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Fatty acid profile and antioxidant properties of oils extracted from *dabai* pulp using supercritical carbon dioxide extraction

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Abstract

The present work was aimed to optimise the supercritical carbon dioxide extraction of *dabai* oil from *dabai* pulp and defatted pulp using RSM in comparison with hexane extraction method. Fatty acid profile and antioxidant properties of the extracted *dabai* oil were also determined. Optimal RSM conditions were 36 MPa pressure, 40°C temperature and 21 min of extraction time. The extracted yields ranged between 14.13% and 15.42%. Supercritical carbon dioxide extracted oils had lower total phenolics, total flavonoids, and antioxidative activities than the hexane-extracted oils. High-performance liquid chromatography results showed that only vanillic, protocatechuic and gallic acids were identified in both hexane and supercritical carbon dioxide extracted oils of defatted *dabai* pulp. The total saturated fatty acids were higher in hexane-extracted *dabai* oil as compared to the supercritical carbon dioxide extracted oil, and vice versa for the unsaturated fatty acid. Although supercritical carbon dioxide extracted *dabai* oils are inferior to the hexane-extracted oils, they are preferred as they are extracted using green technology.

Introduction

Malaysia is known for its rich source of seasonal indigenous fruits, one of which is '*dabai*' fruit that is available only in a few districts in the state of Sarawak. The fruit is scientifically called *Canarium odontophyllum* Miq. *Dabai* is a seasonal fruit and often compared to olives due to their similarity in appearance (Chew *et al.*, 2012). Ripe *dabai* fruit is dark purple in colour. The fruit has an average weight of around 10.0 - 18.0 g and measures about 3.0 - 4.0 cm in length and 2.2 - 3.0 cm in diameter. *Dabai* fruits are also ovoid drupes containing a sub-triangular, three-chambered seed. The endocarp layer is rather thick about 2.5 - 3.5 cm in length and 1.6 - 2.0 cm in diameter, while the yellow fleshy pulp is 3.0 - 5.0 mm in thickness. The skin and flesh of *dabai* are edible, but its seed is usually discarded.

Dabai is a fruit that contains a high amount of antioxidants. Previous studies have proven that besides other health benefits; *dabai* fruits have a considerable amount of antioxidants such as phenolics, flavonoids, anthocyanidins and carotenoids (Prasad *et al.*, 2011; Chew *et al.*, 2011; 2012). The fatty acid profile of hexane-extracted *dabai* pulp oil has been reported by Azlan *et al.* (2010), where the oil has about 44.4% of total saturated fatty acids, 42.8% of total monounsaturated fatty acids and 12.8% of total polyunsaturated fatty acids. The pulp oil also showed a protective effect in healthy experimental rabbits (Shakirin *et al.*, 2012). Moreover, the supercritical carbon dioxide (SCCO2) extracted *dabai* pulp oil had a cholesterol-lowering effect in hypercholesterolemia-induced SPF Sprague-Dawley rats (Kadir *et al.*, 2018).

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There are several methods that can be used for the purpose of extracting plant materials. The typically used methods involve liquid extraction using water and organic solvents by applying techniques like maceration, percolation and Soxhlet extraction (Khajeh, 2011). In recent years, however, the supercritical fluid extraction method has gained attention and has proven itself to be a promising alternative method in extracting oils and other materials. $SCCO_2$ gives many advantages as it is environmentally friendly, safe, non-carcinogenic, non-flammable and non-toxic with modest critical conditions and quite affordable for its quality (Ghafoor *et al.*, 2012). Moreover, its selectivity can be easily modified by adjusting the pressure and temperature to get fractions of desirable amounts, and it can be used in a wide range of biochemical and chemical extraction processes.

Many factors can affect the efficiency of the $SCCO_2$ extraction such as flow rate, extraction time, temperature and pressure. Therefore, in situations where multiple variables have an effect on the output, the response surface methodology (RSM) is used to optimise the process (Xu *et al.*, 2008). RSM has a big advantage which allows estimation of the effects of more than one variable and their interactions on the output variables with fewer trials as compared with the one-factor-a-time approach (Liyana-Pathirana and Shahidi, 2005). The present work aimed to optimise the supercritical fluid extraction of *dabai* pulp by determining the influence of extraction parameters on the yield and antioxidative properties of the extracted oils. These extraction parameters are temperature, time and pressure. A comparison was made between *dabai* pulp oil and defatted pulp oil extracted using both $SCCO_2$ and hexane.

Materials and methods

Materials

About 20 kg of matured *dabai* fruits were supplied by the Semongok Agriculture Research Centre, Sarawak, Malaysia. The *dabai* fruits used for this study were a mixture of two superior clones; namely, Laja and Lulong which can be mainly found in Sarikei, Sarawak. The maturity of the *dabai* fruits used was determined by officers of the Sarawak Department of Agriculture, Malaysia. All fresh ripe fruits were stored in polystyrene boxes and shipped by flight to Universiti Putra Malaysia a day after harvest.

Methanol, sodium acetate trihydrate, ferric chloride and acetonitrile were purchased from Merck (Petaling Jaya, Malaysia). Aluminium chloride, rutin, sodium nitrite, Folin-Ciocalteu reagent, gallic acid, DPPH, potassium hydroxide, and protocatechuic acid were purchased from Sigma-Aldrich (USA). Sodium carbonate, sodium bicarbonate, sodium hydroxide, hydrochloric acid, and petroleum ether were purchased from R&M Chemicals (Semenyih, Malaysia). Ethanol was purchased from Systerm (Shah Alam, Malaysia). Glacial acetic acid and hexane were purchased from Fisher Scientific (Shah Alam, Malaysia). Acetic acid was purchased from HmbG Chemicals (Germany). Carbon dioxide gas with a high purity of 99.99% and nitrogen gas were used in GC analysis.

