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ORIGINAL ARTICLE

The effect of flow rate at different pressures and temperatures on cocoa butter extracted from cocoa nib using supercritical carbon dioxide

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Abstract The effects of flow rate, different pressures and temperatures on cocoa butter extracted from cocoa nib using supercritical carbon dioxide ($scCO_2$) were investigated. The yield was analyzed for total fat content, triacylglycerol (TG) profile, and fatty acid (FA) profile. Extractions were carried out at pressures of 20 and 35 MPa, temperatures of 50 and 60 °C, and CO_2 flow rates of 0.5, 1, 2, 4 mL min^{-1}. The result shows that the yield of cocoa butter extract increased with increasing pressure, temperature, and flow rate and the optimum conditions for the maximum cocoa butter extraction were 35 MPa, 60 °C and 2 mL min^{-1}, repectively. TGs and FAs were found to be similar in composition to those of cocoa butter obtained by conventional methods. The lower molecular weight TGs and FAs showed higher selectivity compared to higher molecular weight TGs and FAs.

Highlights
- Cocoa butter extraction from cocoa nib using supercritical carbon dioxide
- Effects of CO_2 flow rate, pressure, and temperature on cocoa butter extraction
- Cocoa butter quality base on extraction method
- Triglycerides in term of fatty acids constituents.

Keywords Cocoa butter · $scCO_2$ extraction · Pressure · Temperature · Flow rate · Selectivity

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Introduction

Cocoa butter is unique among vegetable fats due to its composition and crystallization behavior. Cocoa bean contains substantial amounts (55 %) of cocoa butter (Saldaña et al. 2002; Rodríguez et al. 2014). There is a high demand for quality cocoa butter, especially for use in foods, nutraceuticals, cosmetics and pharmaceuticals (Asep et al. 2013; Jahurul et al. 2014, 2015). In comparison with the conventional method, supercritical fluid extraction (SFE) is a feasible and a potential method to be implemented in the cocoa industry to produce higher yield and better quality cocoa butter due to its rapid, non-toxic, environmental friendly, contamination-free and easily manipulated conditions (Jahurul et al. 2012; Jinap et al. 2013).

In recent years, there has been increasing interest in the development of supercritical fluid technology and its application. Recently the uses of SFE are widely increasing for the extraction, purification, crystallization, fractionation, and nanoparticle formation in food, nutraceutical and pharmaceutical industries (McHugh and Krukonis 1994; Reverchon and Adami 2006; Campardelli et al. 2012; Jahurul et al. 2012; Knez et al. 2014). Compared to traditional organic solvents, supercritical fluids have been known to be more efficient and environmental friendly extraction fluids (Rodríguez et al. 2014).

Cocoa butter from cocoa bean was extracted using SFE technology by many researchers (McHugh and Krukonis 1994; Li and Hartland 1996; Saldaña et al. 2002; Tan et al. 2008; Asep et al. 2008, 2013; Jinap et al. 2013; Rodríguez et al. 2014). Less than 5 % of the cocoa butter from the nibs were extracted at 48.3 MPa for a period of 8 h at 40 to 60 °C (McHugh and Krukonis 1994). The authors concluded that the extraction of cocoa butter by $scCO_2$ was time consuming and resulted lower yield. In order to facilitate the extraction process, the authors suggested that the addition of ethanol as a cosolvent may enhance the solubility of cocoa butter in $scCO_2$ (Rossi et al. 1989; Mchugh and Krukonis 1994; Li and Hartland 1996; Asep et al. 2013).

In contrast to the numerous patents for coffee decaffeination, only Li and Hartland (1992,1996) have conducted studies on the extraction of cocoa butter by $scCO_2$ using a cosolvent. Nevertheless, they did not optimize SFE process conditions for cocoa butter extraction. In addition, there are limited data reporting cocoa butter extraction optimization with respect to the effects of flow rate and other variables on the composition of TG and FA in butter (Tan et al. 2008).

With respect to cocoa butter extraction using $scCO_2$, flow rate was studied to allow cocoa butter to reach equilibrium solubility in $scCO_2$. The extraction of cocoa butter has been pronounced with $scCO_2$ flow rate. An enhancement in the recovery and solubility of cocoa butter can be achieved using appropriate pressure and temperature. Machmudah et al. (2006) reported that the extraction yield of carotenoids obtained from the optimal processing of Rosehip fruit using $scCO_2$ was increased by flow rate, pressure and temperature. As expected, the extraction rate constant increased with increasing pressure and flow rate. In order to develop cocoa butter extraction processing using $scCO_2$ and to determine the efficiency of extraction process, the variables involved in the extraction process (i.e., flow rate, pressure and temperature of extraction) should be studied. The aim of this study was to investigate the effects of $scCO_2$ extraction process variables, such as flow rate, pressure, and temperature on the yield of cocoa butter. The study also estimates the solubility and selectivity of TG and FA methyl esters.

