

Fatty acid profiles and antioxidant properties of dabai oil

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Received 9 Jun 2016

Accepted 4 Sep 2017

ABSTRACT: Supercritical CO₂ (SC-CO₂) was used to extract dabai oil. Total phenolic content, total flavonoid content, DPPH radical scavenging assay, and gas chromatography analysis were performed to determine the antioxidant properties of SC-CO₂-extracted dabai oil and to compare with hexane-extracted dabai oil. The total phenolic content of SC-CO₂-extracted oil was slightly higher than hexane-extracted oil, whereas the total flavonoid content of SC-CO₂-extracted dabai oil was four times lower than the hexane-extracted oil. The percentage of radical scavenging activity for SC-CO₂-extracted oil was 0.71%, but the hexane-extracted oil had a higher radical scavenging activity of 3%. Analysed using gas chromatography, SC-CO₂-extracted oil had lower total saturated fatty acids than the hexane-extracted oil.

KEYWORDS: *Canarium odontophyllum*, supercritical CO₂, green technology

INTRODUCTION

Canarium odontophyllum Miq. is one of the plants found in the tropical rainforest of Sarawak. The fruit of *C. odontophyllum* is the most commonly consumed by the native people in Sarawak¹. The fruit is well-known as ‘dabai’ among local community². Dabai fruits are white when they are still immature and then turn into a purplish pink and a powdery black colour once it ripens³. Its seed is subtriangular in shape with three chambers⁴. A mixture of two superior clones, namely Laja and Lulong which can be found mainly in Sarikei, Sarawak, were used for this study⁵. The cross section of the Laja clone shows a triangular seed with concave sides while the Lulong clone has a more rounded seed⁵. The locals usually soak the ripe fruit in warm water for 3–5 min to soften the flesh before consumption. Sometimes the fruit is eaten with seasoning such as sugar, salt, pepper, or sauce⁴.

Dabai is an oily fruit having about 25% of fat from only its pulp⁶. The fat content of dabai is comparable to that of avocado. Avocado is another fruit known to be high in fat, and studies

discovered that avocado contains around 15–30% of fat^{7,8}. Dabai oil has similar characteristics with some commercially available edible oils. Literature reveals that plant oil is a better source of dietary lipid because it supplies a lower level of saturated fat and provides important dietary antioxidants like vitamin E, and phenolic compounds⁹. The fatty acid constituents of a plant oil together with phytochemicals in the oil, which are responsible for its functionality plus health promoting properties¹. A few studies have been previously determined for antioxidant properties of the dabai fruit. It was reported that the fatty acid compositions of dabai pulp and kernel oil samples, where palmitic (16:0), oleic (18:1) and linoleic (18:2) acids are the major fatty acids¹. Besides, dabai pulp oil contains a fatty acid profile that is comparable to that of palm oil. Hence dabai pulp oil most probably can be used as an alternative choice as vegetable oil.

In the previous studies, dabai oil was extracted using organic solvents, such as hexane or petroleum ether. A green technology used in the extraction of oil, which is supercritical fluid extraction (SFE), may offer an alternate to produce a toxic-free dabai oil.

Among the modern technologies used in oil extraction, SFE is undoubtedly the most extensively studied for its application. SFE is commonly performed using CO₂ for several practical reasons, such as low critical temperature and pressure ($T_c = 31.1\text{ }^\circ\text{C}$; $P_c = 72.8\text{ atm}$). CO₂ is not toxic and non-flammable, and it is also an inert gas that does not react with the food constituents¹⁰.

Supercritical CO₂ has a polarity akin to liquid pentane and thus is applicable for extraction of lipophilic compounds. Due to the lipophilic characteristic of essential oils from plant, supercritical CO₂ is the best candidate for extraction of these oils¹¹. In this study, we aimed to extract the dabai using SFE and to compare the supercritical CO₂ extracted dabai oil with hexane-extracted dabai oil for their fatty acid compositions. As the dabai fruit has high fat content and is rich in antioxidant properties with potential health benefits; hence we determined the antioxidant properties of supercritical CO₂ extracted together with the fatty acid compositions.

