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Effects of supercritical carbon dioxide extraction parameters on virgin coconut oil yield and medium-chain triglyceride content

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ABSTRACT

The extraction of coconut oil has been performed using supercritical carbon dioxide (SC-CO_2). The extractions were performed at pressure and temperature ranges of 20.7–34.5 MPa and 40–80 °C, respectively. It was observed that almost all (more than 99%) of the total oil could be extracted. Response surface methodology (RSM) was applied to evaluate the effects of the parameters (pressure, temperature and CO_2 consumption) on the extraction yield and medium-chain triglycerides (MCTs), in terms of the fatty acid content in the extracted oil. A correlation was established with *p*-values for both responses significant at the 95% confidence level.

1. Introduction

Coconut (*Cocos nucifera* L.) oil is a natural source of medium-chain triglycerides (MCTs) with approximately 60% of the total oil content being MCTs. The term MCT refers to triglyceride which is composed of a glycerol backbone and three saturated fatty acids with chain length of 6–12 carbons. MCTs have been reported to be beneficial to human health. MCTs are mainly utilised as a nutritional supplement for patients suffering from malabsorption caused by intestinal resection and also as a component of infant feeding formulas (Nandi, Gangopadhyay, & Ghosh, 2005). Marten, Pfeuffer, and Schrezenmeir (2006) reported that MCTs have beneficial effects on weight control and glucose, as well as lipid metabolism. Compared with other triglycerides that mainly contain saturated long chain fatty acids, MCTs have a lower melting point, smaller molecular size, lower solidification temperature and lower energy density. These distinct chemical properties affect the ways in which MCTs are absorbed and metabolized. MCTs have also been reported as tumour inhibitors when consumed in a diet (Cohen & Thomson, 1987), and, if they are mixed with phytosterol and high-oleic canola oil, they can decrease plasma lipid content in overweight men (Rudkowska, Roynette, Nakhasi, & Jones, 2006).

Coconut oil that is extracted from fresh coconut flesh is known as virgin coconut oil (VCO). The extraction involves a process that does not use thermal treatment or food preservatives. Coconut oil obtained from copra, dried coconut, has no taste or fragrance, due to the refining process, whereas VCO has the fragrance and taste of coconut. The absence of heating and chemical treatment in the oil makes it tasty and healthy. The antioxidant activity of VCO is superior to that of regular coconut oil, which is extracted from copra, and also of groundnut oil (Nevin & Rajamohan, 2005). The existing process production of VCO basically is conducted through oil separation from coconut milk (Sukartin & Sitanggang, 2005). Coconut milk can be obtained by either pressing fresh coconut flesh without additional water or grating the coconut flesh followed by extracting the water–oil emulsion with water. The oil can be separated from the emulsion by means of boiling, fermentation, refrigeration or mechanical centrifuge. Separation of the oil from the water–oil emulsion can also be accomplished by breaking the emulsion and creating an oil–oil emulsion, in which pure coconut oil must be added to the coconut milk to extract the oil from the emulsion, and then the oil must be separated from the water and protein with decantation. The existing process requires 24–48 h and produces an oil yield of about 40% of the oil available in the coconut.

Although extraction of natural compounds using SC-CO_2 has been reported as a promising technique by many researchers it is remarkable that very few reports on the extraction of coconut oil from copra using SC-CO_2 have been published. Additionally, there is a lack of fundamental insights into the SC-CO_2 extraction process

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of coconut oil. The effects of temperature and pressure on the extraction time and CO_2 consumption for oil separation from copra were reported by Brannolte, Mangold, and Stahl (1983). Celestino, Ruther, Socorro, and Steven (2006) also analysed the oil content of copra extracted with SC-CO_2. In this study, it was reported that approximately 100% of the coconut oil can be extracted from the copra within 1 h. However, there have been no further studies reported in the literature regarding the effects of extraction conditions on the extraction yield and the MCT content of the extracted oil.