Sample preparation

The frozen *dabai* fruits, previously stored in -80°C, were defrosted at room temperature for 1 h in the laboratory. Later, the fruit samples were prepared by manually removing the seed. The pulp with the peel attached was then mashed together, kept in plastic bags before the bags were wrapped with aluminium foil. All fruit samples were stored at -80°C freezer before freeze-drying. The samples were freeze-dried, and the freeze-dried samples were powdered using a blender. The sample was sieved, kept in an airtight container and stored at -80°C before the extraction process.

$SCCO_2$ extraction

$SCCO_2$ extraction was performed with three independent variables, extraction time (30 - 60 min), pressure (20 - 50 MPa) and temperature (40 - 60°C). The extraction was done using SFT-100 supercritical fluid extractor (Supercritical Fluid Technologies Inc., Cambridge, UK). These values were selected by referring to the studies related to the extraction of olives (Vázquez *et al.*, 2009; Al-Otoom *et al.*, 2014). Response surface methodology (RSM) was used for the optimisation of the extraction process, whereby central composite design (CCD) was chosen with three factors and two blocks. The cartridges were filled with approximately 8.0 g of *dabai* pulp powder. The extraction temperature and pressure were controlled and maintained during the entire process of extraction once the required values were set according to the experimental design. A syringe pump was used to supply pure CO_2 (99.9%) into the system. The CO_2 flow rate used for the system was 2.0 mL/min. The oil extracted was collected in methanol and later evaporated under nitrogen at room temperature. The extraction produced *dabai* oil and defatted *dabai* pulp. The defatted *dabai* pulp was re-extracted using $SCCO_2$ for collecting the remaining oil residue. The collected *dabai* oil was stored in -20°C before further analysis.

Solvent extraction

Approximately 8 g of *dabai* pulp powder was weighed in a conical flask and hexane was added at a ratio of 1:10 (pulp:hexane, w/v). The flask was wrapped with aluminium foil and left on the shaker overnight (24 h). The mixture was filtered using Whatman No. 1 filter paper, and the hexane collected in the filtrate was fully removed using a rotary evaporator with the water bath temperature set at 20°C. The same volume of fresh hexane was then added to the solid residue. The re-extraction of oil was repeated three times using the same solid to solvent ratio. Hexane was removed for the final time, and the oil collected was then re-filtered. The oil obtained was finally stored in -20°C until further analysis.

The by-product of $SCCO_2$ extraction of *dabai* pulp is defatted *dabai* pulp. Before extraction of oil from the defatted *dabai* pulp using hexane, the defatted pulp was first oven dried at 40°C for 48 h. Briefly, 1 g of defatted *dabai* powder was added to 10 mL of 62.25% methanol following an optimised method by Khoo *et al.* (2012). All extracts were stored at -20°C before further analysis.

Estimation of total phenolic content

Total phenolic content (TPC) of the samples was estimated following the method described by Ismail *et al.* (2013). The result was calculated based on a gallic acid standard calibration curve and expressed as mg of GAE per g of the oil extract.

Determination of total flavonoid content

Total flavonoid content (TFC) of the samples was analysed following a method described by Hossain *et al.* (2011) based on the aluminium chloride colorimetric method with minor modifications. Briefly, 0.5 mL of oil was mixed with 0.1 mL of 10% aluminium chloride, 0.1 mL of potassium acetate (1 M) and 4.3 mL of distilled water. The result was expressed as mg quercetin equivalent of the sample (mg QE/g sample).

DPPH radical scavenging activity

The method used to determine the DPPH free radical scavenging activity of *dabai* pulp oil was based on the method described by Ghafoor *et al.* (2012). Antiradical activity (%) of the extract was calculated based on Equation 1:

DPPH radical scavenging activity, DRSA (%) = [(1–Absorbance of sample)/Absorbance of control] × 100 Equation 1)

Determination of fatty acid composition

Fatty acid methyl esters (FAMEs) of the oil samples were prepared following the method described by Azlan *et al.* (2010). The mixture was then centrifuged at 402 g for 10 min. Fatty acid composition of the oil samples was analysed using a GC system equipped with a split-splitless injector. Based on the reported GC conditions, flame ionisation detection (FID) system was used to separate and quantify each FAME component. FAMEs were separated using DB-23 column (60 m × 0.25 mm, i.d. 0.15 mm). Oven temperature was held at 50°C for 1 min, then increased to 175°C at 4°C/min, and later increased to 230°C and held for 5 min. The temperatures of injector and detector were set at 250°C and 280°C, respectively.

Oil sample (1 µL) was injected with a split ratio of 1:50. Helium (1 mL/min) was used as carrier gas and controlled at 103.4 kPa, while hydrogen and air were used for FID and were held at 275.6 kPa. FAME standards were prepared and used as external standards for the identification and quantification of fatty acids. Identification of fatty acids in the oil samples was performed by comparing the retention times of reference standards, and all samples were analysed under the same operating condition as those employed for the FAME standards.

High-performance liquid chromatography analysis

Following the reported high-performance liquid chromatography (HPLC) method (Mokiran *et al.*, 2014), methanolic extract of defatted *dabai* pulp oil was filtered using 0.45 µm membrane filter before being injected into the HPLC system. Phenolic acids in the samples were quantified using an Agilent 1100 HPLC system equipped with a diode array detector (Agilent Technologies, Germany). The stationary phase used was a Lichrospher C_{18} column (250 × 4 mm, i.d. 5 µm) from Merck KGaA (Darmstadt, Germany).

