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Research Article

Optimisation of Process Parameters for Supercritical Carbon Dioxide Extraction of Oil from Gac Seed Kernel Powder

[1,2]Anh V. Le, [2]Paul D. Roach, [2,4]Minh H. Nguyen and [2,3]Sophie E. Parks
[1]Faculty of Bio-Food Technology and Environment, University of Technology (HUTECH), HCMC, Vietnam
[2]School of Environmental and Life Sciences, University of Newcastle,
[3]NSW Department of Primary Industries, Central Coast Primary Industries Centre, Ourimbah, NSW 2258,
[4]School of Science and Health, Western Sydney University, Penrith, NSW 2751, Australia

Abstract: This study aimed to maximize the oil yield from Gac seed kernels using supercritical carbon dioxide (SC-CO_2) extraction. Gac seed kernel powder (4 g) with particle diameters <500 µm was extracted for 32 min. Response surface methodology with central composite design was used to optimize the SC-CO_2 extraction parameters: temperature (60-80°C), pressure (5,000-7,000 psi (34,474-48,263 kPa)) and SC-CO_2 flow rate (1-2.5 mL/min). The oil yield, accurately represented by a second order equation (R^2 = 0.99, p<0.0001), was predicted to be most substantially and significantly influenced by temperature (p<0.0001), followed by pressure (p<0.02) but not by the CO_2 flow rate (p = 0.20). The optimum conditions were predicted to be: temperature of 73°C, pressure of 5,900psi (40,679kPa) and CO_2 flow rate of 1.5 mL/min. The optimum oil yield was predicted to be 34.1±0.8% (g oil/100 g Gac seed kernel powder) and experimentally validated at 33.9±0.5%. The oil was likely high in saturated fat, being solid at room temperature and having a low iodine value, with 33.2±1.1% being unsaponifiable matter.

Keywords: Extraction, *Momordica cochinchinensis*, oil, response surface methodology, supercritical carbon dioxide

INTRODUCTION

Momordica cochinchinensis Spreng. (Gac), a plant of the Cucurbitaceae family, is widely distributed in Asian countries. The seeds of Gac are used as a Chinese traditional medicine called Mubezhi. These seeds are believed to have anticancer, antiviral, immuno-enhancing, anti-inflammatory, antioxidant, gastroprotective, antiulcerogenic, ribosome inactivating and trypsin inhibiting activities (Lim, 2012).

Vietnam is one of the world's largest producers of Gac fruit. A small percentage of the Vietnamese produce is sold in markets/supermarkets but most of it is processed by industry into commercial products such as Gac aril oil and Gac aril powder. The Gac seeds are usually discarded as a waste product after being separated from the aril of the fruit.

Gac seeds contain 35-53% oil (Ishida *et al*., 2004; Matthaus *et al*., 2003), with the long chain saturated fatty acid, stearic acid (60%) and the omega-6 polyunsaturated fatty acid, linoleic acid (20%), being the major components (Ishida *et al*., 2004). Unlike other long chain saturated fatty acids, which increase blood cholesterol levels, stearic acid has been shown to have a neutral effect on blood total and Low-Density Lipoprotein (LDL) cholesterol levels (Grundy, 1994; Hunter *et al*., 2010). In addition, the high percentage of stearic acid in the oil raises its melting point, which is desirable if it is to be used as frying oil or in the confectionary industry. Stearic acid, as a saturated fatty acid, is also relatively stable to oxidative processes (Leyton *et al*., 1987) linoleic acid is also an important component of the oil; It is an essential dietary fatty acid for humans, which is vital to human metabolic processes and it also lowers blood cholesterol levels (Rassias *et al*., 1991).

Vegetable oil is conventionally extracted physically using a mechanical pressing process or chemically with solvents (Norris, 1982). Although the properties of oil extracted by the mechanical pressing process are better than when solvents are used because the oil is less likely to be contaminated with chemical solvents, the extraction rate is low. The extraction rate is high using solvent extraction but the solvent is mixed

Corresponding Author: Anh V. Le, School of Environmental and Life Sciences, University of Newcastle, Ourimbah, NSW 2258, Australia, Tel.: +61 481245586; Fax: +61 2 4348 4145

with the oil, which results in a need for further purification.