In this work, the application of SC-CO_2 extraction of virgin coconut oil was performed, and the extraction parameters were examined. Response surface methodology (RSM) was applied to obtain the interaction effects among the parameters. By analysing the interaction parameters, the relationships between the variables could be understood and the optimum conditions could also be determined. Pressure, temperature and CO_2 consumption were chosen as the parameters. The responses were the extraction yield and the MCT content of the extracted oil. In addition, this study should provide a better understanding of the effects of each parameter and its interaction.

2. Materials and methods

2.1. Materials and chemicals

Coconut as a raw material was locally grown and bought from a market in Pulau Pinang, Malaysia. The coconut was grated and sun-dried to reduce the moisture content from about 50–3%. This was done to avoid clogging the capillary restrictor. The dried sample was ground into particles of sizes ranging between 0.5 and 1.0 mm by sieving, and the particles were used for the extraction. Fatty acid methyl ester standard (Supelco FAME mix GLC-10 and GLC-30), analysis grade *n*-hexane (Merck) and sodium methoxide 1 M methanolic solution (Sigma–Aldrich) were used in the GC analysis and esterification. Carbon dioxide was obtained from Malaysian Oxygen (MOX), Pulau Pinang, Malaysia, with a purity of 99.9%.

2.2. Extraction of coconut oil using the soxhlet method

The total oil content in the sample was extracted by Soxhlet extraction, using *n*-hexane, for an extraction time of 30 h, for comparison with the supercritical extraction. The extraction was carried out in duplicate for 10 g of sample and resulted in 68.53 ± 0.3% oil content, on a dry basis.

2.3. Supercritical CO_2 extraction

Experiments were performed with an extraction apparatus that consisted of a SC-CO_2 extractor (ISCO, Inc., Lincoln, NE; Model SFX 220), a carbon dioxide cylinder, a chiller (B/L-730, YIH DER, Taipei) for CO_2 liquefaction and a high pressure syringe pump (ISCO, Inc.; Model 100 DX) with a maximum operating pressure of 69 MPa. The volume of the extraction vessel was 2.5 ml. The extractor was equipped with a heated capillary restrictor (ISCO, Inc.) that had an outer diameter of 50 μm and a maximum operating temperature of 150 °C. The temperature and pressure were controlled with software (ISCO, Inc.; Model SFX 200) that was integrated into the extractor system.

Extractions were performed at 40 to 80 °C, with a pressure range of 20.7–34.5 MPa and an average CO_2 flow rate of 1.2 ml/min. The temperature-controlled chamber and the pump were set to the desired temperature and pressure. Approximately 2 g of sample were placed into the extraction cell. The extraction vessel was then placed in the extractor unit and was allowed to equilibrate to the desired temperature. Upon reaching the desired temperature, pressurisation was initiated, and the CO_2 flowed through the extraction cell from the bottom to the top of the vessel. The oil was separated from oil-rich CO_2, through an expansion valve and a heated capillary restrictor, and was collected in a vial. In all cases, the restrictor temperature was set at 80 °C. Since the extracted oil, like other triglycerides, has very low vapour pressure, there was no cooling necessary to be applied in the sample collection vial to prevent volatilisation of the oil. The extracted sample was weighed with an analytical balance at a certain time interval as per the experimental design. The extraction yield was determined, based on the amount of the extracted oil divided by the total oil content of the sample (Eq. (1)).

$$\textit{Extraction Yield}(\%) = \frac{\textit{mass of extracted oil}}{\textit{mass of total oil}} \times 100\% \quad (1)$$

The volume of CO_2 consumption was measured under each extraction condition, using a flow meter integrated in the SC-CO_2 extractor, and displayed at the extractor controller monitor. The mass of CO_2 was calculated using the CO_2 density under each condition.

