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RESEARCH ARTICLE

Process optimization of supercritical CO_2 extraction of Roselle using response surface methodology

Wong Lee Peng [a], Siti Hamidah Mohd Setapar [a,c,*], Hasmida Mohd Nasir [b]

[a] Centre of Lipids Engineering and Applied Research (CLEAR), Ibnu Sina Institute for Scientific and Industrial Research, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia
[b] Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia
[c] SHE Empire Sdn Bhd, No 44, Jalan Pulai Ria 2, Bandar Baru Kangkar Pulai, 81300 Skudai, Johor, Malaysia

* Corresponding author: sitihamidah@cheme.utm.my

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Abstract

Hibiscus sabdariffa, commonly known as Roselle, is a native plant in Malaysia that is rich with bioactive compounds. In the present study, supercritical carbon dioxide (SC-CO_2) extraction of Roselle was investigated. The optimum particle size (212µm, 300µm, 425µm, 600µm, and 710µm) to obtain highest yield was pre-determined. The effects of two operating parameters, pressure (20MPa, 25MPa, and 30MPa) and temperature (40 ºC, 60 ºC, and 80 ºC) on extraction yield were studied using response surface methodology (RSM). From the experimental data, the optimum conditions were achieved using particle size 300µm, pressure 27.5MPa, and temperature 50.8 ºC. Using the optimized parameters, the highest extraction yield was predicted to be 163.26 mg-extract/g-dried sample. The validation experimental results were consistent with the predicted values.

Keywords: Roselle (*Hibiscus sabdariffa*), supercritical CO_2 extraction, response surface methodology, fatty acid composition, optimization

INTRODUCTION

In some Asian countries such as China, Malaysia, India, and Sudan, *Hibiscus sabdariffa* L. (Roselle) is one of the popular traditional remedies used to treat many chronic diseases (Tseng *et al*., 2000). While the calyces of Roselle are commonly used to prepare various food products such as beverage, jam, and preserved food, other parts of this plant are not utilized. Recent scientific studies are interested in investigating the benefits of Roselle in different parts and their industrial applications including in cosmetics, personal care, and pharmaceutical products (Wong *et al*., 2014; Mohamed *et al*., 2007; Amin *et al*., 2008). It has been documented in several reports that the Roselle seed extract is rich in proteins, dietary fibre, carbohydrates, and fats (Zikou *et al*., 2011; Tounkara *et al*., 2014; Lessoy *et al*., 2012). It is also high in antioxidant polyphenols, sterols, tocopherols, and flavonoids which exhibit radical activity and unsaturated fatty acids especially linoleic and oleic acids, namely Omega-9 and Omega-6 fatty acids, respectively. These components are potential sources of nutrient and natural antioxidants supply.

There are many conventional methods of oil extraction. Most of the edible oils are extracted using solvent extraction or mechanical press process. A right method for edible oil extraction is one of the important factors for composition isolation and identification. Previously, studies have been conducted to develop a novel extraction process in order to increase the product quality and active natural products quantity (Chuo *et al*., 2016; Mohd-Setapar *et al*., 2014). By applying the mechanical press process, the produced oil retains its properties and benefits, but the extraction rate is very low (Giacomo *et al*., 2015). Another conventional method is Soxhlet where an organic solvent is used to extract bioactive compounds from plant materials. Unfortunately, this approach is time-consuming (up to 10 hours), requires a large amount of solvent or high temperature for extraction and the solvent is easily left in the extracted oil even after the evaporation process (Liza *et al*., 2010). Therefore, novel extraction techniques with high efficiency and eco-environmentally friendly are highly desirable.

In recent years of research in separation, supercritical carbon dioxide (SC-CO_2) extraction has drew attention from researchers and industrial practitioners as a promising alternative to conventional technology. Compared to solvent extraction, SC-CO_2 can perform in varied of temperature levels but usually at low temperature levels with shorter extraction time compared to solvent extraction. Plus, it has high extraction rate and only need a small amount of solvent to operate (Chen *et al*., 2014). The extract obtained from SC-CO_2 extraction is free from chemicals. The CO_2 is a non-active solvent which can protect the extracted compound from thermal degradation. CO_2 is also environmentally friendly solvent which can be released and evaporated immediately into atmospheric as it is non-toxic. SC-CO_2 extraction produces better quality oil, for example oil with acidity. In addition, the extraction minimizes unwanted oxidative reacts on sensitive bioactive compounds, for examples vitamins, polyphenols, tocopherols, sterols, and unsaturated fatty acids (Gomez *et al*., 1996).

