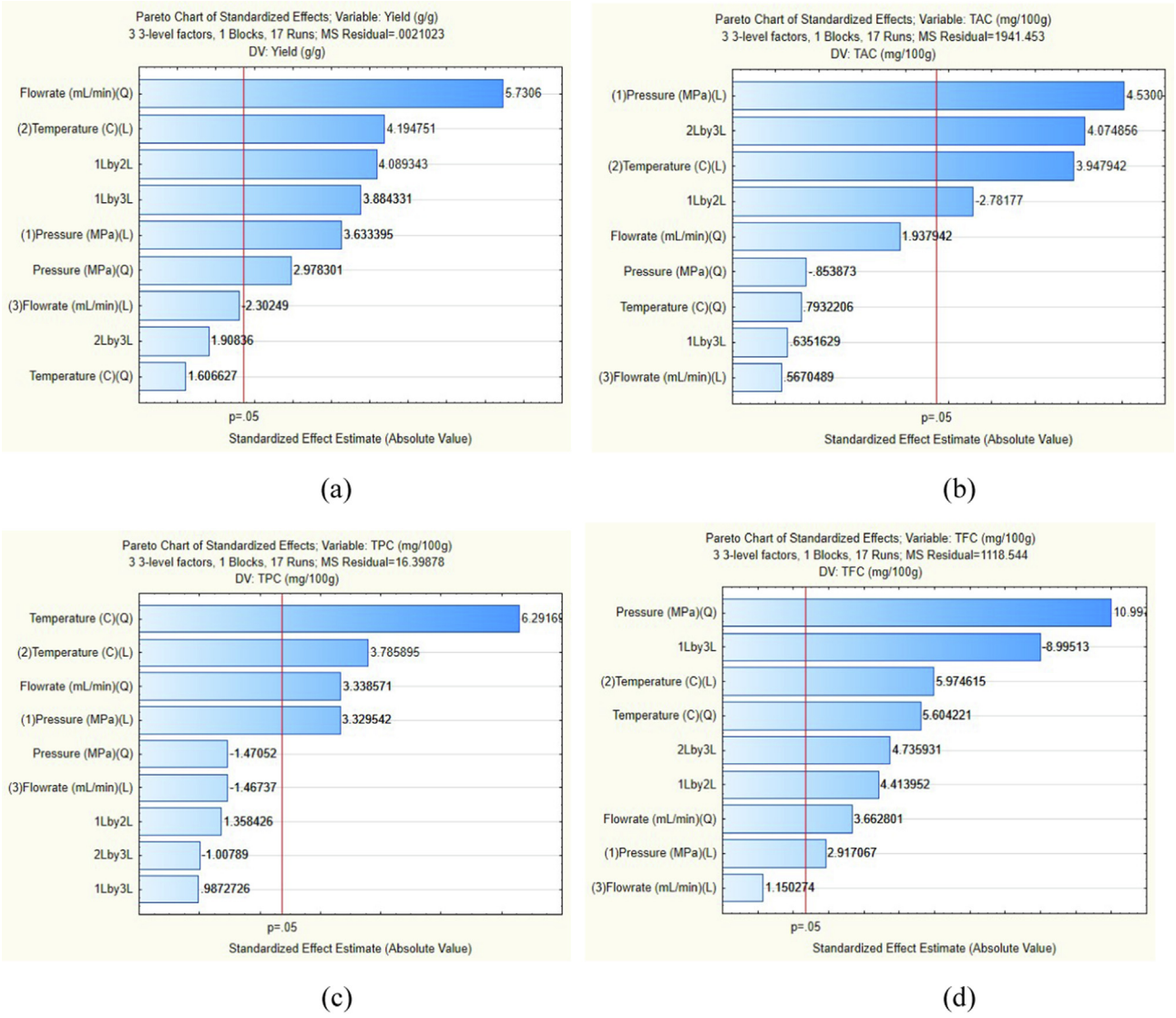

Fig. 3 Predicted vs Actual responses: (a) global yield (b) TAC (c) TPC and (d) TFC on SEE.

hydrogen bonding and van der Waals forces between the analyte and matrix, as well as the softening of the dipole attraction between matrix active sites and analyte molecules. Continuous fluid flow through the extraction cell avoids solid–fluid equilibrium, resulting in higher extraction rates for the dynamic (continuous) mode of SEE over the static mode.