2.4. Analysis

The MCT content of the extracted oil were defined as the medium chain fatty acids (C_6–C_{12}) present in the oil. The fatty acid composition was analysed as fatty acid methyl ester (FAME), using a gas chromatography with flame ionisation detector (GC-17A, Shimadzu, Osaka, Japan). Prior to injection, the extracted oils were converted to FAMEs, according to PORIM Test Method No. p3.4, (1995). Approximately 50 mg of extracted oils were dissolved in 0.95 ml *n*-hexane in a 2 ml vial. The vial was capped and then shaken to dissolve the oil. Then, 0.05 ml sodium methoxide were added to the solution using a micropipette. The vial cap was quickly removed, and the solution was mixed vigorously for 5 s with the help of a vortex mixer. The mixture first became clear and then turbid as the sodium glyceroxide precipitated. After a few minutes, the clear upper layer of methyl ester was pipetted off and analysed by gas chromatography, according to PORIM Test Method No. p3.5, (1995). The GC column was 30 m in length, with a 0.25 μm film coating, 0.25 mm ID, BPX5 phase (non-polar). The column temperature was 150 °C for the first 3 min, was increased to 190 °C for 5 min and then was finally increased to 195 °C. The rate of temperature increase was 5 °C/min. The injection temperature and detector temperature were maintained at 200 °C and 220 °C, respectively. The weight fractions of the FAMEs were determined according to PORIM Test Methods No. p3.5 (1995). This was based on the percentage that is represented by the area of the corresponding peak relative to sum of the area of all the peaks. To get the highest accuracy, a correction factor K_i (Eq. (2)) was applied to convert the percentage of peak area into weight percentage of the FAME components (Eq. (3)). Determination of the correction factor was performed with the help of a chromatogram derived from the analysis of the FAME standard under operation conditions identical with those used for the sample:

$$K_i = \frac{m_i \times \sum A_i}{A_i \times \sum m_i} \quad (2)$$

$$\textit{weight percentage of the component } i = \frac{K_i \times A_i \times 100}{\sum (K_i \times A_i)} \quad (3)$$

where K_i = correction factor of component *i*, m_i = weight percentage of component *i* in the FAME standard solution, A_i = area under the peak corresponding to component *i*.

2.5. Statistical design

A Box–Behnken design of response surface methodology (RSM) was employed to collect the data by three factors and three levels of variable combinations (Myers & Montgomery, 2002). The study was carried out according to the Box–Behnken design, and the experimental points used according to this design are shown in Table 1.

The data were analysed by multiple regressions to fit the quadratic equations to the dependent variables. Statistical analysis was performed using RSM software, Design-Expert 6-0-10 (Stat-Ease Inc., Minneapolis, MN). A mathematical model was established to describe the behaviour of each response. Analysis of variance (ANOVA) was performed to evaluate the adequacy of the generated mathematical models.

3. Results and discussion

3.1. Effect of extraction conditions on oil yield and composition of the oil

Extraction at pressures ranging from 13.8 to 34.5 MPa and a constant temperature of 70 °C shows a variation in yield dependent upon pressure. Only 20% yield was obtained at 13.8 MPa, whereas the extracted yield increased to more than 95% as the pressure increased to 20.7 MPa. At 34.5 MPa, the yield was almost 100%. Therefore, it was not necessary to increase the extraction pressure beyond 34.5 MPa. By extracting the oil at constant pressure and varying temperatures (40–80 °C) and CO_2 consumptions, the ultimate yield could be achieved at different levels of CO_2 consumption.

Fig. 1 shows a typical chromatogram of the FAME from the extracted oil at 20.7 MPa, 60 °C and 60 g CO_2 consumption. The fatty acid constituents obtained in the extracted oil were caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2). The composition of the fatty acid in the extracted oil varied, based on the extraction conditions (Fig. 2).

The target compounds of the extraction were the MCTs. To determine the interactions between the parameters (pressure, temperature and CO_2 consumption), the extraction yield and the MCT content of the extracted oil, a statistical analysis was applied

Table 1
Experimental programme and results of the Box–Behnken design of extraction of MCT content using supercritical carbon dioxide.