In recent years, Supercritical Carbon dioxide (SC-CO_2) extraction of vegetable oil has attracted considerable attention as a promising alternative to the conventional solvent extraction and mechanical pressing processes (Gomes *et al*., 2007; Lu *et al*., 2007). The main reasons are that SC-CO_2 has a higher extraction rate than the mechanical pressing process and that the solvent, CO_2, is non-flammable, non-explosive, cost-efficient, readily available and, because it is a gas, it is easy to remove from the extracted oil.

To maximize the yield of oil by SC-CO_2 extraction, the pressure, temperature and flow rate of the SC-CO_2 need to be optimized. The classical method of optimizing such processes is the single dimensional search, which involves changing one variable while fixing the others at a certain level (one-factor-at-a-time experiment). This is laborious and time consuming especially when the number of variables is large. More importantly, the results of one-factor-at-a-time experiments do not reflect actual changes in the environment as they ignore interactions between factors presented simultaneously. An alternative and more effective approach, which is increasingly being used, is based on statistical methods. One such method is the Response Surface Methodology (RSM), which has been well described (Baş and Boyacı, 2007; Bezerra *et al*., 2008). The RSM has been demonstrated to be a fast, economical and powerful tool for determining the effects of several factors and their interactions, which allows process optimization to be conducted effectively (Baş and Boyacı, 2007).

For the extraction of seed oils, many studies have used RSM to investigate the effect of the SC-CO_2 operating conditions on the yield of oil in order to determine the optimal process conditions for the extraction of oil from a number of seeds, including hemp seed (Da Porto *et al*., 2012), flaxseed (Jiao *et al*., 2008; Özkal, 2009), pomegranate seed (Liu *et al*., 2009a), *Passiflora* seed (Liu *et al*., 2009b; Zahedi and Azarpour, 2011), apricot kernel (Özkal *et al*., 2005a), hazelnut (Özkal *et al*., 2005b) and *Nigellaglandulifera* freyn seed (Zhang *et al*., 2012).

However, to the best of our knowledge, there is no data in the literature on the optimization of the yield of oil from Gac seeds using SC-CO_2 extraction. Thus, the present study aims to determine the optimum SC-CO_2 process parameters, in terms of pressure, temperature and flow rate of SC-CO_2, for the yield of oil from Gac seeds using RSM. Knowledge of the total oil yield with SC-CO_2 extraction will be useful for the design and development of a process for the extraction of oil from Gac seeds in a commercial setting.

MATERIALS AND METHODS

Materials and chemicals: Gac seeds, accession VS7 according to the classification by Wimalasiri *et al.* (2016), were collected from fresh Gac fruits grown in Ho Chi Minh (HCM) city, Vietnam (Latitude: 10.757410; Longitude: 106.673439). After their separation from the fresh fruit, the seeds were vacuum dried at 40°C, de-coated and the kernels were packaged in vacuum-sealed bags and stored at 4°C. Before conducting experiments, the Gac seed kernels were ground into powder of particle sizes less than 500 µm using the 100 g ST-02A Mulry Disintegrator. The powdered Gac kernel particle sizes were measured using the Endecotts Test Sieve (Endecotts, London, England). The powder was then dried in a Dynavac FD3 Freeze Dryer (Sydney, NSW, Australia) for 24 h at -45°C under vacuum at a pressure loading of 10^{-2} m bar (1Pa). There was no detectable moisture in the freeze-dried product as measured according to the standard AOCS Ab 2-49 method (AOCS, 1998). Carbon dioxide (99.9%) was purchased from Coregas Pty. Ltd. (Mayfield, NSW, Australia).