3.4. Statistical data analysis and process effects on TFC

Analysis of variance was used to determine the quadratic effect of the treatments factors, their interactions, and coefficients on the response variables. Additionally, the quadratic model was chosen above the linear and 2FI models because the coefficient (R^2) was greater than 0.8 and the p-value was less than 0.05, as shown in Table 5. Fig. 2 likewise depicts the predicted vs actual data. The Model F-value of 38.75 indicates that the model is statistically significant. Due to noise, there is a 44.92 % chance that an F-value this significant will occur. Significant words are those with *p*-values less than 0.05, as indicated in Table 5. In this case, the coefficients of *A* and *B* are significant model terms. As a result, pressure and temperature are more important than flowrate in enhancing TFC recovery.

At a flowrate of 5 mL/min and a pressure of 6 and 8 MPa, there was a considerable increase in temperature and TFC recovery, as shown in Fig. 7. Due to the predominance of the vapor/sublimation pressure state during the extraction process, the flavonoid are easily diluted in the solvent. Additionally, when the temperature rises, the diffusivity of the solvents increases. Increase the solvating capacity of solvents by raising their diffusivity to carry out the target compounds [1]. Fig. 7 demonstrates that increasing the flowrate was a significant role in increasing the TFC. The absence of an effect of ethanol flow rate on the extraction rate of TFC (Fig. 6) revealed that the extraction behaviour was unaffected by external mass transfer of the TFC from the solid phase's surface to the ethanol phase.