Many factors can affect the efficiency of SC-CO_2 extraction including the condition of the samples, pressure, temperature, and extraction time (Bimkr *et al*., 2013). Therefore, these factors have been collectively studied in this work to validate the optimal extraction conditions. Response surface methodology (RSM), a collection of mathematical and statistical techniques useful for the modelling and analysis of problems in which a response of interest is influenced by several variable, was used in this study. This allows process optimization to be conducted effectively (Norodin *et al*., 2016).

In this study, historical data is applied to observe the effects of the experimental range of pressure (20MPa, 25MPa, and 30MPa) and temperature (40 ºC, 60 ºC, and 80 ºC) on the extraction yield of Roselle extract by SC-CO_2. The objective of this study was to obtain the optimal conditions within the experiment ranges to achieve the highest yield of Roselle extract by using SC-CO_2.

EXPERIMENTAL

Preparation of Roselle sample

The dried Roselle samples were ground to fine powder using miller (Werke, Germany) and sieved (USA STD Test Sieve, Endecott Ltd, England) to particle sizes of 212 µm, 300 µm, 425 µm, 600 µm, and 710 µm. The dried samples were kept in the airtight zipper bag and stored in freezer at the temperature about -20ºC to maintain their freshness.

Determination of particle size

The samples were extracted at fixed conditions of temperature 40 ºC, pressure 30 MPa, CO_2 flow rate 5 ml/min, and extraction time 180 min.

Supercritical carbon dioxide (SC-CO_2) extraction

The extraction of Roselle was performed using lab-scale supercritical fluid extractor (SSI, State College Pennsylvania, US) which consists of Supercritical 24 fluid extractor, constant flow pump, a carbon dioxide cylinder, and a programmable back pressure regulator (Model BP-2080, JASCO, Japan). The schematic diagram of lab-scale supercritical fluid extraction instrument is shown in Fig. 1.

In this extraction method, CO_2 was used as the solvent. In every extraction process, 5 g of sample was used. The samples were put into the extraction vessel and the extraction was performed at range of temperature and pressure shown in Table 1 under dynamic condition of 180 min and CO_2 at constant flow rate of 5 mL/min. After the extraction process, the extract was placed in the vial attached to the restrictor valve.

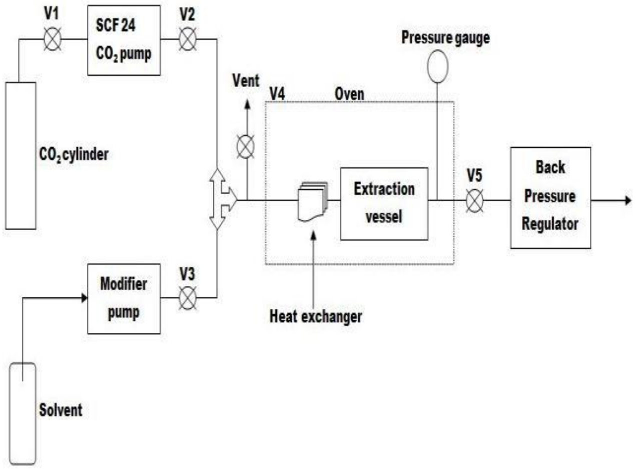

Fig. 1 The schematic diagram of lab-scale supercritical fluid extraction instrument.

Table 1 The range of temperature and pressure for supercritical carbon dioxide extraction.

Parameters	Unit	Range	Increment
Temperature (T)	ºC	$40 \le T \le 80$	20.00
Pressure (P)	MPa	$20 \le P \le 30$	5.00

The amount of extracted oil obtained by SC-CO_2 is calculated using direct measures of the extracted oil as shown in Eq. (1):

$$\text{Percentage of overall extracted oil yield, \%} = (W_{oil}/W_s) \times 100\% \quad (1)$$

where W_{oil} represents the weight of the extracted oil in gram while W_s represents the weight of sample used in gram.

Experimental design

The historical data was applied for investigating the optimal process parameters, namely temperature and pressure for SC-CO_2 extraction of Roselle. The independent variables in the experimental design, temperature and pressure were investigated to optimize the responses oil yield from Roselle. The extraction time and the solvent, CO_2 flow rate were set to constant. The whole experiment design consisted of 9 combinations. The ANOVA tables were generated and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significance of all the terms in the polynomial were analyzed statistically.