Supercritical carbon dioxide extraction of Gac seed oil: The extraction of Gac seed oil with SC-CO_2 was performed using a laboratory-scale Supercritical Fluid Extraction System (Teledyne Isco, Lincoln, NE, USA), which consisted of an SFX 2-10 extractor and two 260D syringe pumps. The extractor was a 10 mL cartridge in which SC-CO_2 flowed downwards. The operation parameters of the system range between 10 and 7,500psi (68.9 and 51,711kPa) for pressure, between ambient and 150°C for temperature and between 0.001 and 107 mL/min for CO_2 flow rate.

Four grams of Gac seed kernel powder, with particle diameters less than 500 µm was added to a 10 mL extraction vial, which was then placed in the extraction vessel. Based on the literature (Zhang *et al*., 2012), the mass ratio of powder to CO_2, ranges from 1:8 to 1:10 and therefore, it was fixed at a ratio of 1:10 for these experiments. The extraction time for each run was determined from the known mass of CO_2 which needed to be passed through the system (~40 g) and this was based on the CO_2 flow rate of the run and the density of the CO_2 at given temperatures and pressures according to the Benedict-Webb-Rubbin (BWR) equation of state proposed by Span and Wagner (1996).

The oil-CO_2 extract was passed through the coaxially heated adjustable restrictor set at 70°C to evaporate the CO_2 and the oil was collected in a test tube. After the desired extraction time (~32 min), the extraction was manually stopped - at which time the oil had stopped flowing. The oil extracts obtained from the SC-CO_2 extractions, done under the different conditions, were then weighed to obtain the Gac seed oil yield for each extraction and expressed as g of oil per 100 g of Gac seed kernel powder.

EXPERIMENTAL DESIGN

The RSM with Central Composite Design (CCD) was employed to investigate the effect of the SC-CO_2

extraction parameters on the yield of oil from the Gac seed kernel powder (Myers *et al*., 2014). Based on preliminary experiments, the three independent parameters namely, extraction temperature (X_1), extraction pressure (X_2) and supercritical CO_2 flow rate (X_3), were tested at three different levels each, as shown in Table 1.

A total of 16 experiments were carried out. The experimental design consisted of eight (2^3) factorial points, six axial points (star points) to form a central composite design and two replicates for the center point. Optimization was performed using an on-face central composite design with an alpha value of ±1.00 for the three factors (Table 1). The experiments were run in random order to minimize the effects of unexpected variability in the observed responses due to extraneous factors. When the optimum extraction conditions were predicted by RSM from the experimental data, three Gac seed kernel powder samples (4 g) were extracted by SC-CO_2 using the predicted optimum conditions for the temperature, pressure and CO_2 flow rate and the oil yield was compared to the predicted oil yield.

The experimental plan was designed and the results were analysed using JMP software version 11 (SAS, Cary, NC, USA). The software was also used to establish the model equation for graphing the three dimensional and two dimensional contour plots of the variable responses and to predict the optimum values for the three response variables. The Student's *T*-test, conducted using the SPSS statistical software version 20 (IBM, Armonk, NY, USA), was used to compare the observed oil yields to the predicted oil yields, after optimization. Values were taken to be statistically significant at $p<0.05$.

Physical and chemical properties of Gac seed oil: Oil extracted from the Gac seed kernel powder at the optimum conditions was subjected to physical and chemical characterization. The state (solid or liquid) of the oil at room temperature and the color when liquid were noted by visual inspection. The procedures for determination of the other physical and chemical indices were carried out following the official AOCS methods (AOCS, 1998) as follows: Specific gravity: AOCS Cc 10a-25, Refractive index: AOCS Cc 7-25, Slip melting point: AOCS Cc 3-25, Free fatty acids: AOCS Ca 5a-40, Peroxide value: AOCS Cd 8-53, Saponification value: AOCS Cd 3-35, Unsaponifiable matter: AOCS Ca 6a-40, Iodine value: AOCS Cd 1-25, Insoluble impurities: AOCS Ca 3-46, Moisture and volatile matter: AOCS Ca 2c-25.