Materials and Methods

Materials

Cocoa liquor was purchased from K.L. Kepong Sdn. Bhd., Port Klang, Malaysia. Liquid CO_2 with a purity of 99.9 % was obtained from Malaysian Oxygen (MOX), Petaling Jaya, Selangor, Malaysia. Chromatography grade solvent was used in high pressure liquid chromatography (HPLC) and gas chromatography (GC) analysis. Standard of triglycerides, fatty acid methyl esters, ethanol, isopropanol, acetone, ethanol, ethylene glycol, petroleum ether, methanol and acetonitrile were purchased from Sigma, Merck, and Fisher (Malaysia).

Supercritical fluid extraction (SFE) method for studying the effects of flow rate, pressure and temperature

This experiment used supercritical fluid extraction with pure CO_2 as a solvent ($scCO_2$). The SFE apparatus consisted of an Intelligent HPLC pump (model PU-1580, Jasco Corporation, Tokyo, Japan) fitted with a cooling jacket to deliver CO2. The detailed procedure for SFE process was discussed in our previous report (Asep et al. 2013). The liquid carbon dioxide was compressed to the desired pressure of 20 and 35 MPa and then continuously pumped through the extractor at 0.5 (f1), 1.0 (f2), 2.0 (f3) and 4.0 (f4) mL min^{-1} of flow rates.

The volume of gas that flowed through the system was calculated by the following equation:

$$\text{Weight of } CO_2\text{gas used(g)} = t \times f \times \delta \quad (1)$$

where.

*t*is the extraction time (min),

*f*is the CO_2 gas flow rate (mL min^{-1}), and

$\delta$is the density of CO_2 gas at 20 and 35 MPa and 60 °C (g/mL).

Determination of yield

The determination of the total yield of the extracted cocoa butter was carried out according to the method described in our previous report (Asep et al. 2013).

Determination of triglycerides (TG)

Triglycerides composition was determined by HPLC according the AOCS method (1993). The detailed procedure for triglycerides analysis was discussed in our previous report (Asep et al. 2013).

Determination of fatty acid methyl esters (FAMEs)

The fatty acids (FA) composition of fat mixtures was determined as fatty acid methyl esters (FAMEs) using gas chromatography and Restek Rtx-2330 column (30 m; 0.25 mm; 0.2 μm). The detailed procedure for fatty acid analysis was discussed in our previous report (Asep et al. 2013).

Selectivity (α)

The effects of $scCO_2$ process variables such as flow rate, pressure, and temperature on TG profile and fatty acid (FA) composition of cocoa butter extracted was determined by estimating the selectivity (α) as function of extraction time. As shown in the following equation, the selectivity was calculated based on the mass balance of TG and FA concentration in the sample and the extract phase:

$$(\boldsymbol{\alpha}) = \frac{[(X_1)_{Ex}] / [(X_1)_S]}{[(X_2)_{Ex}] / [(X_2)_S]} \quad (3)$$

where $(X_1)_{Ex}$ and $(X_2)_{Ex}$ denote as concentration of two different TGs or FAs in the extract phase (supercritical fluid); while $(X_1)_S$ and $(X_2)_S$ are considered as concentration of two different TGs or FAs in the solid phase (soxhlet extraction) (Li and Hartland 1992; AOCS 1993; Rossi 1996). In this research, the selectivity (α) of TG was calculated by comparison of TG relative to 1-palmitoyl-3-stearoyl-2-oleoyl-glycerol (POS) (C52) as the major TG of cocoa butter. Similarly, the selectivity (α) of FA was calculated by comparison of FA relative to stearic acid (C18:0) as the major FA of cocoa butter.

Statistical analysis

Statistical analysis was carried out using the Statistical Analysis System (SAS 1999) Version 8 (TS M1, SAS Institute Inc., Cary, NC, USA) for analysis of variance (ANOVA) and significant differences between means for all treatments (Duncan's multiple range test) at a level of $P < 0.05$.

Results and Discussion

Effects of flow rate, pressure, and temperature on cocoa butter yield

In an attempt to investigate the effect of solvent flow rate on cocoa butter extraction from cocoa liquor, a set of experiments was carried out at 50 and 60 °C temperature, 20 and 35 MPa pressure and the flow rate of supercritical carbon dioxide ($scCO_2$) was varied at 0.5 (f1), 1.0 (f2), 2.0 (f3) and 4.0 (f4) mL min^{-1}. Figures 1(a), 2(a), 3(a) and 4(a) show the