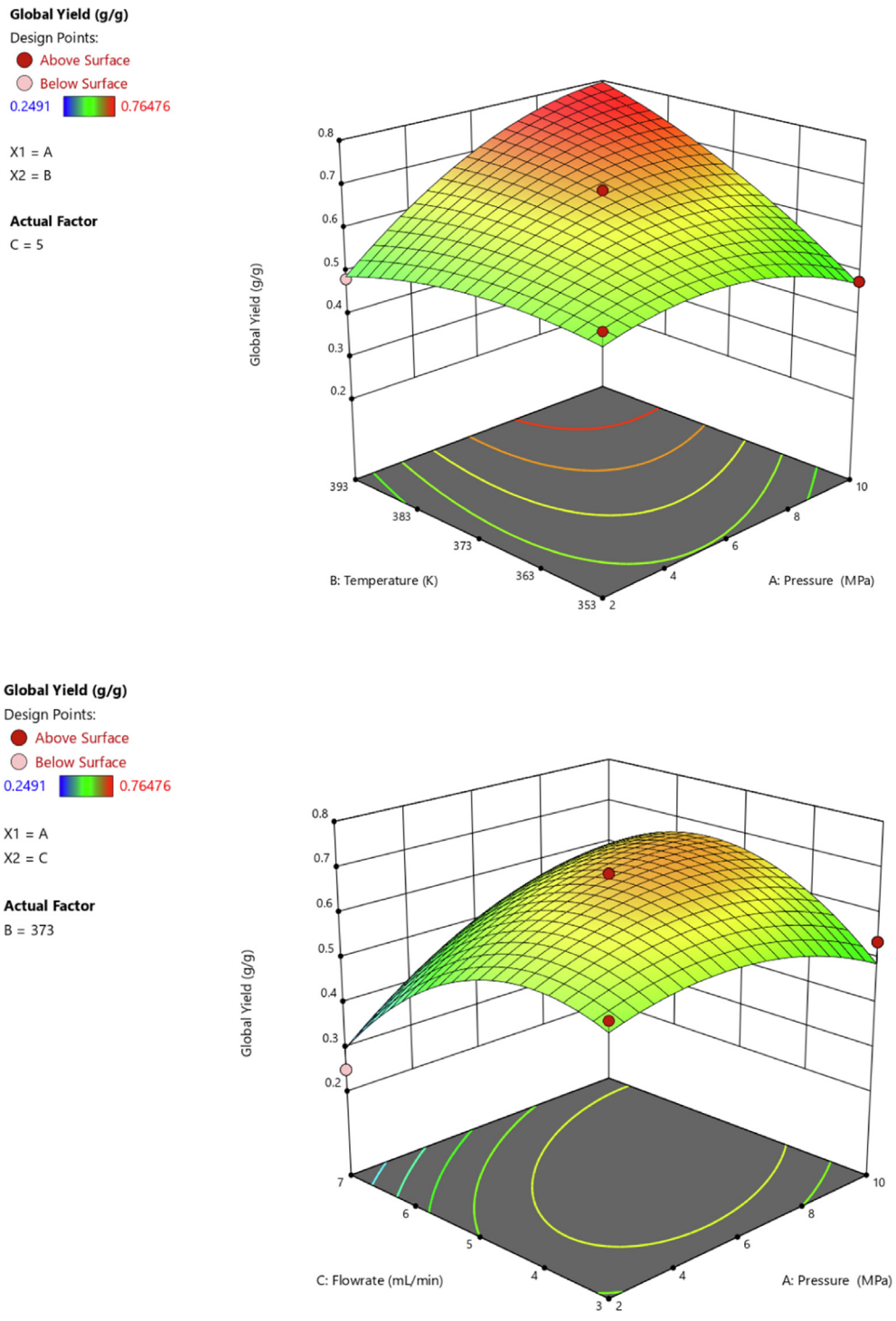

Fig. 4 3D response of the effect of variables on global yield (g/g).

3.5. Multiple responses optimization

Multiple optimizations were performed to obtain the optimum conditions for maximum multiple responses (global yield, anthocyanin, TPC and TFC). The optimal conditions were 8.74 MPa, 383.51 K, and 5.21 mL/min, providing a global yield of 0.765 g/g, anthocyanin concentrations of 921.43 mg/100 g, TPC concentrations of 40.57 mg/100 g, and TFC concentrations of 559.14 mg/100 g. This results is compared with the previous studies related to the extraction of anthocyanin by other extraction method as shown in Fig. 8. Table 6 shows the validation of the optimization in SEE extraction, where the error of between predicted and actual data is below the 10 %. Therefore, this optimization data can be applied for the scale up process.

According to Idham et al. [9], the optimal conditions for supercritical carbon dioxide ($SCCO_2$) extraction were