Analysis of phenolic acid was performed following the method described by He and Xia (2007). Mobile phases used consisted of 0.5% acetic acid (v/v) (A) and methanol (B). The gradient elution profile applied was: 0 - 20 min, linear gradient from 0% to 90% B; 20 - 25 min, 90% B isocratic; 25 - 30 min, linear gradient from 90% to 0% B. The column temperature and flow rate were set at 30°C and 0.6 mL/min, respectively. The phenolic compounds were identified by comparing the UV-Vis absorption spectra and the retention time with the phenolic standards.

Table 1. Response surface design and experimental values for the response variables

Run Order	Block	Pressure (MPa)	Temperature (°C)	Time (min)	Yield (g)	Yield (%)	TPC (mg GAE/g oil)
1	1	20.00	40.00	30.000	0.64	8.00	3.841
2	1	50.00	40.00	60.000	1.12	14.00	3.687
3	1	50.00	60.00	30.000	0.78	9.75	3.687
4	1	50.00	60.00	60.000	1.81	22.63	4.228
5	1	35.00	50.00	45.000	0.83	10.38	3.648
6	1	35.00	50.00	45.000	1.20	15.00	3.919
7	1	35.00	50.00	45.000	0.88	11.00	4.074
8	1	35.00	50.00	45.000	1.35	16.88	3.919
9	1	50.00	40.00	30.000	0.76	9.5	3.841
10	1	20.00	60.00	30.000	0.47	5.88	4.576
11	1	20.00	40.00	60.000	0.36	4.50	4.770
12	1	20.00	60.00	60.000	0.23	2.88	4.770
13	2	35.00	50.00	45.000	1.46	18.25	3.648
14	2	35.00	50.00	20.505	1.41	17.63	3.725
15	2	35.00	50.00	69.495	1.28	16.00	3.725
16	2	10.50	50.00	45.000	0.13	1.63	0.000
17	2	35.00	33.67	45.000	1.30	16.25	3.532
18	2	59.49	50.00	45.000	1.95	24.38	3.803
19	2	35.00	50.00	45.000	1.05	13.13	4.383
20	2	35.00	66.33	45.000	0.82	10.25	3.803

Statistical analysis

All values of oil yield and TPC were expressed as means ± standard error of the means. Response surface model was designed, and statistical analysis was performed using the Minitab version 15 (Minitab Inc., PA, USA). Theoretical validation of the model was performed by using 2-sample *t*-test between theoretical and practical values of the predicted dependent variable at $p < 0.05$.

Results and discussion

Optimisation of $SCCO_2$ extraction

Response surface methodology (RSM) was applied in the optimisation study for extracting *dabai* oil using $SCCO_2$ extractor. Based on the central composite design, the RSM generated 20 extraction conditions to be applied for extracting *dabai* oil from the fruit samples. Three factors that were analysed were pressure (MPa), temperature (°C) and time (min). The yield percentage (%) and total phenolic acid (mg GAE/g oil) of the oil samples under each extraction condition are tabulated in Table 1.

Effect of $SCCO_2$ parameters on oil yield and TPC

Based on Table 1, the five highest yields (%) were achieved at run order 18 (highest), 4, 13, 14 and 8, whereas the lowest yield was obtained from run order 16. The two highest yields were obtained from the extraction condition using 50 MPa and 59.49 MPa which were the two highest pressures used. Meanwhile, looking at the trend in terms of extraction temperature, all five highest oil yields were extracted using temperature of 50°C. According to He *et al.* (2007), temperature and pressure are the two most important attributes for $SCCO_2$ extraction. In fact, pressure has the highest impact on the properties of the supercritical fluid in terms of density or hydrogen bond intensity.

The five highest TPC results were obtained from the run order 11 (highest), 12 (highest), 10, 19 and 4, whereas the lowest TPC was obtained from the run order 16. As observed from Table 1, run orders 11 and 12 produced the *dabai* oil with the highest phenolic content which was both 4.77 mg GAE/g oil. Both *dabai* pulp and defatted *dabai* pulp were extracted using the same optimal extraction conditions for pressure and time (20 MPa and 60 min), but only the temperatures differed (40°C and 60°C, respectively). There was no particular trend observed for the extraction temperature and extraction time affecting TPC for these five highest values. However, a trend was observed in terms of the extraction pressure; whereby out of the five different pressures used (10.50, 20, 35, 50 and 59.49 MPa) and applying extraction pressure of 20 MPa