RESULTS AND DISCUSSION

Fitting of the model for prediction of oil yield: The RSM model generated the following second-order polynomial formula:

$$\begin{aligned}Y_{(\%)} &= 33.726 + 2.184X_1 - 0.722X_2 \\ &-0.317X_3 + 0.129X_1X_2 - 0.561X_1X_3 \\ &-0.961X_2X_3 - 3.938X_1^2 - 2.778X_2^2 - 0.733X_3^2\end{aligned} \quad (1)$$

where,
$Y_{(\%)}$ = Oil yield (g oil/100 g Gac seed)
X_1 = Temperature (°C)
X_2 = Pressure (psi)
X_3 = CO_2 flow rate (mL/min)

It is necessary to test the reliability of the RSM mathematical model in predicting variances and

Table 1: Uncoded and coded levels of independent variables used in RSM design

Coded levels (X)	Temperature (X_1, °C)	Pressure (X_2, psi)	Flow rate (X_3, mL/min)
-1	60	5,000	1.00
0	70	6,000	1.75
+1	80	7,000	2.50

Table 2: Experimental and predicted data for the yield of Gac seed oil obtained from the central composite experiment design

	Factors			Oil yield (g oil/100 g powder)	
Trial no.	X_1	X_2^*	X_3	Experimental	Predicted
1	60	7,000	1.00	24.51	23.96
2	60	7,000	2.50	21.96	22.52
3	60	5,000	1.00	23.43	23.74
4	60	5,000	2.50	26.28	26.15
5	60	6,000	1.75	27.79	27.60
6	70	7,000	1.75	29.95	30.26
7	70	6,000	1.00	32.52	33.31
8	70	6,000	1.75	33.80	33.73
9	70	6,000	1.75	34.10	33.73
10	70	6,000	2.50	33.24	32.68
11	70	5,000	1.75	31.72	31.67
12	80	7,000	1.00	29.63	29.71
13	80	7,000	2.50	26.39	26.03
14	80	6,000	1.75	31.56	31.97
15	80	5,000	2.50	28.64	29.14
16	80	5,000	1.00	29.59	28.97

X_1: temperature (°C); X_2: pressure (psi); X_3: CO_2 flow rate (mL/min); * 5,000, 6,000 and 7,000 psi are 34,474, 41,369 and 48,263 kPa, respectively

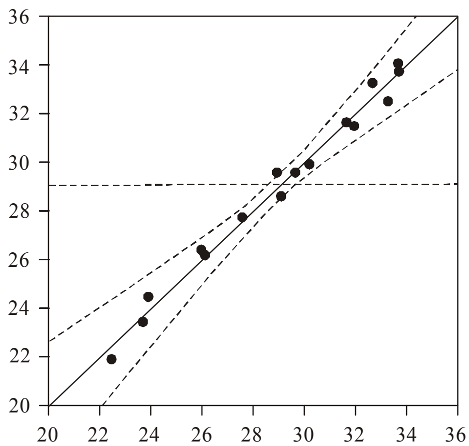

Fig. 1: Correlation (p<0.0001; R^2 = 0.99) between the predicted and actual total oil yields from Gac seed kernel powder extracted under the conditions listed in Table 2

Table 3: Analysis of variance values for model fitting

Sources	Values
Lack of fit	0.56
R^2	0.99
Adjusted R^2	0.97
PRESS	41.80
F Ratio of model	12.57
p of Model > F	0.21
X_1	p<0.0001
X_2	p<0.02
X_3	p = 0.20
X_1X_2	p = 0.62
X_1X_3	p = 0.06
X_2X_3	p<0.01
X_1^2	p<0.0001
X_2^2	p<0.001
X_3^2	p = 0.14

accurately representing the real interrelationships between the selected parameters. Therefore, a goodness of fit analysis was undertaken using the oil yields obtained from all the experiments listed in Table 2 in relation to the RSM design.