MATERIALS AND METHODS

Sample preparation

Fresh dabai fruits were obtained from the selected dabai plantations in Sibul, Sarawak with the help of Sarawak Agriculture Research Centre. The dabai used for this study was a mixture of two superior clones, namely, Laja and Lulong which can be found mainly in Sarikei, Sarawak. The maturity of the dabai fruits used were determined by professional farmer of the dabai plantation and all fresh ripe fruits were packed in airtight chilling containers and shipped to Putra University Malaysia on the same day of fruit collection. All fruits were immediately stored at $-80\text{ }^\circ\text{C}$ freezer before sample preparation. Dabai kernel was removed, chilled and freeze dried. The lyophilised dabai pulp was ground into a powder form of 20 mesh particle size. The lyophilised powder was kept in an airtight container at $-20\text{ }^\circ\text{C}$ until further analyses.

Supercritical fluid extraction

Extraction of dabai oil was performed using a bench-top supercritical CO₂ extractor from Supercritical Fluid Technologies Inc. (Model SFT-100). The extraction was carried out at a temperature of $24\text{ }^\circ\text{C}$, pressure of 60 MPa, and 40 min extraction time. Approximately 10.0 g of dabai powder was inserted into the extraction vessel. Extraction was carried out by injection the CO₂ into the extraction chamber. After 40 min, the oil was collected in an amber

bottle. The oil obtained was kept in $-20\text{ }^\circ\text{C}$ until further analyses.

Solvent extraction

Approximately 8 g of dabai pulp powder was weighed into a conical flask and hexane was added at a ratio of 1:10 (pulp:hexane). The mixture was shaken well, and then the flask was wrapped in aluminium foil and stored at $4\text{ }^\circ\text{C}$ overnight (24 h). The hexane fraction was filtered using a Whatman No. 4 filter paper, and hexane was removed using a rotary evaporator and re-extracted with hexane. The extraction was repeated three times using the same ratio of 1:10. The oil obtained was stored in $-20\text{ }^\circ\text{C}$ until further analyses.

Total flavonoid content

Total flavonoid content (TFC) was determined based on a method described by previous literature¹² with some modifications. Approximately 500 μl of dabai oil, 1.5 ml of ethanol, 100 μl of Al(NO₃)₃, 100 μl of potassium acetate (1 M), and 2.8 ml of distilled water were mixed into a test tube. The mixture was left to stand for 40 min at room temperature ($25\text{ }^\circ\text{C}$). The mixture was then centrifuged at 3000 rpm (1500g). Clear part of the mixture was collected and then absorbance was read at 415 nm using a spectrophotometer. The standard calibration curve was plotted based on absorbance versus concentrations of quercetin standard. TFC was calculated for each sample, and the result was expressed as mg quercetin equivalent of sample (mg QE/g sample).

Total phenolic content

Total phenolic content (TPC) was determined based on a colourimetric method¹³. About 0.5 g dabai oil was dissolved in 1 ml of hexane, and the phenolic compounds were extracted with 0.6 ml methanol/water (60:40 v/v) using an orbital shaker for 2 min. The mixture was centrifuged at 3500 rpm (2100g) for 10 min, and the hexane phase was re-extracted with 0.6 ml methanol/water (60:40 v/v). The methanolic extract solutions were pooled, and an aliquot of 0.2 ml of the methanolic phase was diluted with distilled water to a total volume of 2.5 ml.

Next, 0.25 ml of Folin-Ciocalteu reagent was added. After 3 min of incubation at room temperature ($25\text{ }^\circ\text{C}$), 0.5 ml of Na₂CO₃ solution (35%, w/v) was added to the mixture, which was finally top-up with distilled water to 5 ml. Absorbance of the solution was measured exactly after 2 h

against a blank sample using the spectrophotometer at a wavelength of 725 nm. A calibration curve was obtained based on different concentrations of gallic acid standard, and the result was expressed as mg gallic acid equivalent per g sample (mg GAE/g sample).