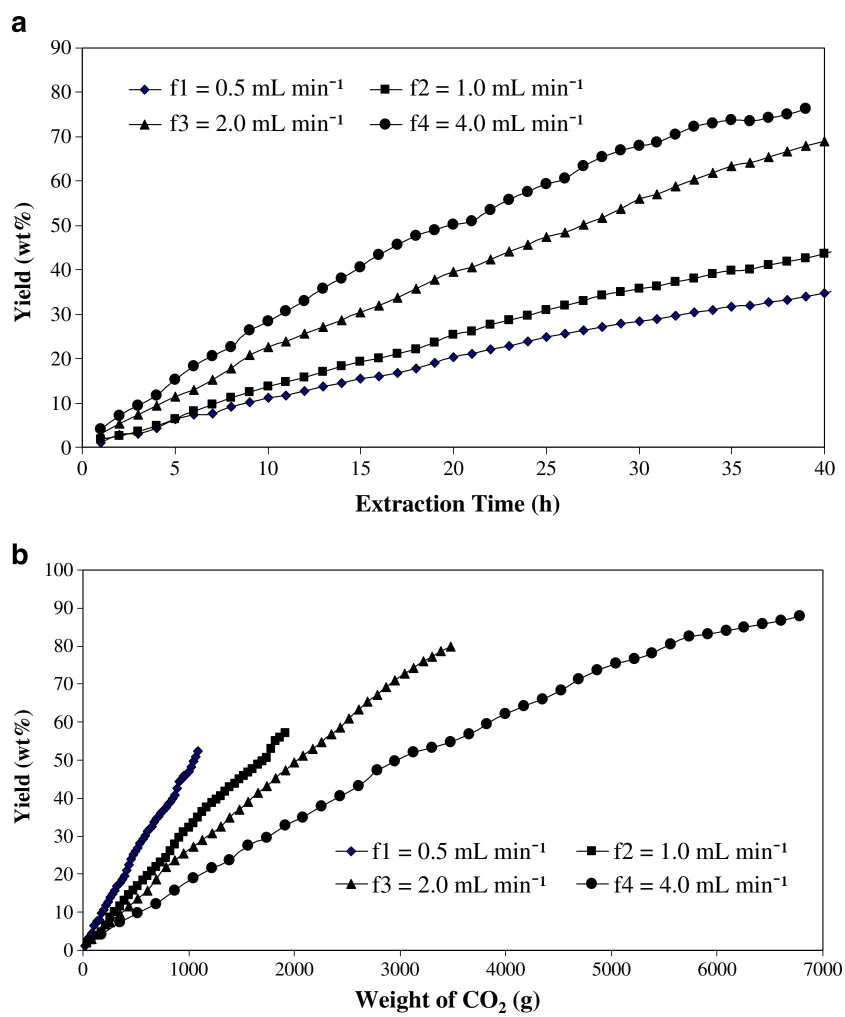


Fig. 1 Effect of flow rate on cocoa butter extraction from cocoa liquor using $scCO_2$ at 20 MPa and 50 °C, **a** yield (wt%) versus extraction time (h), **b** yield (wt%) versus weight of CO_2(g). Standard deviations of the reported results are in the range of 0.08 to 1.21, 0.07 to 1.06, 0.09 to 1.14 and 0.09 to 1.11 for f1, f2, f3 and f4 curves, respectively