The results from the analysis of variance of the central composite design are shown in Fig. 1 and Table 3. Figure 1 shows the correlation between the predicted and the experimental values listed in Table 2 while Table 3 presents the values for the analysis of variance of the model.

Figure 1 shows that the fit between the experimental values and the model oil yield outputs was highly significant (p<0.0001). Furthermore, the coefficient of determination (R^2) for the model was 0.99, which indicated that 99% of the experimental data for the oil yield predictively matched against the model data.

Table 3 shows that the lack-of-fit value (0.56) was also not significant (p>0.05), which indicated that the generated model adequately explained the variation in the experimental data and that the model was an accurate representation of the actual relationship between the extraction parameters and the oil yield.

In addition, the Predicted Residual Sum of the Squares (PRESS) for the model, which is a measure of how well the predictive model fits each point in the design, was 41.80, the F value of the model was 12.57 and the experimental and predicted values did not differ significantly (p = 0.21) from each other (Table 3). This further showed that the mathematical model accurately predicted the amount of oil that could be extracted from the dried Gac seed kernel powder using the SC-CO_2 system when the values for the temperature in ^{o}C (X_1), the pressure in psi (X_2) and the CO_2 flow rate in mL/min (X_3) were varied as shown in Table 2.

Response surface analysis: From Eq. (1), it can be seen that the oil yield from the Gac seed kernel powder had a complex relationship with the three independent variables. This can also be seen in the three-dimensional response surface curves and their corresponding contour plots as shown in Fig. 2A to 2C, with each illustrating the relationship between two of the independent variables and the oil yield.

Figure 2A shows the response surface curve and its contour plot for the combined effects of temperature and pressure on the oil yield and their interaction at a fixed CO_2 flow rate of 1.75 mL/min. The effect showed a response that could typically be modelled using a quadratic Eq. (1) with temperature having a more marked effect on the oil yield than pressure with both having significant effects (Table 3). However, the interaction between the temperature and the pressure was not significant (p = 0.62) (Table 3).

At the low end of the temperature levels, the model predicted that the oil yield would substantially increase as the temperature increased but that the effect would plateau at approximately 73°C and that the oil yield would then decrease with further increases in temperature (Fig. 2A). At the low end of temperatures, the positive effect is most likely due to an increased mass transfer speed of the solutes into the liquid CO_2 as the temperature is increased. However, at high temperatures, the density of the CO_2 is likely to be reduced, with a consequent reduction in solute solubility (Clifford and Clifford, 1999).

At the low end of the pressure values, the model predicted that the oil yield would increase as the pressure was increased but that the effect would plateau at approximately 5,900psi (40,679 kPa) and that the oil yield would then decrease with further increases in pressure (Fig. 2A). At the low end of pressures, the positive effect is most likely due to an improvement in solute solubility in the liquid CO_2, which results from an increase in the CO_2 density as the pressure is increased in the SC-CO_2 system (Zhang *et al*., 2012). However, when the pressure is increased to high levels, a reduction in the diffusivity and mass transfer