DPPH assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was conducted based on the electron-transfer reaction method as described by previous literature¹⁴. Briefly, 1 ml of dabai oil extract was mixed with 2 ml of 10 µg/ml DPPH solution in methanol. The mixture was then shaken vigorously and left to stand at room temperature for 5 min. The decrease in absorbance was recorded at 517 nm using methanol as a control. The DPPH radical scavenging activity of the extract was calculated as $1 - (\text{absorbance of sample} / \text{absorbance of control})$.

Determination of fatty acid composition

Fatty acid methyl esters of the studied oil samples were prepared based on the method described by a previous study¹. Briefly, 0.1 g dabai oil was weighed into a test tube. The sample was dissolved by adding 10 ml of hexane and 100 µl of KOH (2 M). The test tube was capped and vortexed for 30 s. The mixture was then centrifuged at 402g for about 10 min; the extracts were transferred into an autosampler vial and analysed by the gas chromatography-flame ionization detector to determine the fatty acid composition of the dabai oil.

Gas chromatography condition

The GC system (Agilent 6890, Wilmington DE, USA) used was equipped with a split-splitless injector. Hewlett-Packard EL-980 flame ionization detection (FID) system was used to separate and quantify each FAME component which were separated using the DB-23 column (60 m × 0.25 mm, i.d. 0.15 µm polyethylene glycol film) (Muskegon, MI, USA). Oven temperature was set at 50 °C for 1 min and further increased to 175 °C at 4 °C/min and increased to 230 °C which was held for 5 min. The injector and detector temperature used were 250 °C and 280 °C. Oil sample of 1 µl was injected with split ratio of 1:50 at column temperature of 110 °C. Helium (1 ml/min) was used as carrier gas controlled at 103.4 kPa while hydrogen and air was used for FID and held at 275.6 kPa. FAME standards were used for the identification of the fatty acids.

Statistical analysis

Triplicate determinations were done for each analysis. All data were expressed as mean ± standard deviation. Significant differences in the fatty acid profiles and antioxidant properties between hexane and supercritical CO₂ (SC-CO₂) extractions were analysed by one sample *t*-test using SPSS 21, where the statistical significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

Yield

The result showed that there was a significant difference ($p < 0.05$) in the oil yield between hexane and SC-CO₂ extraction methods employed. The percentage of oil (51%) obtained by hexane extraction was almost twice as high as the SC-CO₂ extraction (23%). Unlike the SC-CO₂ extraction method, the lipid soluble substances together with triacylglycerols are basically more soluble in hexane¹⁵.

A previous study revealed that extraction of oil from oleaginous fungus, *Mortierella alpine*, using hexane had a higher percentage of oil extracted than the SC-CO₂ method¹⁵. Hexane also differs from CO₂ because it is non-selective for triglycerides, causing it to extract almost all available fatty acids, pigments, phospholipids and unsaponifiable substances along with triglycerides¹⁶. The fact explains why the yield of organic solvent extraction is higher than that of SC-CO₂ extraction¹⁶.

Pressure plays a significant role in affecting the percentage of extracted oil. A high pressure can significantly increase the oil yield¹⁷. At lower extraction pressure, the solubility of oil is affected by the vapour pressure of oil. Also, CO₂ acts as an ideal gas that does not have special properties of a solvent at lower pressure. At higher pressures, the solubility of oil increases due to the increase density of CO₂¹⁷. The increase in CO₂ density will reduce the distance between the molecules of CO₂ and oil, and the interaction between these molecules increase, thus increase the solubility of oil in CO₂.

Total phenolic content

TPC of dabai oils extracted using hexane and SC-CO₂ was determined based on the colourimetric method. Dabai oil extracted by SC-CO₂ demonstrated a non-significantly higher TPC (0.733 ± 0.009 mg GAE/g) than the hexane-extracted oil (0.679 ± 0.002 mg GAE/g), with $p = 0.069$. The result is in contrast to the finding reported by a previous study that phenolic compounds were not detected in SC-CO₂-extracted peach palm

pulp (*Bactris gasipaes*)¹⁸. A possible explanation for this finding is the hydrophobic nature of SC-CO₂ that solubilised and extracted almost all non-polar molecules. Hence SC-CO₂ extraction is not an ideal way to extract phenolic compounds^{19,20}.