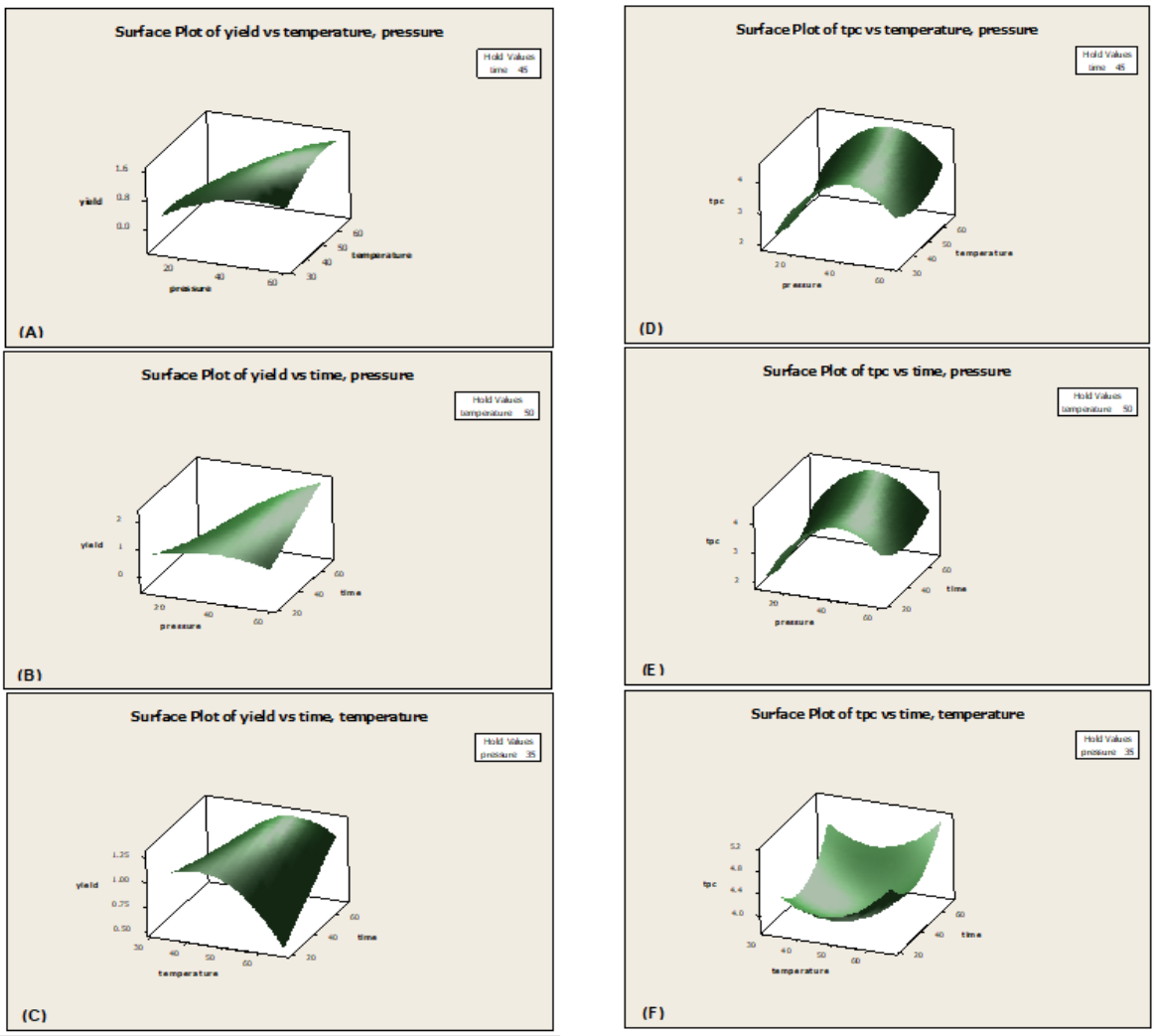

Figure 1. Surface plot of: (A) yield against pressure and temperature; (B) yield against time and pressure; (C) yield against temperature and time; (D) TPC against pressure and temperature; (E) TPC against time and pressure and (F) TPC against temperature and time.

resulted in a higher TPC for the *dabai* pulp oil. Some samples had specific extraction pressures, by which increasing pressure did not improve the recovery of TPC (Esquível *et al.*, 1999).

Analysis of response surface and effect of process variables

Surface plots were plotted for yield and TPC against the factors studied (pressure, temperature, and time). As can be seen in Figure 1A, as the temperature gradually rose from 30°C up to 60°C, so did the extraction pressure increase the extraction yield. Wang *et al.* (2012) reported that at a given temperature, the oil yield significantly increased with increasing temperature, and the effect can be clearly seen at the lower temperatures and pressures. When the pressure is raised, solubility of the $SCCO_2$ increases, which in turn results in its enhanced solubility.

Figure 1B shows the surface plot of yield against time and pressure at a constant temperature of 50°C. Based on the plot, the time factor undeniably had a positive linear effect on the yield. However, the effect of pressure varied. The increase in pressure managed to increase the yield up to a certain point; but after that, the yield dropped when the pressure was further increased.

Figure 1C shows the surface plot of yield against temperature and time at a constant pressure of 35 MPa. The time factor showed a positive linear effect on oil yield as the extraction time increased from 20 min to 60 min in higher temperature conditions. At lower temperatures, extraction time did not affect the extraction yield. Looking into the other parameter, increasing the temperature was found to reduce the yield of oil. However, as extraction time increased, the reduction in oil yield respective to the temperature became lower.

Figure 1D shows the surface plot of TPC against pressure and temperature at a constant extraction time of 45 min. There were two distinguished trends observed in the TPC as the temperature increased. The pressure caused a rise in TPC as it increased from 20 MPa to 40 MPa; thereafter, higher pressure only reduced TPC. At the constant extraction time of 45 min, the TPC showed an increase but only at a small rate starting from 30°C to around 50°C. Further increase in temperature continued to increase the TPC with the maximum content observed at 60°C.

Figure 1E shows the surface plot of TPC against time and pressure at a constant temperature of 50°C. Based on the figure, it can be seen that an increase in pressure led to an increase in TPC until 40 MPa. The pressure level higher than 40 MPa reduced the TPC of samples. Meanwhile, the time factor did not show any major difference in the TPC obtained.

The final surface plot, as seen in Figure 1F, was of TPC against temperature and time at a constant pressure of 35 MPa. Starting with the lower temperatures, TPC was seen to drop a bit. After a certain point, a rise in temperature of up to 60°C was able to increase TPC. It was also observed that the longer the extraction time, the more TPC was extracted.