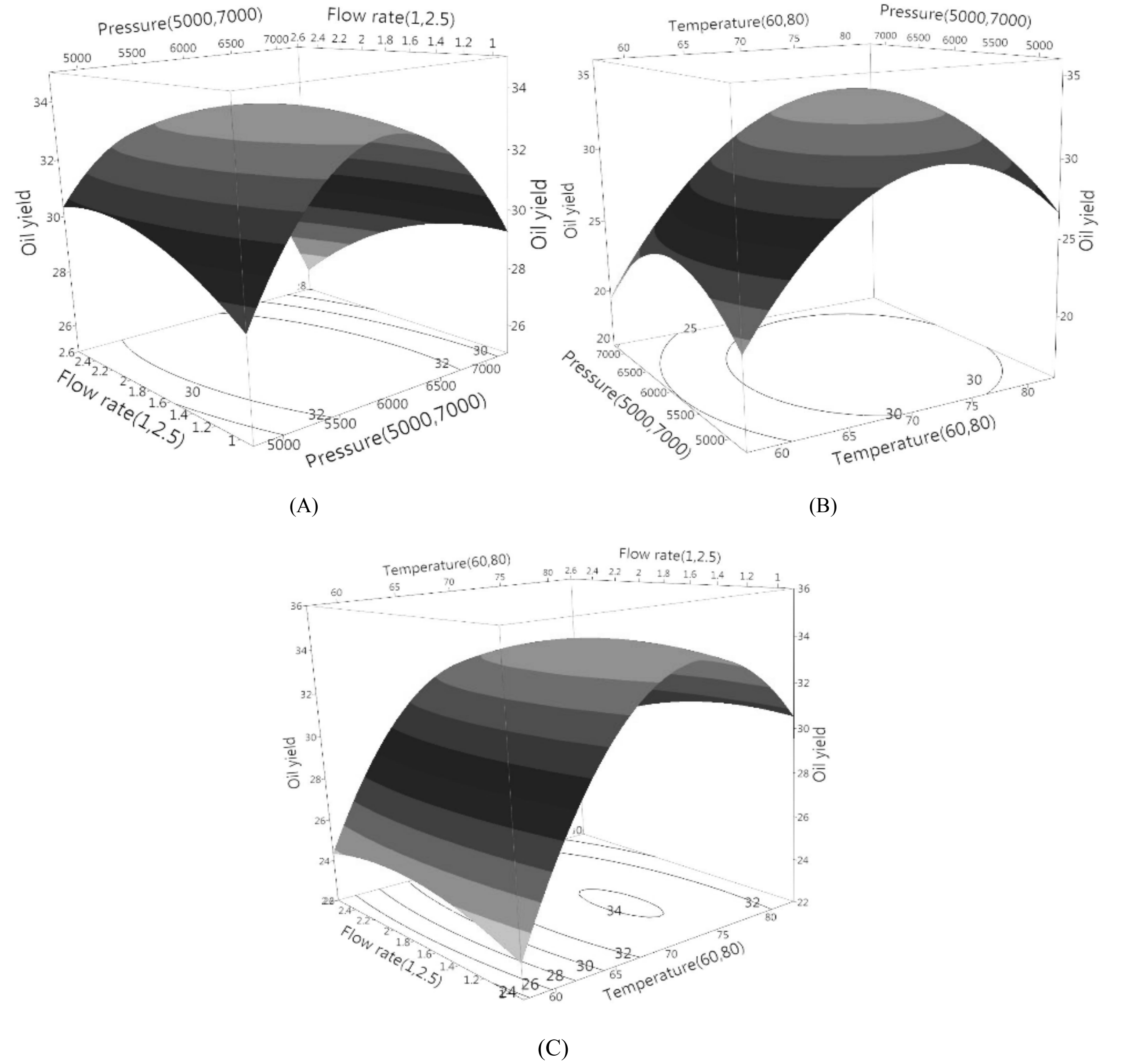

Fig. 2: Response surface curve and its contour plot for; (A): the effects of temperature and pressure at a constant CO_2 flow rate of 1.75 mL/min; (B): the effects of pressure and CO_2 flow rate at a constant temperature of 70°C; (C): the effects of temperature and CO_2 flow rate at a constant pressure of 6,000psi (41,369 kPa) on the oil yield

coefficient of the liquid CO_2 will occur, which can offset the increase in the extraction rate caused by higher CO_2 densities (Clifford and Clifford, 1999).

Figure 2B shows the response surface curve and its contour plot for the combined effects of pressure and CO_2 flow rate on the oil yield and their interaction at a fixed temperature of 70°C. The effect of the extraction pressure and flow rate showed a response that could typically be modelled using a quadratic Eq. (1) with pressure having a significant effect ($p<0.02$) on the oil yield while the CO_2 flow rate did not ($p = 0.20$) (Table 3). The interaction between the pressure and the CO_2 flow rate was also not significant ($p = 0.62$) (Table 3).