There are several other ways to increase the amount of phenolics extracted from plants. An increase in operation time can facilitate extraction of higher molecular weight compounds, such as polyphenols because these compounds need a longer time to be solubilised in the extraction solution¹⁹. Besides that the addition of co-solvents such as ethanol has been found successfully to improve the SC-CO₂ extraction of phenolic compounds in grape samples²¹.

On the other hand, an increase in extraction pressure can improve the extraction yield. A higher phenolic content can be obtained in the extract of berries pressing wastes by applying a higher pressure (200 bar or higher) during supercritical extraction¹⁹. Different sample matrices of plants might need different optimal extraction pressures for extracting phenolic components. Furthermore, other organic compounds could also be extracted at a higher extraction pressure, resulting in slight increase in TPC of dabai oil extracted using SC-CO₂.

Total flavonoid content

TFC of dabai oil extracted using hexane and SC-CO₂ was also determined based on colorimetric method. The TFC of hexane-extracted dabai oil (53.45 ± 0.07 mg QE/g) was significantly higher than the SC-CO₂-extracted dabai oil (12.6 ± 1.1 mg QE/g). A previous study revealed that TFC was not detected in the SC-CO₂ extract of peach palm pulp sample¹⁸. This finding explains the low TFC in SC-CO₂-extracted oil. The amount of phytochemicals obtained is largely affected by the extraction temperature and pressure using SC-CO₂²². Extraction time may also influence the extraction yield, especially within the first 90 min of extraction²³.

DPPH radical scavenging activity

DPPH radical scavenging assay was used to evaluate the antioxidant activity of the dabai oil extracts. The extract of hexane-extracted dabai oil (3%) exhibited a significantly higher radical scavenging activity ($p < 0.01$) than the extract of SC-CO₂-extracted dabai oil (0.71%). The extraction of oil using Soxhlet extraction showed a low antioxidant profile, which could be due to the weak interaction between hexane and antioxidant components²⁴. The fact may explain that the extract of hexane-extracted

dabai oil has little radical scavenging activity. However, a modifier such as ethanol added to the extraction solvent could increase the DPPH antiradical power²¹.

Conversely, a previous study reported that *Rosmarinus officinalis* extract obtained from SC-CO₂ extraction has higher DPPH radical scavenging activity (62%) than the hexane extract (57%)²⁵. A possible explanation for the low radical scavenging activity of the extract of SC-CO₂-extracted dabai oil is the high pressure applied during SC-CO₂ extraction, and it might also be due to the hydrophobic characteristic of CO₂ that reduced the extractability of hydrophilic antioxidant compounds. Thus the SC-CO₂-extracted oil extract has a low percentage of antioxidant activity²⁶. Interestingly, DPPH radical scavenging activity is strongly correlated with TFC²⁷. Hence the results obtained from this study also revealed a correlation between TFC and DPPH radical scavenging activity.

Fatty acid profile

Fatty acid compositions (mg/g) of dabai oil obtained from hexane and SC-CO₂ extraction are shown in Tables 1–3. The results also revealed that both hexane and SC-CO₂-extracted oils have high percentages of total saturated fatty acid (SFA), 90% and 86%, respectively. Among the SFA, palmitic acid (16:0), heneicosylic acid (21:0) and margaric acid (17:0) are the most abundant. The major MUFAs in the extracted dabai oil were myristoleic acid (C14:1) and heptadecenoic acid (C17:1), while brassic acid (C22:2), eicosadienoic acid (C20:2), and gamma-linolenic acid (C18:3n6) were the main PUFA in the extracted oil.