Standard error	Pressure (Mpa) (x_1)	Temperature (°C) (x_2)	CO_2 consumption (g) (x_3)	Yield (weight%)	MCT content (weight%)
1	20.7	40	35	94.56	59.47
2	34.5	40	35	96.25	57.99
3	20.7	80	35	40.29	67.27
4	34.5	80	35	99.41	59.07
5	20.7	60	10	30.21	65.49
6	34.5	60	10	93.37	62.00
7	20.7	60	60	92.37	60.39
8	34.5	60	60	99.08	56.71
9	27.6	40	10	73.69	62.40
10	27.6	80	10	63.30	64.27
11	27.6	40	60	95.64	58.94
12	27.6	80	60	99.32	58.07
13	27.6	60	35	98.28	59.12
14	27.6	60	35	97.30	56.49
15	27.6	60	35	97.08	56.76
16	27.6	60	35	97.64	56.35
17	27.6	60	35	96.55	56.35

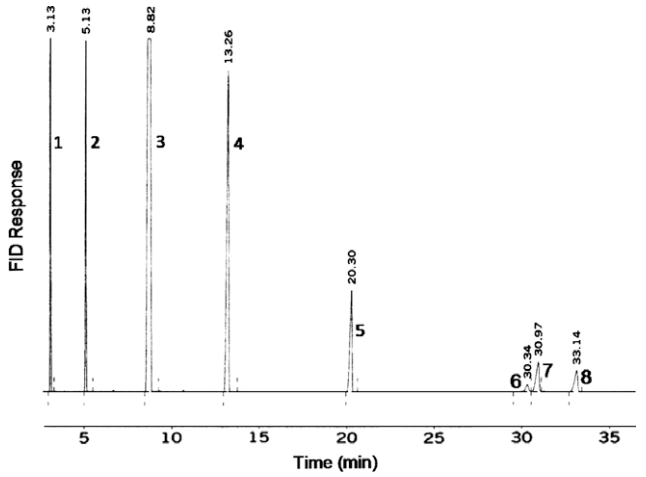

Fig. 1. Typical chromatogram of the FAMEs from the extracted oil (20.7 MPa, 60 °C and 60 g CO_2 consumption). Peaks: (1) caprylic ($C_{8:0}$), (2) capric ($C_{10:0}$), (3) lauric ($C_{12:0}$), (4) myristic ($C_{14:0}$), (5) palmitic ($C_{16:0}$), (6) linoleic ($C_{18:2}$), (7) oleic ($C_{18:1}$), (8) stearic ($C_{18:0}$). The medium-chain triglyceride (MCT) content of the extracted oil was calculated based on the FAMEs with carbon numbers C_8–C_{12}.

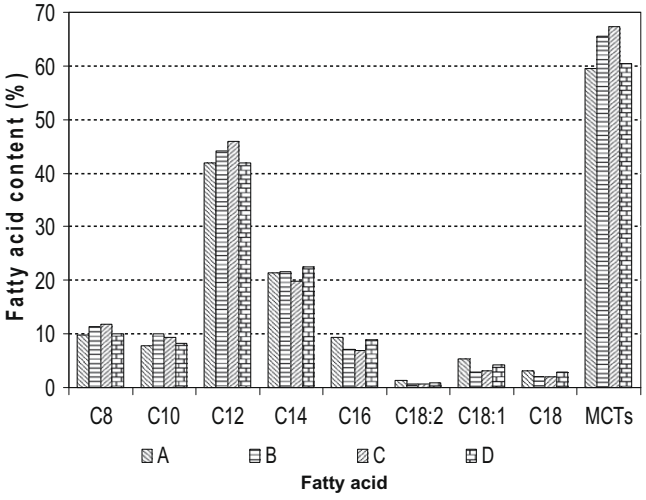

Fig. 2. Fatty acid composition of the extracted oil under different extraction conditions. (A: 20.7 MPa, 40 °C, 35 g CO_2 consumption, B: 20.7 MPa, 60 °C, 10 g CO_2 consumption, C: 20.7 MPa, 80 °C, 35 g CO_2 consumption, D: 20.7 MPa, 60 °C, 60 g CO_2 consumption.)

(Myers & Montgomery, 2002; Palma & Taylor, 1999). The experimental data were obtained, according to the response surface methodology (RSM) design shown in Table 1.

A second-order polynomial model was applied to express the responses as a function of the chosen variables.

$$y_s = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{12}x_1x_2 + b_{22}x_2^2 + b_{13}x_3 + b_{23}x_2x_3 + b_{33}x_3^2 \quad (4)$$

where y_s represents the dependent variables (extracted yield and the MCT content), x_s represents the independent variables (pressure, temperature and CO_2 consumption) and b_s represents the coefficients. Multiple regression analysis was performed to determine the regression coefficients of the model (Table 2).