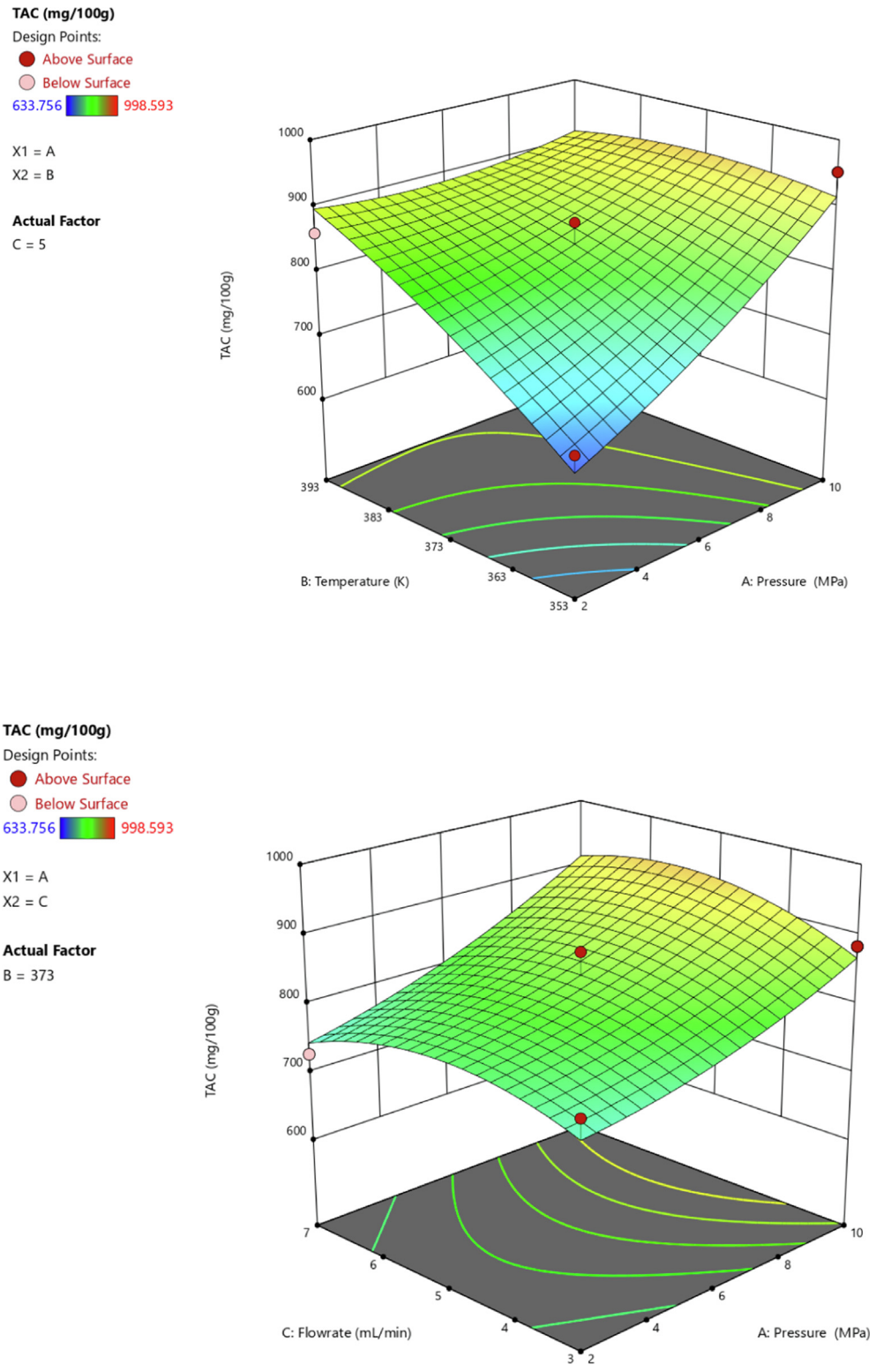

Fig. 5 3D response of the effect of variables on total anthocyanin content (TAC) (mg/100 g).

27 MPa, 58 °C, and a cosolvent ratio of 8.86 % at a maximum anthocyanin concentration of 1197 mg/100 g. Additionally, Redzuan et al. [22] discovered that Optimizing Anthocyanin Extracts from Roselle (*Hibiscus sabdarifa*) Petals Using the Ultrasonic-Assisted Extraction (UAE) Method. The results indicate that a particle size of 0.125 mm, a solvent concentration of 10:1 mL/g solid, and a 15-minute extraction time resulted in the highest percent mass yield (64.72 %), TAC concentration (70.97 mg/100 g), and AA concentration (90.05 %). The results indicate that the SEE contains more anthocyanin