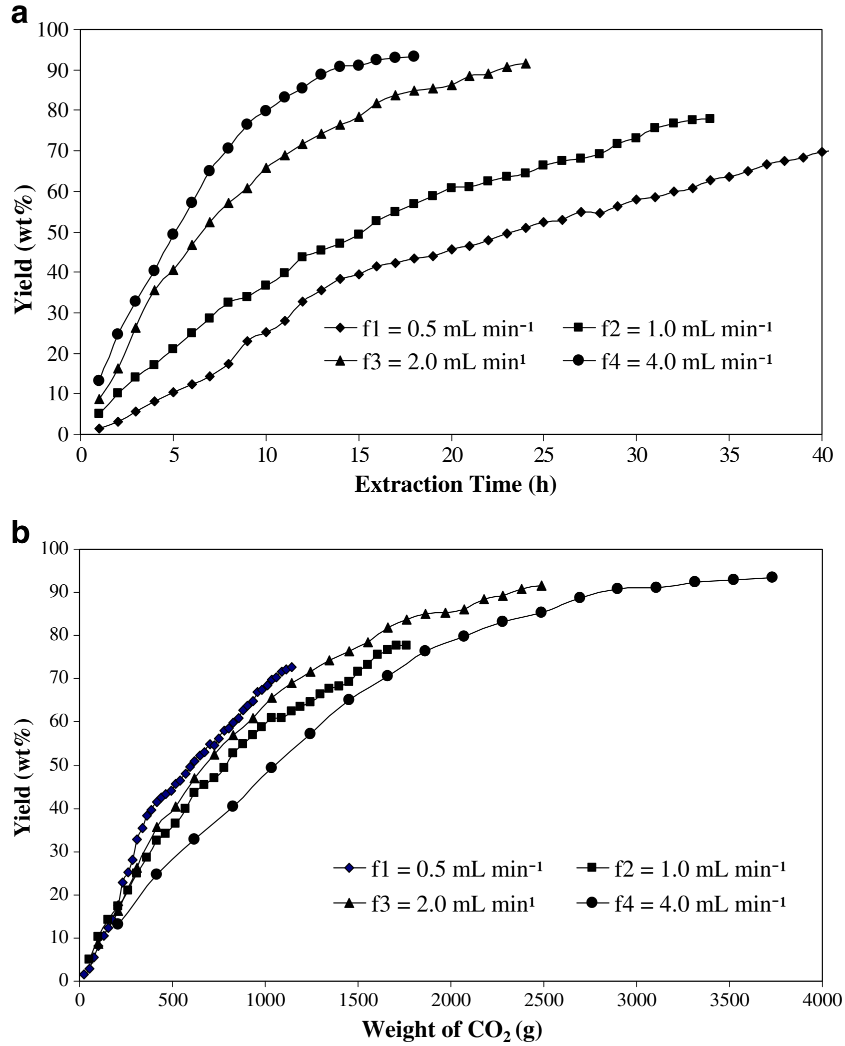


Fig. 2 Effect of flow rate on cocoa butter extraction from cocoa liquor using scCO2 at 35 MPa and 50 °oC, a yield (wt%) versus extraction time (h), b yield (wt%) versus weight of CO2(g). Standard deviations of the reported results are in the range of 0.09 to 1.40, 0.07 to 1.04, 0.10 to 1.18 and 0.08 to 1.21 for f1, f2, f3 and f4 curves, respectively

plots for the effect of the $scCO_2$ flow rate on the yield of cocoa butter extracted as a function of extraction time at 20 MPa, 35 MPa, 50 °C and 60 °C, respectively. It can be observed that cocoa butter yield significantly increased with the CO_2 flow rate. At 20 and 35 MPa, a flow rate of 4.0 mL min^{-1} (f4) showed the highest yield, followed by 2.0, 1.0 and 0.5 mL min^{-1}. At 20 MPa, 60 °C and 20 h extraction time, a flow rate of 4.0 mL min^{-1} resulted in the highest yield of 54.80 %, followed by 2.0, 1.0 and 0.5 mL min^{-1} yielding 42.23, 27.76 and 22.48 % of cocoa butter, respectively. An increase in pressure up to 35 MPa at 60 °C, resulted in significant increase in the yield at 20 h extraction time. A flow rate of 4.0 mL min^{-1} resulted in nearly 100 % yield, followed by 2.0, 1.0 and 0.5 mL min^{-1}, which produced yields of 89.40, 66.22 and 50.88 %, respectively. Therefore, it can be observed that cocoa butter extraction yield increases with $scCO_2$ pressure. In addition, it can be observed from the results that cocoa butter yield significantly increased with increase in extraction temperature. The $scCO_2$ extraction process at the 60 °C produced a higher cocoa butther yield compared to 50 °C. Jun-ping et al. (2012) reported that the extraction yield of seed oil from *Nigella glandulifera* Freyn using $scCO_2$ was increased with increasing flow rate, pressure and temperature.

Figures 1(b), 2(b), 3(b) and 4(b) show the effect of the flow rate on cocoa butter yield as a function of the amount of CO_2 used at pressures of 20 and 35 MPa and temperatures of 50 and 60 °C, respectively. Figures 1(b) and 3(b) show that at 20 MPa, the amount of cocoa butter extracted significantly decreased with CO_2 flow rate using the same amount of CO_2. At 20 MPa and 60 °C, when 1000 g CO_2 was used for cocoa butter extraction, the highest amount of the extract obtained was 47.08 % at a flow rate 0.5 mL min^{-1}, followed by 32.32, 26.36 and 18.16 % yield at 1.0, 2.0 and 4.0 mL min^{-1}flow rate, respectively. Furthermore, Fig. 4(b) shows that the use of 1000 g CO_2 for the extraction of cocoa butter at 35 MPa and 60 °C, resulted in higher yields as compared to those under other