In this study, the hexane-extracted dabai was found to contain a higher total SFA than the SC-CO₂-extracted oil. In contrast, the SC-CO₂-extracted oil had higher total MUFA and total PUFA than the hexane-extracted oil. Among the types of SFA, arachidic acid, behenic acid, tricosylic acid, and lignoceric acid were not detected in the SC-CO₂-extracted dabai oil. The result indicated that a long-chain arachidic acid and all very long-chain fatty acids were not found in the SC-CO₂-extracted dabai oil. Conversely, hexane-extracted dabai oil consisted a very long-chain behenic acid. Also, the hexane-extracted oil had a higher percentage of total medium-chain fatty acids (3%) than the SC-CO₂-extracted oil (2%).

Among the types of MUFA, SC-CO₂-extracted dabai oil had higher percentages of myristoleic acid, pentadecylic acid, oleic acid, and gondoic acid than

Table 1 Saturated fatty acid (SFA) content of hexane-extracted and SC-CO₂-extracted dabai oil.

Common name	Fatty acid	Hexane		SC-CO ₂	
		mg/g oil	%*	mg/g oil	%*
Butyric acid	C4:0	0.148 ± 0.028	0.78	0.206 ± 0.141	1.09
Caproic acid	C6:0	0.442 ± 0.056	2.32	0.218 ± 0.009	1.15
Caprylic acid	C8:0	0.110 ± 0.014	0.58	0.094 ± 0.003	0.50
Capric acid	C10:0	0.038 ± 0.003	0.20	0.066 ± 0.006	0.35
Undecylic acid	C11:0	0.008 ± 0.006	0.04	0.019 ± 0.005	0.10
Lauric acid	C12:0	0.033 ± 0.006	0.17	0.057 ± 0.008	0.30
Tridecylic acid	C13:0	0.024 ± 0.001	0.13	0.038 ± 0.012	0.20
Myristic acid	C14:0	0.032 ± 0.002	0.17	0.060 ± 0.049	0.32
Pentadecylic acid	C15:0	ND	0	0.021 ± 0.001	0.11
Palmitic acid	C16:0	12.6 ± 2.2	66.1	11.096 ± 0.274	58.7
Margaric acid	C17:0	1.563 ± 0.245	8.21	1.994 ± 0.584	10.5
Stearic acid	C18:0	0.069 ± 0.045	0.36	0.098 ± 0.004	0.52
Nonadecylic acid	C19:0	0.307 ± 0.050	1.61	0.324 ± 0.105	1.71
Arachidic acid	C20:0	0.231 ± 0.176	1.21	ND	0
Heneicosylic acid	C21:0	1.566 ± 0.252	8.22	1.920 ± 0.537	10.2
Behenic acid	C22:0	0.026 ± 0.001	0.13	ND	0
Tricosylic acid	C23:0	ND	0	ND	0
Lignoceric acid	C24:0	ND	0	ND	0
Total SFA		17.187	90.2	16.211	85.7

* The percentage of fatty acids is calculated based on the total fatty acids determined by gas chromatographic analysis. ND = not detected.

Table 2 Monounsaturated fatty acid (MUFA) content of hexane-extracted and SC-CO₂-extracted dabai oil.

Common name	Fatty acids	Hexane		SC-CO ₂	
		mg/g oil	%*	mg/g oil	%*
Myristoleic acid	C14:1	0.437 ± 0.070	2.29	0.571 ± 0.107	3.02
Pentadecylic acid	C15:1	0.105 ± 0.012	0.55	0.121 ± 0.008	0.64
Palmitoleic acid	C16:1	ND	0	ND	0
Heptadecenoic acid	C17:1	0.382 ± 0.053	2.01	0.352 ± 0.047	1.86
Oleic acid	C18:1n9t	0.019 ± 0.001	0.10	0.021 ± 0.001	0.11
Gondoic acid	C20:1n9	ND	0	0.050 ± 0.019	0.26
Erucic acid	C22:1n9	ND	0	ND	0
Nervonic acid	C24:1n9	0.121 ± 0.054	0.64	0.100 ± 0.072	0.53
Total MUFA		1.064	5.59	1.215	6.42

* The percentage of fatty acids is calculated based on the total fatty acids determined by gas chromatographic analysis. ND = not detected.

the hexane-extracted oil. Palmitoleic acid and erucic acid were not detected in both hexane and SC-CO₂-extracted dabai oils. The percentages of all individual type of PUFAs of SC-CO₂-extracted dabai oil were higher than the hexane-extracted oil. Hence we found no docosahexaenoic acid in the hexane-extracted dabai oil. Furthermore, a lower ratio of omega-6/omega-3 was also calculated for the SC-CO₂-extracted dabai oil (1.1:1) than the hexane-extracted oil (3.4:1), as well as other vegetable oils (> 5:1). The omega-6/omega-3 ratio in food is important for prevention of several chronic diseases²⁸.