The experimental data were fitted to the following second-order polynomial model and regression coefficients were obtained; the generalized second-order polynomial model proposed for the response surface analysis was given as Eq. (2):

$$Y = \beta_0 + \sum\beta_i x_i + \sum\beta_{ii} x_i^{\,2} + \sum\beta_{ij} x_i x_j \quad (2)$$

where Y represents the response variables; β_0 is a constant, β_i, β_{ii}, and β_{ij} are the linear, quadratic and interactive coefficients, respectively; X_i and x_j are the levels of the independent variables.

Response surface methodology (RSM) was used to determine the optimum conditions. The experimental design and statistical analysis were carried out using the Design Expert 6.0.4.

RESULTS AND DISCUSSION

Effect of particle size on oil yield

Particle size is a key factor that can determine the satisfactory of SC-CO_2. Theoretically, the extraction yield increases as the particle size of the sample is decreased because of the increased surface area of a sample. Fig. 2 shows the cumulative extraction yield which results from varied particle size of Roselle. The experiment was done as described, at fixed parameters of temperature, pressure, and extraction time.

The results show that as Roselle particle size decreased from 0.71mm to 0.30mm, the percentage of oil yield increased from 2.05 to 15.85%. According to Del Valle and Uquiche (2002), extracting the samples in smaller particle size will result in higher oil yield than using sample in larger particle size. It is because the surface area of oil seed materials increased during the grinding process (Pan *et al*., 2013). Furthermore, the higher extraction efficiencies can be achieved when the grinding process ruptures the cell walls, thus expose more surface area for mass transfer and in quantity of soluble fraction of the oil to be extracted.

For Roselle with particle size of 0.2mm, it shows the lower oil yield than sample with particle size of 0.3mm. This may due to the size of particle is too small and tends to clump together and forming larger surface area of extraction materials. In this investigation, 0.3mm has been determine as the best particle size of Roselle since it gave the highest percentage of oil yield which is 15.85% compared to other particle sizes.

Response Surface Analysis

The effect of temperature and pressure on Roselle yield obtained using SC-CO_2 extraction was investigated using historical data which are shown in Table 2. The results were analyzed by using analysis of variance, ANOVA (Table 3). From the model ($p < 0.0055$), coefficient of determination ($R^2 = 0.9861$) shows that the quadratic model developed to predict the Roselle yield is significant.

The ANOVA shows highly significant impacts of temperature ($p < 0.001$), pressure ($p < 0.0093$), and the interaction between the variables ($p < 0.05$) on the Roselle oil yield. Fitting a regression surface to the experimental results, Eq. (3) is applicable to predict the achievable extraction yield (Y) as a function of the studied process variables:

$$Y\ (mg/g) = 148.93 - 31.15\ X_1 + 17.31\ X_2 - 27.64\ X_1^2 - 21.84\ X_2^2 - 11.51\ X_1\ X_2 \qquad (3)$$

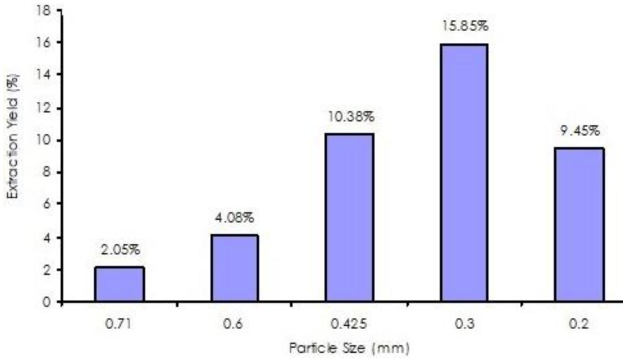

Fig. 2 Effect of particle size of sample on extraction yield.

Table 2 Historical data of independent variables and their corresponding Roselle yield.

Run	X_1 Temperature (°C)	X_2 Pressure (MPa)	Y Roselle Yield (mg/g)
1	40	20	98.06
2		25	153.9
3		30	161.68
4	60	20	114.32
5		25	151.76
6		30	137.02
7	80	20	61.64
8		25	85.84
9		30	79.24

Where Y is the response (yield), X_1 and X_2 are the value of factors, temperature and pressure respectively. The minus sign in front of the factor in the Eq. (3) indicates reduce the Roselle yield, while the plus sign increases the yield.