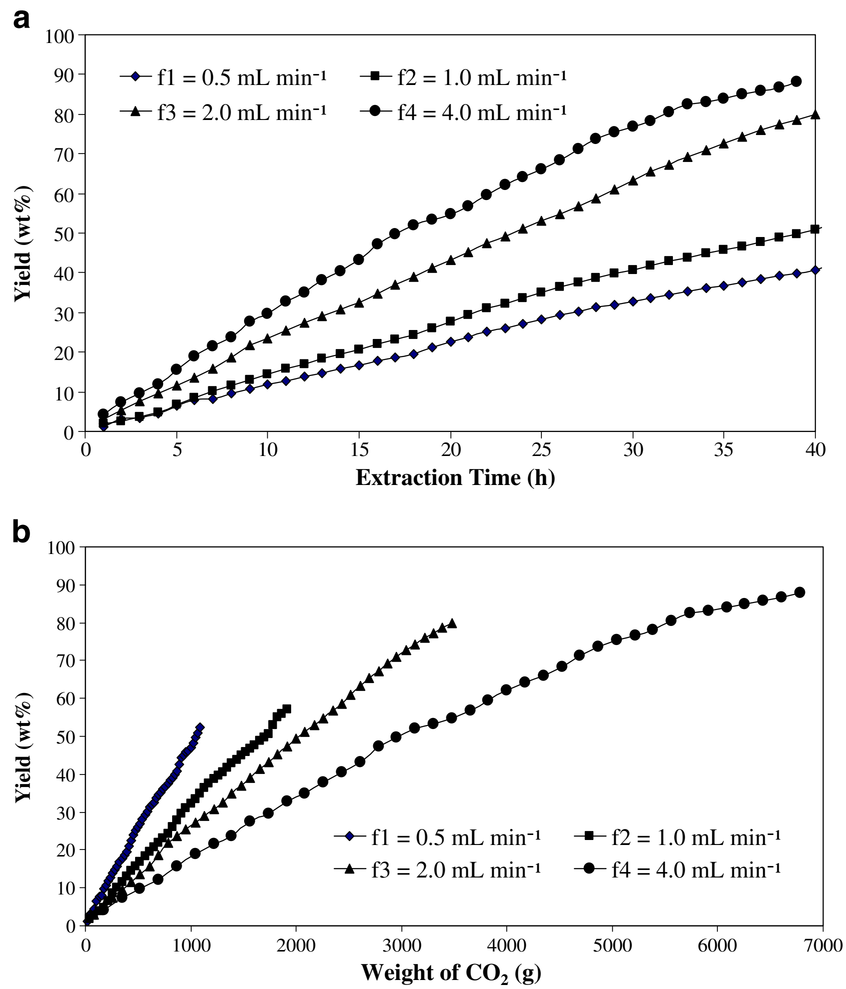


Fig. 3 Effect of flow rate on cocoa butter extraction from cocoa liquor using $scCO_2$ at 20 MPa and 60 °C, **a** yield (wt%) versus extraction time (h), **b** yield (wt%) versus weight of CO_2(g). Standard deviations of the reported results are in the range of 0.08 to 1.27, 0.06 to 1.09, 0.10 to 1.44 and 0.09 to 1.10 for f1, f2, f3 and f4 curves, respectively

conditions (20 MPa, 50 °C; 20 MPa, 60 °C; 35 MPa, 50 °C), with the highest amount of extracts obtained being 76.04 % at flow rate of 0.5 mL min^{-1}, followed by 67.06, 64.73, 46.88 % at 2.0, 1.0 and 4.0 mL min^{-1}, respectively

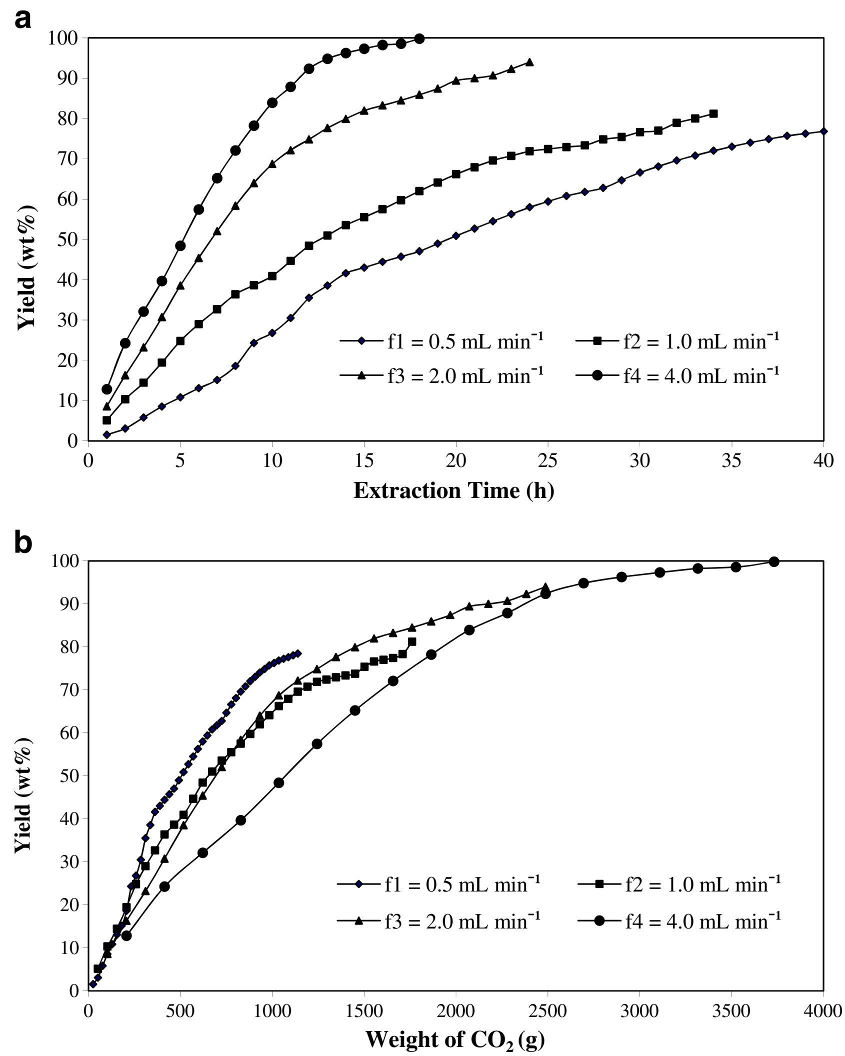
On the other hand, from Fig. 4(b), it can be observed that a 70 % yield was obtained when 837.5 g of CO_2 was used at a flow rate of 0.5 mL min^{-1}, followed by 1158.4, 1075.1, and 1595.2 g at 1.0, 2.0, and 4.0 mL min^{-1}, respectively. A flow rate of 0.5 mL min^{-1} consumed less CO_2 than the other flow rates. CO_2 consumption at a flow rate of 2.0 mL min^{-1} was very close to that at a flow rate of 1.0 mL min^{-1}. Interestingly, the graph of the optimum flow rate of 2.0 mL min^{-1} at 35 Mpa was observed to overlap (crossover) with that of 1.0 mL min^{-1} flow rate; indicating that the equilibrium solubility limit had been achieved, and the interaction between solute and solvent may have been optimum.