The model predicted that the oil yield would increase as the pressure was increased at the low end of values but that the effect would plateau at approximately 5,900psi (40,679 kPa) and that the oil yield would then decrease with further increases in pressure (Fig. 2B). At the low end of the pressure values, the model predicted that the oil yield would increase as the CO_2 flow rate was increased but that the effect would plateau at approximately 1.5 mL/min (Fig. 2B). However, at the median values for the pressure around 5,900psi (40,679kPa), the model predicted that the CO_2 flow rate would have little effect on the oil yield and that at the high end of the pressure values, the oil yield would decrease as the CO_2 flow rate was increased (Fig. 2B).

Figure 2C shows the response surface curve and its contour plot for the combined effects of temperature and CO_2 flow rate on the oil yield and their interaction at a fixed pressure of 6,000psi (41,369kPa). The effect showed a response that could typically be modelled using a quadratic Eq. (1) with temperature having a significant effect on the oil yield while the CO_2 flow rate did not (Table 3). In addition, the interaction between the temperature and the CO_2 flow rate was not significant ($p = 0.062$) (Table 3).

The model predicted that the oil yield would substantially increase as the temperature increased but that the effect would plateau at approximately 73°C and that the oil yield would then decrease with further increases in temperature (Fig. 2C). However, at the low and optimal values (~73°C) for the temperature, the model predicted that the CO_2 flow rate would have little effect on the oil yield and that at the high end of the temperature values, the oil yield would decrease as the CO_2 flow rate was increased (Fig. 2C).

From these three-dimensional response surface curves (Fig. 2A to 2C) and the analysis of variance values for the model (Table 3), it is evident that the extraction temperature was predicted to have the most substantial and significant effect on the oil yield during the SC-CO_2 extraction of the Gac seed kernel powder, followed by the extraction pressure. However, the predicted effect of the CO_2 flow rate was not significant (Table 3). Furthermore, there were no significant interactions between the extraction temperature with the pressure or the CO_2 flow rate but the interaction between the pressure and the CO_2 flow rate was significant (Table 3).

Optimization of supercritical carbon dioxide extraction of Gac seed oil: Based on the predictive model in Eq. (1), the response surface curves (Fig. 2) and the predictive plots shown in Fig. 3, the optimum conditions for the extraction of the oil from the Gac seed kernel powder were determined to be: temperature = 73°C, pressure = 5,900psi (40,679kPa) and CO_2 flow rate = 1.5mL/min. Under these conditions, the maximum predicted oil yield was 34.1±0.8% (g oil/100 g Gac seed kernel powder).

To validate the optimum conditions predicted by the model, three Gac seed kernel powder samples (4 g) were extracted by SC-CO_2 for 32 min at 73°C, 5,900psi (40,679kPa) and 1.5 mL/min. The result showed that the experimental value for the oil yield was 33.9±0.5%, which was not significantly different ($p = 0.72$) from the predicted value of 34.1±0.8%.

Physical and chemical properties of the Gac seed oil: The Gac seed oil, extracted using the optimum SC-CO_2 extraction conditions, was light green yellow in colour

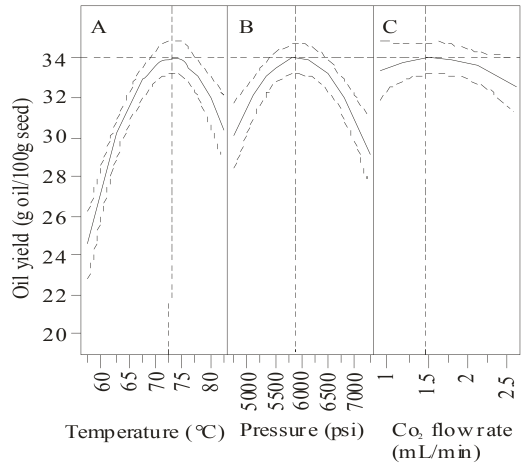

Fig. 3: Determination of the optimum values for temperature (A, 73°C), pressure (B, 5,900psi) and CO_2 flow rate (C, 1.5 mL/min).

as observed visually when it eluted in the liquid state from the extraction system. However, the oil was solid at room temperature and had a slip melting point of 24.8±0.2°C. The specific gravity of the oil was 0.90±0.01 and its refractive index was 1.45±0.01. Its free fatty acid content, expressed in terms of acid value, was 1.47±0.02 mgKOH/g oil, a value that is within the allowable limits for edible oils (CODEX, 1999).