The effects of pressure, temperature and CO_2 consumption on extraction yield and MCT content can be seen in the sample graph (Fig. 3). In Fig. 3a, it was observed that at pressures lower than 28.3 MPa, the extraction yield decreased with increasing temperature, while at higher pressures (greater than 28.3 MPa), the yield

Table 2
Estimated coefficients of second-order response models for SFE.

	Extraction yield (%)	Extracted oil MCT content (%)
Intercept	−77.2497	88.4694
x_1	9.9870	−1.6310
x_2	−1.9022	−0.0206
x_3	3.4872	−0.2067
x_1x_1	−0.1993	0.0374
x_2x_2	−0.0131	0.0039
x_3x_3	−0.0146	0.0028
x_1x_2	0.1040	−0.0122
x_1x_3	−0.0818	−0.0003
x_2x_3	0.0070	−0.0014

increased with temperature. This was most likely due to the solubility crossover phenomenon, which often occurs in solute-CO_2 systems. The phenomenon was also noticed in a VCO–CO_2 system (Setianto, Norulaini, Hanipah, Zulhilmi, & Omar, 2008). It was reported that a solubility cross-over region occurs at pressures ranging from 27 to 29.5 MPa. At pressures lower than 27 MPa, increasing temperature causes the solubility of the oil in CO_2 to decrease at constant pressure. However, at pressures higher than 29.5 MPa, increasing temperature causes the solubility of the oil in CO_2 to increase at constant pressure. The extraction yield is further affected by the physical condition of the sample. Reduction of the sample particle size liberates most of the oil from oil bearing cells as "free oil", as a result of the influence of the oil solubility being greater than that of internal mass transfer. Fig. 3b shows a contour plot of the interaction of the pressure and the CO_2 consumption. It is clear that the increase in CO_2 consumption resulted in an increase in the yield. At a constant temperature (60 °C) and certain CO_2 consumption level, increasing pressure provided higher extraction yields (Fig. 3a). The MCT content increased with increasing temperature, while it decreased with increasing pressure (Fig. 3c). The increase in CO_2 consumption reduced the MCT content of the extracted oil (Fig. 3d). The trend of the MCT content was contrary to that of the yield. This might be due to the shorter chain triglycerides being extracted more easily than the longer chain triglycerides; consequently, the oil extracted earlier in the process has a higher MCT content than the fractions extracted later.

The ANOVA for the extraction yield and the extracted oil MCT content are shown in Table 3. The calculated values of F were compared to the table value of $F_{(p-1,n-p,\alpha)}$. The ANOVA showed that the calculated value of F for the yield response (20.52) and that of the MCT content response (6.44) were greater than the table (experi-