Esquível *et al.* (1999) revealed that certain samples have their suitable extraction pressures; raising the pressure will not provide any improvement in the recovery of antioxidants. This explains the trends observed in Figure 1B, whereby the increase in pressure only increased the yield to a certain point. The time factor showed a positive linear effect on oil yield as seen in Figure 1B and Figure 1C, but at lower temperatures, the effect of time did not show a major effect on the oil yield.

Wang *et al.* (2012) mentioned that the influence of extraction temperature on $SCCO_2$ extraction is not easy to be determined when compared with extraction pressure because the increasing temperature can alter the oil yield. As the temperature increases, CO_2 density will decrease, which causes a reduction in solvent power. Conversely, a rise in temperature also increases the solute vapour pressure, which enhances $SCCO_2$ solubility. Therefore, the solubility of $SCCO_2$ can either increase, remain constant or decrease with a rise in temperature at constant pressure and all these depend on whether the solute vapour pressure or solvent density dominates (Thana *et al.*, 2008; Wei *et al.*, 2009). However, it is not advisable to use high pressure as this may induce a negative quadratic effect.

The increase in extraction temperature damages the particle cell walls, which leads to increased mass transfer rate. This is how it causes an increase in the availability of extractable bioactive compounds (Machmudah *et al.*, 2006). However, a further increase in temperature will not help in most cases as it may result in lower recovery of bioactives in the sample due to the vaporisation or decomposition of volatiles that happens at higher temperatures (Cacace and Mazza, 2003) and because of the lower density of CO_2 (Maran *et al.*, 2014).

Fitting the response surface models

In the present work, there were two end variables being studied; oil yield and TPC. The oil yield was estimated by the second-order polynomial equation as follows:

$$Y = (-0.024)X_1 + (0.054)X_2 + (-0.061)X_3 + (-0.001)X_1^2 + (-0.001)X_2^2 + (-0.000)X_3^2 + (0.001)X_1X_2 + (0.001)X_1X_3 + (0.001)X_2X_3 - 1.100$$

(Equation 2)

Based on Equation 2, Y represents oil yield of the applied pressure (X_1), temperature (X_2) and extraction time (X_3). The R^2 value for this equation was 0.852. This model can be used to predict the yield percentage of *dabai* oil extracted using $SCCO_2$ with high accuracy.

On the other hand, the TPC was also estimated by the second order polynomial, as shown in the following equation:

$$Y = (-0.203)X_1 + (-0.150)X_2 + (-0.054)X_3 + (-0.002)X_1^2 + (0.002)X_2^2 + (0.001)X_3^2 + (-0.000)X_1X_2 + (-0.000)X_1X_3 + (-0.000)X_2X_3 + 4.415$$

(Equation 3)

Based on the equation, Y represents TPC of the applied pressure (X_1), temperature (X_2) and extraction time (X_3). The R^2 value for this equation was 0.482.

The optimum condition, which was predicted to provide the best yield percentage and TPC, was then generated using Minitab 16. The generated condition for the best extraction of *dabai* oil using $SCCO_2$ was extraction pressure of 36 MPa, extraction temperature of 40°C and extraction time of 21 min. The targeted yield percentage and TPC for the optimum condition were 1.14% and 4.18 mg GAE/g oil, respectively.

The RSM was verified by comparing the experimental values of extraction yield and TPC to the predicted values obtained from the optimised model. The comparison was performed based on 2-sample *t*-test. *Dabai* oil extraction was performed based on five different experiments by applying the optimised extraction conditions. The mean value of oil yield obtained was 1.11 ± 0.23%, and the mean value of TPC was 7.84 ± 0.47 mg GAE/g oil. The result showed that there was no significant difference

between experimental and predicted values for oil yield at $p \geq 0.05$. However, there was a significant difference between experimental and predicted values of TPC ($p < 0.05$). The findings confirmed that the model fit the experimental design.

Extraction yield

In optimised extraction conditions, the result showed that the extraction yields (%) for *dabai* oil extracted with $SCCO_2$ and hexane were 14.13 ± 0.88% and 15.42 ± 2.75%, respectively. Independent sample t-test was used to determine the difference between the two samples. The results of the t-test indicated that there was no significant difference between the two means ($p \geq 0.05$).

Unlike previous studies, the $SCCO_2$ extracted *dabai* oil had a higher yield than hexane-extracted oil. For instance, Nisha *et al.* (2012) reported that more oil was yielded from the organic solvent extraction method than the $SCCO_2$ method during the extraction of Mortierella alpina oil. $SCCO_2$ can only extract neutral lipids from lipid mixture and a co-solvent must be used to extract phospholipids (Catchpole *et al.*, 2009). On the other hand, lipid-soluble materials along with the triacylglycerols are soluble in hexane and therefore higher lipid yield was obtained when hexane was used as the solvent (Nisha *et al.*, 2012).

Table 2. TPC, TFC, DPPH (%) and selected phenolic acids of dabai pulp and defatted dabai pulp oils.