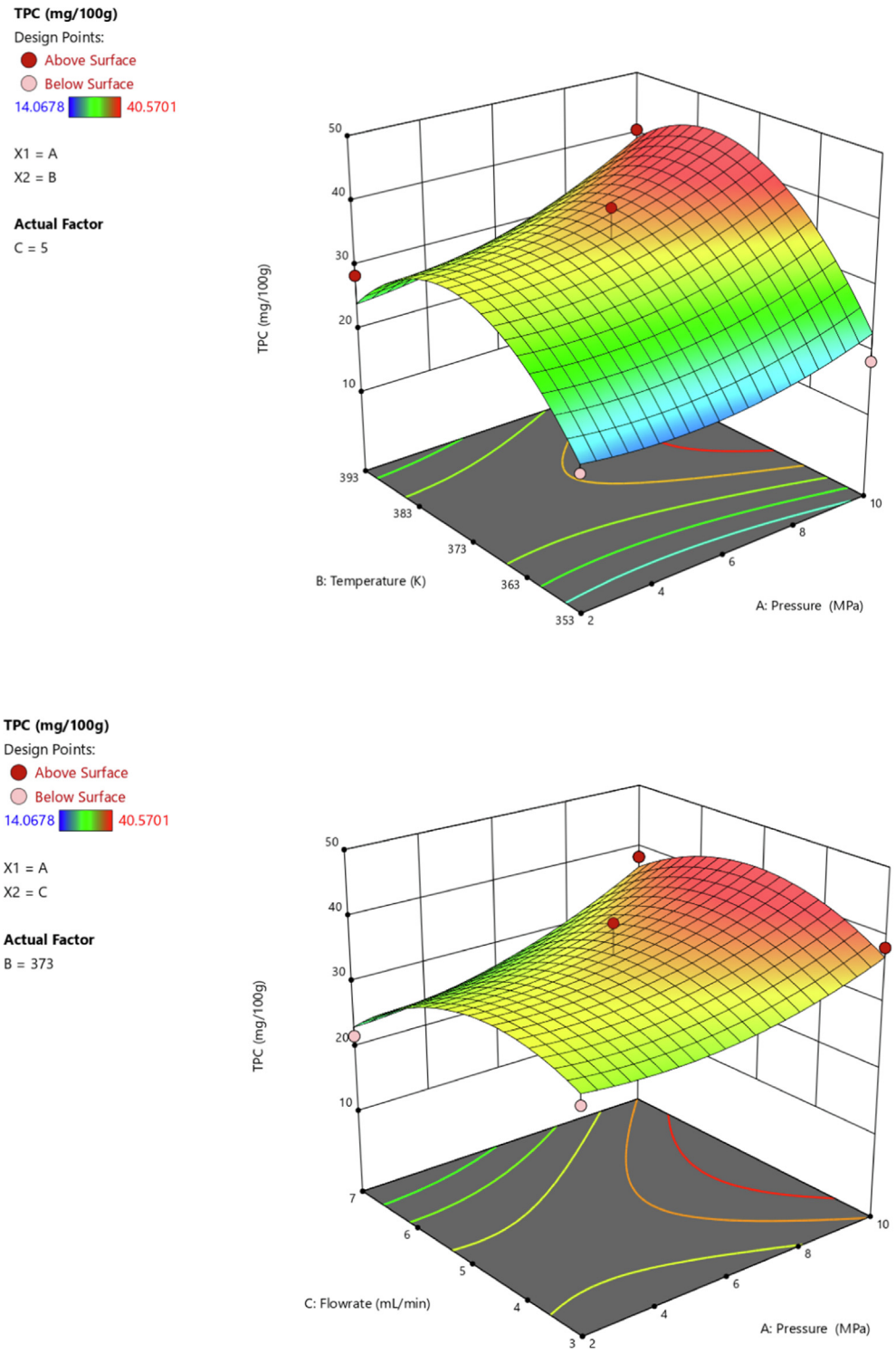

Fig. 6 3D response of the effect of variables on total phenolic compounds (TPC) (mg/100 g).

than the UAE, however this approach produces less anthocyanin than $SCCO_2$. This is because supercritical carbon dioxide employs a higher pressure solvent, which increases the density and diffusivity of solvent to extract the anthocyanin. However, elevated pressure conditions result in increased production and safety costs. As a result, this approach (SEE) may be used in place of the conventional method for extracting anthocyanin.