Influence of factors

The best way to visualize the influence of the process variables is to generate the three-dimensional surface response plot as illustrated in Fig. 3. The response surface and contour plots showing the effect of temperature and pressure on the oil yield at the fixed extraction time of 180 min.

From Eq. (3), it shows that the coefficient of pressure (X_2) is larger than that of temperature (X_1) and X_1 X_2, which indicates that the pressure has a dominant effect over the temperature. Extraction yields were increased from 98.06 to 151.76mg/g with an increase in pressure level from 25 to 30MPa because the raising of the extraction pressure leads to a higher fluid density which increases the solubility of the analytes. This is observed at low temperature levels, 40 to 60 °C, and afterward the extraction yield decreases as the temperature further increased to 80 °C. This results trend happened when the mass transfer speed increased with initial temperature rise, causing an increase of solubility of extract. The extraction yield is directly affected by the solubility of oil in supercritical fluid and the balance between CO_2 density and the oil vapor pressure (Ara *et al*., 2014). At high pressure, the influence of the temperature on the solubility of oil is predominated by the oil vapor pressure effect and then the solubility of oil increases with the increase of temperature (Zhao *et al*., 2011). While the CO_2 reduced density as the temperature continuously rise, causing the decrease of solubility and hence the yield decreased. The highest yield of 161.68mg/g was obtained at the temperature as low as 40 °C. Similar yield trend as a function of temperature was observed in the extraction of other fenugreek seed (Gu *et al*., 2017) and red pepper (Gu *et al*., 2016).

The correlation graph in Fig. 4 shows that high correlation exists between the experimental and predicted values. Each point is close to the regression line which indicates the good fit of the model. In this study, from the experimental data the lower temperature combined with higher pressure is preferred. The highest yield can be obtained at the optimal conditions at pressure 27.5MPa and temperature 50.8 °C.

Source [a]	Sum of Square	Df	Mean square	F-value	P-value [b]	R^2
Model	10634.45	5	2126.89	42.59	0.0055	0.9861
X_1	5823.18	1	5823.18	116.61	< 0.001	
X_2	1799.89	1	1799.89	36.04	0.0093	
X_1 X_2	529.46	1	529.46	10.60	0.0473	
X_1^2	1527.94	1	1527.94	30.60	< 0.001	
X_2^2	953.97	1	953.97	19.10	0.0222	
Residual	148.81	3	49.94			
Lack of fit		3				
Pure Error		0				
Cor. total	10784.26	8				

[a] X_1: Temperature; X_2: Pressure

[b] $p<0.01$ highly significant; $p<0.05$ significant; $p>0.05$ not significant

Df: Degree of freedom

R^2: Coefficient determination

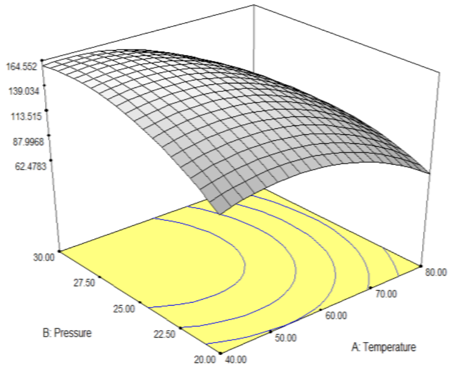

Fig. 3 Response surface plots for Roselle seed extract (mg-extract/g-dried sample) as a function of pressure and temperature.

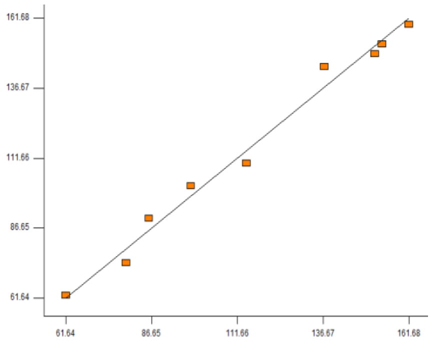

Fig. 4 Correlation graph of predicted and experimental yield (mg/g).

CONCLUSION

This work studied the process parameters of SC-CO_2 extraction of Roselle. The Roselle yield was optimized by response surface methodology by using the extraction temperature and pressure. The optimization of process parameters find the best extraction condition and it was predicted that the highest yield (163.26mg/g) of Roselle seed extract can be obtained at the optimum extraction parameters of pressure 27.5MPa and temperature 50.8 ºC.

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