Antioxidant parameters	Hexane	$SCCO_2$
Dabai pulp oil		
TPC (mg GAE/g oil)	8.64 ± 1.19[a]	7.89 ± 0.95[a]
TFC (mg QE/g oil)	3.56 ± 0.05[a]	1.58 ± 0.03[b]
DPPH (% DRSA)	46.01 ± 0.08[a]	41.14 ± 0.10[b]
Defatted dabai pulp oil		
TPC (mg GAE/g DW)	23.32 ± 1.32[a]	5.58 ± 0.03[b]
TFC (mg QE/g DW)	1.49 ± 0.08[a]	1.24 ± 0.13[a]
DPPH (% DRSA)	84.72 ± 0.24[a]	77.49 ± 1.99[b]
Vanillic acid (mg/g DW)	0.11 ± 0.13[a]	0.10 ± 0.00[a]
Protocatechuic acid (mg/g DW)	0.17 ± 0.00[a]	0.16 ± 0.00[b]
Gallic acid (mg/g DW)	0.14 ± 0.00[a]	0.14 ± 0.00[a]

*Different superscript lowercase letters indicate a significant difference between hexane and supercritical carbon dioxide ($SCCO_2$) extractions. GAE: gallic acid equivalent; QE: quercetin equivalent; DRSA: DPPH radical scavenging activity; DW: dry weight.

Antioxidant properties

Table 2 shows the TFC, TPC and DPPH radical scavenging activity of *dabai* pulp and defatted *dabai* oils obtained from both extraction methods studied. TPC of the defatted *dabai* pulp oil extracted by hexane (23.32 ± 1.32 mg GAE/g DW) was significantly higher than the SCCO2 extracted oil (5.58 ± 0.03 mg GAE/g DW). However, TPC of the *dabai* pulp oil extracted by hexane was 8.64 ± 1.19mg GAE/g DW and by $SCCO_2$ was 7.89 ± 0.95 mg GAE/g DW. No significant difference was observed between the TPC of *dabai* pulp oils extracted by hexane and $SCCO_2$ at $p \geq 0.05$.

TFCs of the defatted *dabai* pulp oil extracted by hexane and $SCCO_2$ were 1.49 ± 0.08 and 1.24 ± 0.13 mg QE/g DW; these TFCs in the two extraction methods were not significantly different at $p \geq 0.05$. However, for the *dabai* oil samples, the TFC of the oils extracted by hexane (3.56 ± 0.05 mg QE/g oil) was doubled in comparison with the amount of the oil extracted with $SCCO_2$ (1.58 ± 0.03 mg QE/g oil). A significant difference was observed for the TFC between these two extraction methods ($p < 0.05$).

$SCCO_2$ extraction of oil has its limitation. As the phenolic compounds obtained from the hexane-extracted oil of *dabai* pulp were not significantly higher than those in the $SCCO_2$ extracted oil, it was concluded that the amounts of polyphenols in the pulp oil extracted by both $SCCO_2$ and hexane were similar. On the other hand, the TPC in the oil of defatted *dabai* pulp extracted from hexane was far higher than that in the $SCCO_2$ extracted oil.

Due to limited studies in this area, we can only hypothesise that the oil extracted by $SCCO_2$ contained a low amount of non-polar antioxidants as determined by Folin-Ciocalteu reagent method. On the contrary, Koubaa *et al.* (2016) reported that the TPC in the cherry seed oil extracted with $SCCO_2$ was significantly higher than the TPC in the oil extracted by hexane. The result obtained might be due to the fact that the cherry seed oil contains phytochemicals which have a high solubility in CO_2 when compared with organic components extracted from defatted pulp oil.

The determination of TPC in the defatted *dabai* pulp oil has never been performed. Therefore, the present work demonstrated the first attempt to study the phenolic compounds in defatted *dabai* pulp oil. Moreover, $SCCO_2$ extraction is not a good way in extracting oil and non-polar antioxidants as compared to hexane. As reported by Wajs-Bonikowska *et al.* (2017), blackberry extracted by hexane had higher extraction yield and phytochemicals than the $SCCO_2$ extracted oil. Furthermore, the defatted pulp collected after $SCCO_2$ extraction still contained a remarkable amount of unextracted oil. In contrast, $SCCO_2$ has its unique properties, such as selectivity and non-toxicity, which are not observed in hexane (Phillips and Robey, 1989).

For DPPH assay, the results were expressed as percentages of DRSA of the oil samples. The defatted *dabai* pulp oil showed a high value, whereby the oil obtained from hexane and $SCCO_2$ extractions had DRSA values of 84.72 ± 0.24% and 77.49 ± 1.99%, respectively. Independent sample *t*-test showed that there was a significant difference between the mean values of these two samples ($p < 0.05$). On the other hand, the percentage of DRSA for the *dabai* pulp oil was much lower than that of the defatted *dabai* pulp oil. When compared with the defatted pulp sample, the result showed that it was almost half of the DRSA. The hexane and $SCCO_2$ extracted *dabai* pulp oils had DRSA values of 46.01 ± 0.08% and 41.14 ± 0.10%, respectively. A significant difference was found for the percentage of DRSA between the oils extracted by hexane and $SCCO_2$ ($p < 0.05$). $SCCO_2$ method has many disadvantages, whereby polar compounds are difficult to be extracted using $SCCO_2$ (Laroze *et al.*, 2010). Non-polar and semi-polar compounds can easily be dissolved in CO_2 (Kiran *et al.*, 2012). This is because the solvent power of the compound is high. The power will also reduce with an increase in molecular weight of the compound. Supercritical fluids such as CO_2 can also result in less volatile, higher molecular weight and higher polarity compounds if the pressure increases.