3.6. Solubility models for global yield and anthocyanin

As indicated in Table 7, the global yield and anthocyanin solubility varied from 5.08 E to 3 to 6.37 E-2 g/L and 1.49 E-3 to 9.83 E-3 g/L, respectively. Table 7 also includes experimental and modelling data of SEE extraction. Correlation results for the models fitted to the solubility of global yield and anthocyanin, as well as the AARD and RMSE of the model, are

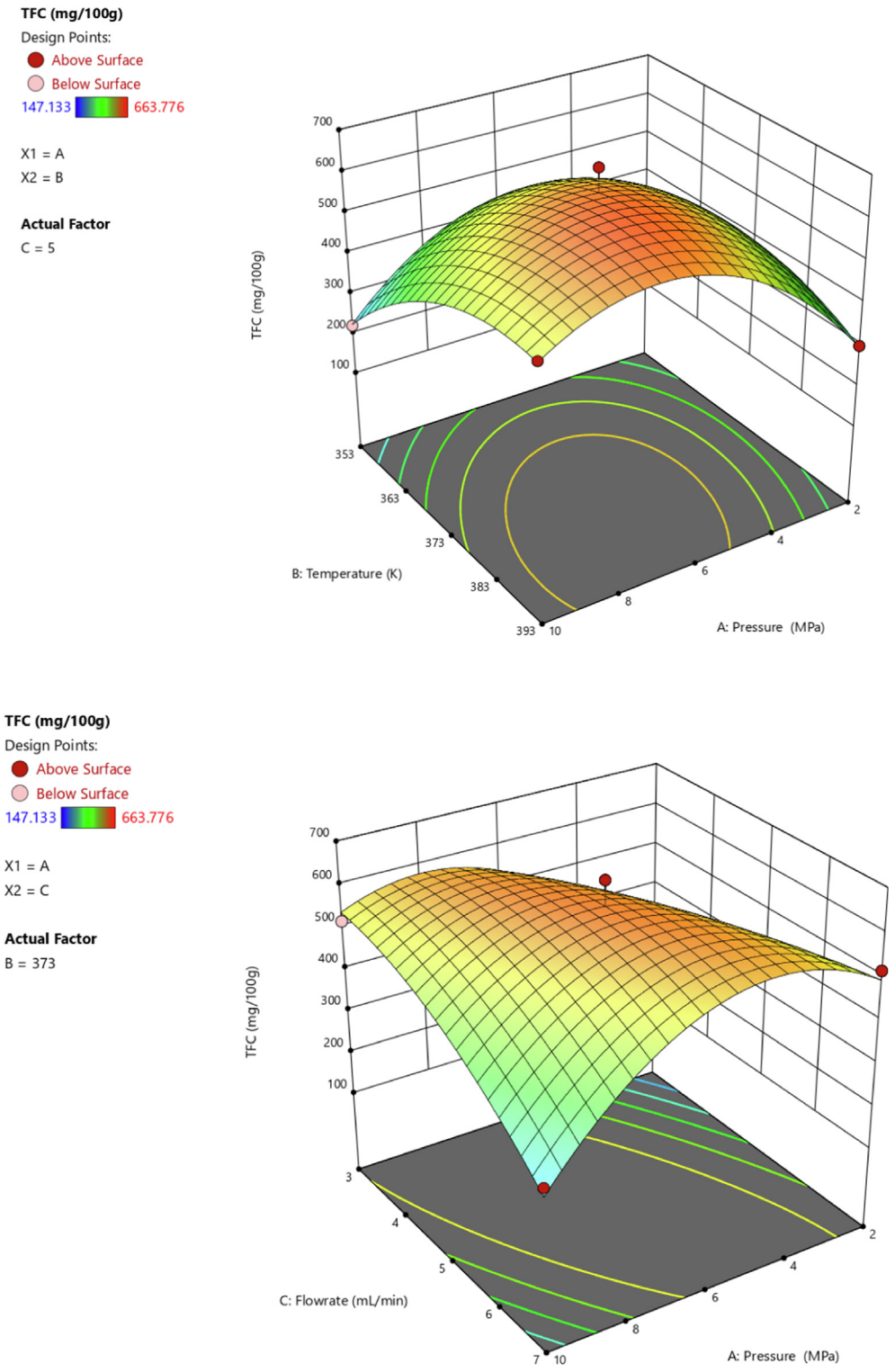

Fig. 7 3D response of the effect of variables on total phenolic compounds (TFC) (mg/100 g).