A previous study reported that dabai pulp has a high total saturated fatty acids¹. In this study, we conclude that the total MUFA and total PUFA in the SC-CO₂-extracted dabai oil were higher than the hexane-extracted oil. On the contrary, a study reported that flaxseed oil extracted using organic solvent contained higher SFA than the SFE method²⁹. The conventional lipid extraction method including hexane extraction may cause deterioration of PUFA due to the high processing temperature³⁰. SFE has been used for preserving PUFA because the extraction can be conducted at a lower temperature³⁰.

Table 3 Polyunsaturated fatty acid (PUFA) content of hexane-extracted and SC-CO₂-extracted dabai oil.

Common name	Fatty acids	Hexane		SC-CO ₂	
		mg/g oil	%*	mg/g oil	%*
Linoleic acid	C18:2n6c	ND	0	ND	0
Linolelaidic acid	C18:2n6t	ND	0	ND	0
Alpha-linolenic acid	C18:3n3	0.037 ± 0.001	0.19	0.065 ± 0.005	0.34
Gamma-linolenic acid	C18:3n6	0.126 ± 0.003	0.66	0.189 ± 0.002	1.00
Eicosadienoic acid	C20:2	0.319 ± 0.065	1.68	0.329 ± 0.022	1.74
Dihomo-γ-linolenic acid	C20:3n6	ND	0	ND	0
Eicosatrienoic acid	C20:3n3	ND	0	ND	0
Arachidonic acid	C20:4n6	ND	0	ND	0
Eicosapentaenoic acid	C20:5n3	ND	0	ND	0
Brassic acid	C22:2	0.315 ± 0.359	1.65	0.804 ± 0.331	4.25
Docosahexaenoic acid	C22:6n3	ND	0	0.102 ± 0.047	0.54
Total PUFA		0.797	4.18	1.489	7.87

* The percentage of fatty acids is calculated based on the total fatty acids determined by gas chromatographic analysis. ND = not detected.

Interestingly, a study reported no significant difference in the fatty acid compositions of grape seed oil extracted using SC-CO₂ and hexane¹⁶. However, the study revealed that different extraction time applied in the SC-CO₂ extraction could slightly alter the fatty acid composition of grape seed oil. This observation could be due to the fractionation of oil by depressurisation, which allowed the production of various grades of oil with different compositions of fatty acids. Freeze-drying of a plant sample before lipid extraction can ensure effective moisture removal and enable a higher lipid retrieval due to better cell wall disruption¹⁵.

CONCLUSIONS

Dabai pulp oil had a high SFA, especially extracted using hexane. Regarding extraction yield, the organic solvent was proven to be a more preferred extraction solvent for extracting fatty acids compared to SC-CO₂. SC-CO₂-extracted dabai pulp oil had higher percentages of total medium-chain fatty acid and contained slightly higher TPC than the hexane-extracted oil. However, it had lower TFC and radical scavenging activity than the hexane-extracted oil. The fatty acid profile of this SC-CO₂-extracted oil was superior to the hexane-extracted oil because it contained higher percentages of MUFA and PUFA. In a nutshell, SC-CO₂ extraction is potentially a more acceptable extraction method due to the quality of fatty acids obtained. CO₂ is also environmentally friendly and produces a non-toxic dabai oil. In the future, phytochemical profile of dabai oil is needed to be determined for ensuring the quality of SC-CO₂-extracted dabai pulp oil.

Acknowledgements: This study was financially supported by the university's research grant (Geran Penyelidikan Pembangunan Inovasi, vote 9449700), Universiti Putra Malaysia.

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