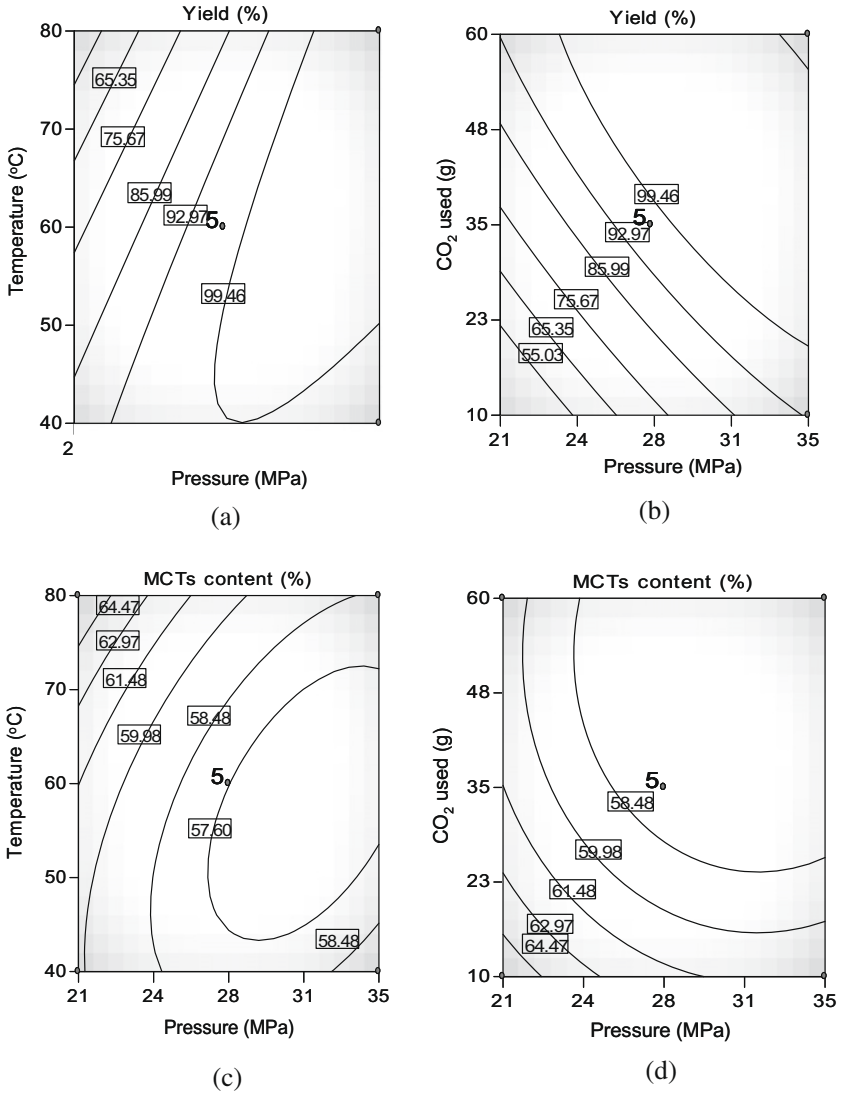

Fig. 3. (a) Effects of pressure and temperature on extraction yield using 35 g CO_2. (b) Effects of pressure and CO_2 consumption on extraction yield at 60 °C. (c) Effects of pressure and temperature on extracted oil MCT content using 35 g CO_2. (d) Effects of pressure and CO_2 consumption on oil MCT content at 60 °C.

Table 3
Analysis of variance (ANOVA) for extraction yield and extracted oil MCTs content.

Source	Degrees of freedom	Sum of squares	Mean square	F	R^2	$F_{(0.05)}$
Extraction yield						
Regression	9	7140.38	793.38	20.52	0.96	3.68
Residual	7	270.59	38.66	–	–	–
Total	16	7410.98	–	–	–	–
Extracted oil MCTs content						
Regression	9	151.84	16.87	6.44	0.90	3.68
Residual	7	18.33	2.62	–	–	–
Total	16	170.17	–	–	–	–

mental) value of F ($F_{(9,7,0.05)}$ = 3.68). If the calculated value of F exceeds the table value of F, the null hypothesis is rejected at the level of significance, and it is inferred that the coefficient estimates are not all zero (that is, one or more coefficients convey information about the model) and the variation verified that the model is significantly greater than the unexplained variation (noise) (Wonnacott & Wonnacott, 1990). Therefore, the p-value for the yield and the MCT content responses is below 0.05. Accordingly, it could be construed that, at the 95% confidence level, the models were significant for both of the responses. The value of r^2 measures the total variation of the observed values about the mean determined by the fitted model. The values of r^2 for the yield and the MCTs content responses were 0.96 and 0.90, respectively; hence, the r^2 value in this study signifies a good correlation between the experimental data and the predicted values. Therefore, the fitted model obtained can be used to describe the effects of the factors within the experimental ranges.

4. Conclusions

The extraction of coconut oil was performed using SC-CO_2 at various pressures, temperatures and CO_2 consumption levels. This process can be successfully performed, extracting about 99% of the total oil. The extraction yield and the MCT content of the extracted oil varied with the extraction conditions. A mathematical model based on statistical analysis was established. The correlation between the extraction conditions and the extraction yield as well as the MCT content applied in this study may be remarkable and should be considered for scaling-up the VCO extraction process.

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