Determination of phenolic acids in defatted dabai pulp oil

Figure 2 shows the HPLC chromatogram of the defatted *dabai* pulp oil obtained from hexane and $SCCO_2$ extraction methods. Detection at 280 nm gave the optimal peak height and resolutions when compared with the other wavelengths tested. As shown in Table 2, the concentrations of vanillic acid, protocatechuic acid and gallic acid of the defatted *dabai* pulp oil extracted with hexane were 0.11 ± 0.13, 0.17 ± 0.00 and 0.14 ± 0.00 mg/g DW, respectively. The results also show that the concentrations of vanillic acid, protocatechuic acid and gallic acid in the defatted pulp oil collected from $SCCO_2$ extraction were 0.10 ± 0.00, 0.16 ± 0.00 and 0.14 ± 0.00 mg/g DW, respectively.

Based on the results obtained from the independent sample t-test analysis, the concentrations of gallic acid and vanillic acid showed no significant differences between the defatted *dabai* pulp oil extracted from both extraction methods. On the contrary, a significant difference was observed in the protocatechuic acid content between the defatted *dabai* pulp oil extracted from the two extraction methods ($p < 0.05$). Although both protocatechuic acid and vanillic acid share the same structure of

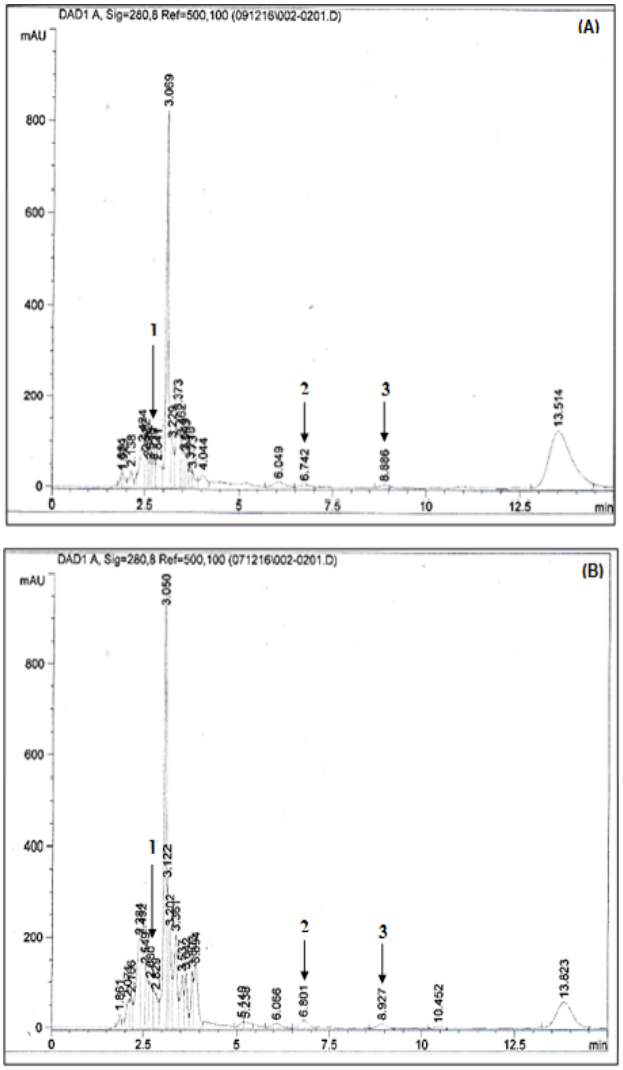

Figure 2. HPLC chromatograms of defatted pulp from (A) hexane and (B) supercritical carbon dioxide ($SCCO_2$) extraction.

dihydroxybenzoic acid, vanillic acid is formed from the methylation of protocatechuic acid (Greathouse and Rigler, 1940). The non-methylated structure of protocatechuic acid makes it less soluble in hexane.

A previous study by Chew *et al.* (2012) investigated the phenolics content of *dabai* fruits from four different divisions, namely Kanowit, Kapit, Song and Sarikei. The studied *dabai* fruit (whole fruit) contained phenolic acids of catechin, epigallocatechin gallate, epicatechin, epicatechin gallate, apigenin, ellagic acid, vanillic acid and ethyl gallate. Only one was similar to the present work which was vanillic acid. Khoo *et al.* (2012) studied the phenolics content of defatted *dabai* pulp and peel extracts from different extraction media. The results obtained in the present work are similar to the findings reported by Chew *et al.* (2012), where the phenolic acids found were ellagic acid, epigallocatechin, catechin, epicatechin, epicatechin gallate, vanillic acid, apigenin, protocatechuic acid and methyl

Table 3. Fatty acid composition of dabai pulp oils.