The peroxide value of the oil was very low at 0.12±0.01meqO_2/kg oil, which showed that oxidization was largely avoided during the course of the SC-CO_2 extraction. The moisture and volatile matter was also very low at 0.08±0.01% and insoluble impurities were not detectable in the oil. These results also demonstrated the advantage of the SC-CO_2 extraction over conventional extraction methods. The low peroxide and moisture values also suggest that the SC-CO_2 extracted Gac seed oil may be able to be stored for long periods without deterioration.

The saponification value was 189.4±3.1 mgKOH/g oil and its content of unsaponifiable matter was relatively high at 33.2±1.1%. Therefore, relatively high amounts of non-glyceride fat-soluble matter was extracted from the Gac seed komel powder under the conditions used for the SC-CO_2 extraction. This is in agreement with the findings of Akihisa *et al*. (1986, 1988), which revealed that there are high percentages of sterols and triterpene alcohols in the Gac seed; these compounds may well be extracted by the SC-CO_2 process. Another study by Kan *et al*. (2006) also reported the presence of potentially important bioactive compounds, such as karounidiol, β-sitosterol, pentacyclic triterpene and their derivatives, as constituents of the unsaponifiable matter of Gac seed oil.

The iodine value of the oil was 55.2±1.7 g I_2/100 g oil, which indicated that the oil was likely to be high in saturated fatty acids as it had an iodine value similar to that of palm oil (Haryati *et al*., 1997), which is high in the saturated fatty acid, palmitic acid. However, unlike palm oil, the Gac seed oil is more likely to primarily contain stearic acid as shown previously (Ishida *et al*., 2004). Therefore, in comparison to palm oil, Gac seed oil, if eaten, is less likely to contribute to the buildup of LDL cholesterol in humans (Grundy, 1994). However, like palm oil, its low iodine value further adds to its likely stability by making it a non-drying oil, an oil which is not likely to harden on exposure to air. Also, the high content of unsaponifiable matter is likely to have contributed to its low iodine value.

CONCLUSION

The RSM was successfully applied to the SC-CO_2 parameters to optimize the extraction of oil from Gac seed kernel powder. A statistically significant multiple regression relationship between the independent variables of the SC-CO_2 extraction (temperature, pressure and CO_2 flow rate) and the response variable (oil yield) was established using a second order polynomial model to represent the relationship among the selected parameters.

The model, the response surface plots and the analysis of variance indicated that two of the three SC-CO_2 parameters, the temperature and pressure but not the CO_2 flow rate, significantly and mainly independently influenced the oil yield. The optimum process parameters were predicted to be: a temperature of 73°C, a pressure of 5,900psi (40,679 kPa) and a CO_2 flow rate of 1.5 mL/min. Under these conditions, the maximum predicted oil yield was 34.1±0.8% (g oil/100 g Gac seed kernel powder). The adequacy of the predictive model was verified by validation experiments, which showed that the experimental values agreed with the predicted values for the oil yield under these optimum SC-CO_2 extraction conditions.

The extracted oil was likely to be high in saturated fatty acids because it was solid at room temperature and had a low iodine value. It also had a high percentage of unsaponifiable matter. Therefore, it would most likely need to be further refined to remove this matter before it can be used as an edible oil. Alternatively, the oil can be further analyzed to confirm the presence of bioactive compounds, which may make it useful as a medicinal oil.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this study.

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