From Fig. 4(b), we can see that the flow rate of 2.0 mL min^{-1} shows a better yield than 1.0 and 4.0 mL min^{-1}. Although 0.5 mL min^{-1} resulted in the highest yield with a consumption of 1000 g CO_2, it required 38.5 h; thus, the extraction time used was longer as compared to 1.0 and 2.0 mL min^{-1} flow rates, which required extraction time of 19.5 and 9.5 h, respectively. Although 4.0 mL min^{-1} showed the highest yield, the yield can be decreased by the residence time of CO_2 in the extraction vessel, and therefore, the time of contact between the solvent and sample was shorter; however, 4.0 mL min^{-1} require more CO_2 (1595.2 g CO_2 at 70 % yield) for extraction. Therefore, 2.0 mL min^{-1} was considered the optimum CO_2 flow rate that can provide the greatest effect on the cocoa butter extraction performance.

Effects of flow rate, pressure, and temperature on triacylglycerol

Triacylglycerols (TGs) are the major component present in cocoa butter, making up 92 to 96 % of the lipid composition (Jahurul et al. 2013). Tables 1 and 2 present the results of the TG analysis of cocoa butter obtained at 0.5 to 4.0 mL min^{-1} flow rate, 20 and 35 MPa pressure and 50 and 60 °C

Fig. 4 Effect of flow rate on cocoa butter extraction from cocoa liquor using scCO2 at 35 MPa and 60 °oC, a yield (wt%) versus extraction time (h), b yield (wt%) versus weight of CO2(g). Standard deviations of the reported results are in the range of 0.09 to 1.43, 0.09 to 1.27, 0.11 to 1.55 and 0.07 to 1.19 for f1, f2, f3 and f4 curves, respectively



temperature using $scCO_2$. The results show that cocoa butter extracted from various treatments had three main TGs; POS, 1,3-dipalmitoyl-2-oleoyl-glycerol (POP) and 1,3-distearoyl-2-oleoyl-glycerol (SOS). The percentage of each TG composition was significantly different ($P < 0.05$) among various flow rates and pressures, except for POS. POS was identified as the major TG component in 42.26 to 45.12 % cocoa butter yields, followed by SOS and POP with 27.05 to 30.67 % and 21.05 to 23.64 %, respectively. These findings were in good agreement with previously reported data on the typical TG composition of cocoa butter (Chaiseri and Dimick 1989; Lipp et al. 2001; Spangenberg and Dionisi 2001; Ali et al. 2001; Tan et al. 2008; Asep et al. 2008, 2013; Jinap et al. 2013). Cocoa butter contains high amounts of symmetrical monounsaturated TG, in particular 1,3-dipalmitoyl-2-oleoylglycerol (POP), 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS) and 1,3-distearoyl-2-oleoyl-glycerol (SOS). These TGs are of particular interest, since they are mainly responsible for the desired properties of chocolate, i.e. crystallization and melting behavior.

As can be seen in Tables 1 and 2, at the same pressure and temperature, POP and POS generally decrease with flow rate and extraction time; in contrast, SOS increase with flow rate and extraction time. When the pressure and temperature are higher, POP and POS decrease with an increase in flow rate and extraction time; however, SOS increase with flow rate and extraction time. In general, POP, POS and SOS showed higher solubility at a higher pressure of 35 MPa and a temperature of 60 °C compared to 20 MPa and 50 °C. This result may be explained by the fact that the increase in pressure and temperature cause an increase the TG solubility. These results show the progressive extraction of cocoa butter; the more soluble POP triglyceride (MW slightly smaller) is easily extracted in the early stages, followed by POS, and the less soluble SOS (MW slightly larger) is removed in the late stages. This behavior is supported by Rossi et al. (1989) who also investigated the composition of cocoa butter by $scCO_2$ of various cocoa products and, observed minor differences in the

Table 1 Triacylglycerol composition (area %)[α] of cocoa butter extracted by supercritical carbon dioxide ($scCO_2$) for effect of flow rate (f) with different pressures at 50 °C