Common name	Carbon chain	Concentration*	
		Hexane	$SCCO_2$
Butyric acid	4:0	0.13 ± 0.02a	0.11 ± 0.00a
Caproic acid	6:0	0.76 ± 0.01a	0.03 ± 0.00b
Caprylic acid	8:0	0.04 ± 0.02a	0.02 ± 0.00a
Capric acid	10:0	0.02 ± 0.00a	0.01 ± 0.00b
Undecylic acid	11:0	ND	ND
Lauric acid	12:0	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}
Tridecylic acid	13:0	0.03 ± 0.00^{a}	1.16 ± 0.04^{b}
Myristic acid	14:0	0.02 ± 0.00^{a}	0.03 ± 0.00^{b}
Myristolic acid	14:1 n-5	0.48 ± 0.01^{a}	0.02 ± 0.00^{b}
Pentadecylic acid	15:0	0.04 ± 0.00^{a}	0.01 ± 0.00^{b}
Pentadecenoic acid	15:1 n-1	0.11 ± 0.00^{a}	0.41 ± 0.02^{b}
Palmitic acid	16:0	0.02 ± 0.00^{a}	0.01 ± 0.00^{b}
Palmitoleic acid	16:1 n-7	0.31 ± 0.00^{a}	0.71 ± 0.03^{b}
Margaric acid	17:0	1.70 ± 0.02^{a}	0.08 ± 0.01^{b}
Heptadecenoic acid	17:1	0.41 ± 0.00^{a}	1.24 ± 0.05^{b}
Stearic acid	18:0	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}
Elaidic acid	18:1 n-9t	0.02 ± 0.00^{a}	0.01 ± 0.00^{b}
Oleic acid	18:1 n-9c	0.01 ± 0.00^{a}	0.06 ± 0.00^{b}
Linolelaidic acid	18:2 n-6t	0.66 ± 0.38	ND
Linoleic acid	18:2 n-6c	0.04 ± 0.00	ND ND
Arachidic acid	20:0	0.08 ± 0.00	ND
y-Linolenic acid	18:3 n-6	0.12 ± 0.00	ND
Eicosanoic acid	20:1 n-9	0.51 ± 0.00	ND
Linolenic acid	19:0	ND	ND
Heneicosylic acid	21:0	1.71 ± 0.02	ND
Eicosadienoic acid	20:2	0.37 ± 0.01	ND
Behenic acid	22:0	0.03 ± 0.00	ND
Dihomo-y-linolenic acid	20:3 n-6	ND	ND
Erucic acid	22:1 n9	0.05 ± 0.00	ND
Eicosatrienoic acid	20:3 n-3	ND	ND
Arachidonic acid	20:4 n-6	ND	ND
Tricosylic acid	23:0	0.22 ± 0.29	ND
Brassic acid	22:2	0.06 ± 0.01	ND
Lignoceric acid	24:0	ND	ND
Eicosapentaenoic acid (EPA)	20:5 n-3	0.07 ± 0.00	ND
Nervonic acid	24:1 n9	0.14 ± 0.06	ND
Docosahexaenoic acid (DHA)	22:6 n-3	ND	ND
Total saturated fatty acid		4.93 ± 0.59	1.60 ± 0.34
Total monounsaturated fatty acid	1.32 ± 0.25		ND
Total polymonounsaturated fatty acid	2.03 ± 0.20		2.45 ± 0.49

*The fatty acids concentration was expressed as mg/g oil; $SCCO_2$: supercritical carbon dioxide; ND: not detected.

gallate. Protocatechuic and vanillic acids were two of the phenolic acids also found in the present work. In addition to phenolic compounds, $SCCO_2$ extracted *dabai* oil also contained volatiles and other aromatic antioxidants (Khoo *et al.*, 2019).

Fatty acid profile of dabai pulp oil

The fatty acid content of *dabai* pulp oil samples extracted with both extraction methods was determined using gas chromatography (GC-FID). The fatty acid compositions and total fatty acid content of both oil samples extracted from hexane and $SCCO_2$ are shown in Table 3. The hexane-extracted *dabai* pulp oil (8.28 ± 0.44 mg/g oil) contained a higher fatty acid content than the $SCCO_2$ extracted oil (4.05 ± 0.41 mg/g oil). The results also showed that the percentage of SFA of the hexane-extracted oil was higher than that of the $SCCO_2$ extracted oil (39.51%). Meanwhile, the sum of UFA of the $SCCO_2$ extracted oil (60.49%) was far higher than that of the hexane-extracted oil (40.46%).

In general, the hexane-extracted *dabai* pulp oil contained higher fatty acid content than the $SCCO_2$ extracted *dabai* pulp oil. Pradhan *et al.* (2010) explained that using hexane as the solvent will extract most of the neutral lipids in a plant sample. Hence, the higher lipid yield might be attributed to the extracted neutral and polar lipids. Pradhan *et al.* (2010) obtained a higher yield of flaxseed oil from solvent (Soxhlet) method when compared with the $SCCO_2$ method. Similarly, the present work also obtained higher yield percentage for hexane-extracted *dabai* oil when compared with the $SCCO_2$ extracted oil and a higher number of fatty acids were detected in hexane-extracted *dabai* oil when compared with the $SCCO_2$ extracted oil.

Azlan *et al.* (2010) determined the fatty acid content of *dabai* pulp oil extracted using Soxhlet extraction. The percentages of total saturated, monounsaturated and polyunsaturated were 44.43%, 42.82% and 12.76%, respectively. The trend is nearly similar to the results obtained in the present work, whereby UFA percentage was the highest (59.54%), followed by MUFA (25.52%) and PUFA (15.94%). Despite the similar trend, the values differ between the two studies probably because the samples used were *dabai* fruits from different cultivars and also from different geographic regions of Sarawak.

Conclusion

The present work demonstrated that solvent extraction produced *dabai* pulp oil and defatted *dabai* pulp oil with higher values of TPC, TFC and DPPH radical scavenging activity. The extraction yield was lower in the hexane-extracted *dabai* pulp oil when compared with the $SCCO_2$ extracted oil. Although the overall results are not comparable with those of many other related studies, this might point out that $SCCO_2$ could not extract all non-polar antioxidants from *dabai* fruit as compared to hexane. The amounts of vanillic, protocatechuic and gallic acids in the defatted *dabai* pulp oil extracted by both extraction methods did not differ much. In terms of fatty acid composition, the present work found that the hexane-extracted *dabai* pulp oil showed higher fatty acid content than the $SCCO_2$ extracted oil. Also, the hexane-extracted *dabai* pulp oil produced a higher amount of SFA, whereas the $SCCO_2$ extracted *dabai* pulp oil had higher UFA than the hexane counterpart. The results indicated that the hexane extraction is superior in terms of fatty acids yield, but $SCCO_2$ is superior in terms of the favourable fatty acid composition.

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