shown in Table 8. Due to the lower percentage of AARD and RMSE, global yield and anthocyanin solubility data were satisfactorily connected using the Del Valle Aguilera (DVa) model. Instead of using the Chrastil model, the DVa model data were used to determine the solubility characteristics of global yield and anthocyanin.

In this study, the coefficient of an is negative for global yield, indicating that an endothermic reaction ($+\Delta H$) is the optimal condition for solubility. This is because a positive value for H implies that the reaction should be accelerated using a high-heat technique. As a result, as the temperature rises, the solubility of subcritical ethanol increases, allowing

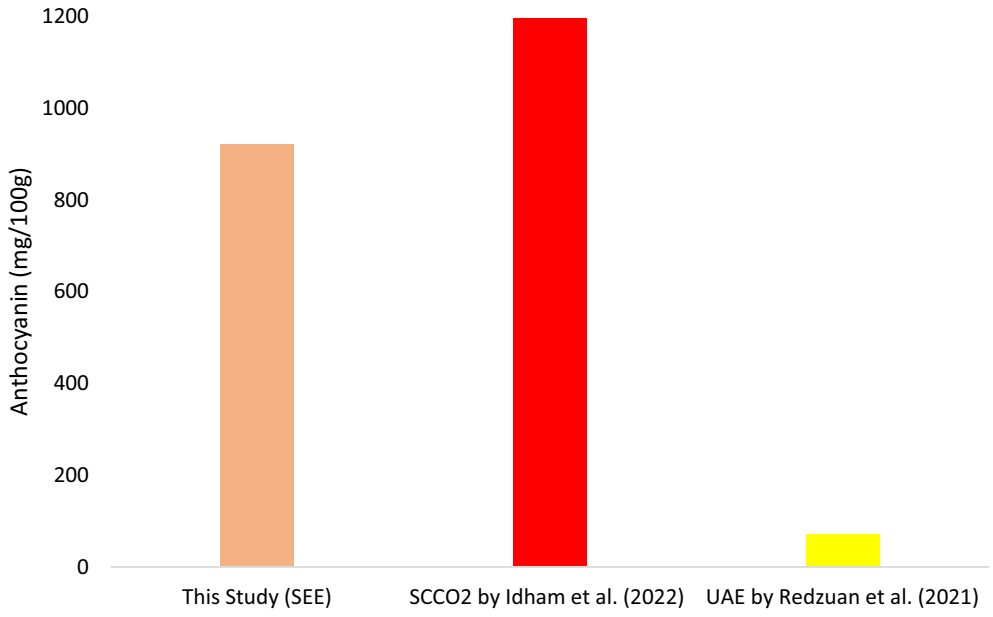

Fig. 8 Comparison of the anthocyanin recovery between this study and previous study.

Table 7 Experimental and calculated solubility of global yield and anthocyanin in subcritical ethanol.