Sample[β]	5 h Extraction Time				10 h Extraction Time				15 h Extraction Time			
	POP	POS	SOS	Others[γ]	POP	POS	SOS	Others[γ]	POP	POS	SOS	Others[γ]
f1 (0.5 mL min^{-1}; 20 MPa)	22.75 ± 0.33	44.31 ± 0.54	27.99 ± 0.70	4.95 ± 0.06	22.67 ± 0.39	44.10 ± 0.65	28.36 ± 0.33	4.87 ± 0.06	22.89 ± 0.31	42.64 ± 0.56	29.67 ± 0.54	4.80 ± 0.04
f2 (1.0 mL min^{-1}; 20 MPa)	22.34 ± 0.41	44.21 ± 0.53	28.61 ± 1.15	4.83 ± 0.13	22.13 ± 0.43	44.01 ± 0.62	29.10 ± 0.51	4.76 ± 0.14	22.87 ± 0.82	42.35 ± 0.53	30.10 ± 0.78	4.69 ± 0.09
f3 (2.0 mL min^{-1}; 20 MPa)	22.47 ± 0.93	44.13 ± 0.97	28.53 ± 0.69	4.86 ± 0.43	22.07 ± 0.92	43.67 ± 1.08	29.48 ± 0.31	4.78 ± 0.44	22.43 ± 1.68	42.26 ± 0.87	30.60 ± 0.43	4.71 ± 0.25
f4 (4.0 mL min^{-1}; 20 MPa)	22.01 ± 1.35	44.08 ± 1.20	28.61 ± 0.52	5.30 ± 0.23	21.74 ± 1.49	43.36 ± 1.49	29.96 ± 0.38	4.94 ± 0.27	22.19 ± 2.78	42.28 ± 1.34	30.67 ± 0.61	4.87 ± 0.17
f5 (0.5 mL min^{-1}; 35 MPa)	22.86 ± 1.34	44.64 ± 1.33	27.66 ± 0.68	4.84 ± 0.68	22.85 ± 1.79	44.27 ± 1.80	28.11 ± 0.61	4.77 ± 0.95	22.78 ± 2.59	44.03 ± 1.95	28.50 ± 1.17	4.69 ± 0.40
f6 (1.0 mL min^{-1}; 35 MPa)	23.01 ± 1.49	44.20 ± 1.40	27.85 ± 1.47	4.94 ± 0.53	22.71 ± 1.29	44.06 ± 1.23	28.37 ± 0.85	4.86 ± 0.48	22.64 ± 1.22	43.99 ± 0.87	28.58 ± 1.07	4.79 ± 0.13
f7 (2.0 mL min^{-1}; 35 MPa)	23.20 ± 1.32	43.65 ± 0.92	28.25 ± 1.17	4.90 ± 0.72	22.45 ± 1.55	43.96 ± 1.09	28.76 ± 0.92	4.83 ± 0.87	22.46 ± 1.83	43.96 ± 1.04	28.82 ± 1.56	4.76 ± 0.33
f8 (4.0 mL min^{-1}; 35 MPa)	23.64 ± 0.20	42.56 ± 0.40	28.73 ± 0.65	5.07 ± 0.05	22.04 ± 0.19	43.77 ± 0.39	29.19 ± 0.26	5.00 ± 0.05	22.43 ± 0.18	43.51 ± 0.40	29.14 ± 0.27	4.93 ± 0.05

[α] Means value ± standard deviation of three replications

[β] Cocoa butter extracted obtained by $scCO_2$ at 50 °C with different flow rate and pressure

[γ] PLiO, PLiP, POO, SOO, and SOA where P = palmitic, O = oleic, S = stearic, Li = linoleic, A = arachidic

selectivity of the process for TG of cocoa butter, namely, POP (C50), POS (C52) and SOS (C54). At 50 °C and 40 MPa, the average selectivity of POP relative to POS was 1.05, whereas that of SOS relative to POS was 0.94. Consequently, Rossi et al. (1989) hypothesized that shorter TGs are more easily extracted than longer ones.

Ragunath et al. (1992, 1993), Hassan et al. (2000, 2001), Markom et al. (2001), Norulaini et al. (2004) and Zaidul et al. (2006) fractionated triglycerides of fatty oil components based on their carbon number using $scCO_2$ at a temperature range of 40 to 80 °C and pressures up to 50 MPa. Researchers reported a low solubility of TGs rich cruide palm oil in $scCO_2$ at relatively low temperature and low pressure while at any temperature and pressure the short chain fatty acids are eluted fast followed by medium and long chain.

Arul et al. (1994) reported that the solubilities of short-, medium- and long chain TGs increased with pressure, temperature and flow rate of CO_2. Another important factor that must be considered is the pressure dependence of the dissolving power of supercritical fluids (Medir and Giralt 1982; Bling and Franck 1983). At constant temperature the volatility or fugacity of the TGs phase increases with pressure. Perry et al. (1949) reported that the vapor pressure of TG decreases with their carbon number. Therefore, the short chain TGs are, in general, more volatile than the long chain molecules. The concentration of long-chain TG is higher than the short chain in milk fat at $scCO_2$ reported by Arul et al. (1994).