Run			Experimental data		Chrastil model		DVa model	
	S_{exp} Global Yield (g/L)	S_{exp} Anthocyanin (g/L)	Ln S_{exp} Global Yield	Ln S_{exp} Anthocyanin	Ln S_{mod} Global Yield	Ln S_{mod} Anthocyanin	Ln S_{mod} Global Yield	Ln S_{mod} Anthocyanin
1	2.29E-02	2.71E-03	−3.78	−5.91	−3.61	−5.57	−3.72	−5.57
2	1.52E-02	2.04E-03	−4.19	−6.20	−3.64	−6.08	−3.50	−5.74
3	2.76E-02	3.31E-03	−3.59	−5.71	−3.61	−5.70	−3.61	−5.70
4	6.37E-02	8.70E-03	−2.75	−4.74	−3.61	−5.70	−3.61	−5.70
5	2.75E-02	3.51E-03	−3.59	−5.65	−3.61	−5.70	−3.61	−5.70
6	1.92E-02	3.81E-03	−3.96	−5.57	−3.61	−5.57	−3.72	−5.57
7	4.14E-02	8.34E-03	−3.19	−4.79	−3.61	−5.57	−3.72	−5.57
8	2.70E-02	3.36E-03	−3.61	−5.70	−3.61	−5.70	−3.61	−5.70
9	6.21E-02	8.40E-03	−2.78	−4.78	−3.64	−6.08	−3.50	−5.74
10	2.68E-02	3.34E-03	−3.62	−5.70	−3.61	−5.70	−3.61	−5.70
11	2.66E-02	3.35E-03	−3.63	−5.70	−3.61	−5.70	−3.61	−5.70
12	1.16E-02	1.81E-03	−4.45	−6.32	−3.61	−5.70	−3.61	−5.70
13	5.08E-03	1.49E-03	−5.28	−6.51	−3.61	−5.70	−3.61	−5.70
14	5.98E-02	9.83E-03	−2.82	−4.62	−3.61	−5.70	−3.61	−5.70
15	2.66E-02	3.56E-03	−3.63	−5.64	−3.64	−6.08	−3.50	−5.74
16	1.94E-02	3.44E-03	−3.94	−5.67	−3.64	−6.08	−3.50	−5.74
17	7.79E-03	1.29E-03	−4.86	−6.65	−3.61	−5.57	−3.72	−5.57

Table 8 Correlation data of the models fitted the solubility of global yield and anthocyanin in subcritical ethanol.

Bioactive compounds	Model	k_1	a	b	c	AARD (%)	RMSE
Global yield	Chrastil	0.53	−4.34	−7.07	–	11.84	0.68
	DVa	−0.26	−692	−15.71	−0.03	11.54	0.64
Anthocyanin	Chrastil	4.11	851	−35.1		7.28	0.67
	DVa	−1.2	849	−15.69	−0.02	7.15	0.60

for the extraction of the global yield. However, because the anthocyanin has a positive coefficient of a, the low-heat technique is required to extract the beneficial components. This is because higher temperatures destroy anthocyanin molecules [10,21]. The coefficient of k has no effect on the pressure/density due to the incompressible solvent in this investigation. This is because the Chrastil/DVa model is frequently utilised when compressible solvents such as supercritical carbon dioxide are involved. As a result, temperature effects on the solubility of global yield/anthocyanin in subcritical ethanol are substantial.

4. Conclusion

Anthocyanin, phenolic, and flavonoid recovery from roselle (*Hibiscus sabdarifa*) via subcritical ethanol extraction (SEE) is an unexplored research area. The subcritical ethanol extraction (SEE) method is more basic and capable of attaining high extraction rates in a short amount of time. Depending on the operational conditions, SEE may have diverse effects on product production and quality (pressure, temperature, flowrate, residence time and particle size). Based on this reseach, the optimal conditions were 8.74 MPa, 383.51 K, and 5.21 mL/min, providing a global yield of 0.765 g/g, anthocyanin concentrations of 921.43 mg/100 g, TPC concentrations of 40.57 mg/100 g, and TFC concentrations of 559.14 mg/100 g. Global yield, anthocyanin phenolic and flavonoid recovery is enhanced by high pressure and temperature conditions. The flowrate of ethanol is not significant to enhance the global yield and its interest compounds. The DVa model fits global yield and anthocyanin solubility in SE efficiently since it has the lowest average absolute relative deviation (AARD), which are 11.54 and 7.19 %, respectively. As a result, this approach (SEE) may be used in place of the conventional method for extracting anthocyanin.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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