Effects of flow rate, pressure, and temperature on fatty acid methyl ester

The results of fatty acid methyl esters obtained from gas chromatography analysis are shown in Tables 3 and 4. Palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1) where the three main fatty acids in the cocoa butter extracted using $scCO_2$, with C18:0 of 32.53 to 38.92 % being the major component, followed by C18:1 with 30.62 to 33.06 % and C16:0 with 25.94 to 31.49 %. It can be seen from Tables 3 and 4 that the FA compositions of cocoa butter were significantly affected ($P < 0.05$) by flow rate with different pressures and temperatures. These findings are in good agreement with the typical FA compositions of cocoa butter obtained by Chaiseri and Dimick (1989), Lipp et al. (2001), Spangenberg and Dionisi (2001), Ali et al. (2001), Tan et al. (2008), Asep et al. (2008, 2013) and Jinap et al. (2013).

In addition, at the same pressure, C16:0 and C18:2 generally decreased with an increase in flow rate and extraction time, whereas C18:0 and C18:1 increased with the increase in flow rate and decreased with an increase in extraction time.

The results also show that when the pressure increased, in general C16:0 decreased with an increase in flow rate and

Table 2 Triacylglycerol composition (area %)[α] of cocoa butter extracted by supercritical carbon dioxide ($scCO_2$) for effect of flow rate (f) with different pressures at 60 °C

Sample [β]	5 h Extraction Time				10 h Extraction Time				15 h Extraction Time			
	POP	POS	SOS	Others[γ]	POP	POS	SOS	Others[γ]	POP	POS	SOS	Others[γ]
f1	22.83	44.70	27.54	4.93	23.41	44.12	27.07	4.88	23.12	43.96	28.09	4.83
(0.5 mL min^{-1}; 20 MPa)	±0.25	±0.56	±0.65	±0.07	±0.37	±0.45	±0.35	±0.07	±0.21	±0.45	±0.38	0.05
f2	22.80	44.66	27.72	4.82	23.22	43.97	28.04	4.77	23.10	43.66	28.52	4.72
(1.0 mL min^{-1}; 20 MPa)	±0.31	±0.54	±1.22	±0.19	±0.43	±0.41	±0.62	±0.19	±0.55	±0.41	±0.65	0.13
f3	22.70	44.58	27.87	4.85	22.89	43.82	28.48	4.80	22.43	43.12	29.69	4.75
(2.0 mL min^{-1}; 20 MPa)	±0.77	±1.00	±0.73	±0.60	±1.02	±0.72	±0.37	±0.57	±1.22	±0.67	±0.37	0.39
f4	22.01	44.33	28.36	5.30	21.64	43.58	29.82	4.97	21.97	42.70	30.41	4.92
(4.0 mL min^{-1}; 20 MPa)	±1.11	±1.23	±0.55	±0.33	±1.65	±0.99	±0.59	±0.34	±2.02	±1.03	±0.65	0.26
f5	22.98	45.12	27.05	4.85	23.47	44.41	27.32	4.80	23.24	44.12	27.89	4.75
(0.5 mL min^{-1}; 35 MPa)	±1.10	±1.57	±0.72	±0.96	±1.98	±1.53	±0.94	±1.22	±1.89	±1.93	±1.26	0.63
f6	22.91	44.75	27.39	4.95	22.75	44.44	27.91	4.90	22.52	43.99	28.63	4.85
(1.0 mL min^{-1}; 35 MPa)	±1.36	±1.65	±1.55	±0.75	±1.58	±1.04	±1.31	±0.62	±0.98	±0.86	±1.14	0.21
f7	22.85	44.70	27.52	4.92	22.69	44.39	28.05	4.87	22.47	43.94	28.77	4.82
(2.0 mL min^{-1}; 35 MPa)	±1.21	±1.09	±1.24	±1.01	±1.90	±0.93	±1.42	±1.13	±1.47	±1.03	±1.67	0.51
f8	21.05	44.24	29.61	5.10	21.61	43.93	29.41	5.05	21.88	43.49	29.63	5.00
(4.0 mL min^{-1}; 35 MPa)	±0.20	±0.40	±0.65	±0.05	±0.19	±0.39	±0.26	±0.05	±0.18	±0.40	±0.27	0.05

[α] Means value ± standard deviation of three replications

[β] Cocoa butter extracted obtained by $scCO_2$ at 60 °C with diferent flow rate and pressure

[γ] PLiO, PLiP, POO, SOO, and SOA where P = palmitic, O = oleic, S = stearic, Li = linoleic, A = arachidic

extraction time; however, C18:1 and C18:2 in general increased with an increase in flow rate and a decrease in extraction time; and the C18:0 percentage increased with an increase in flow rate and extraction time. It is interesting to note that the solubility of FAs increased with an increase in pressure and temperature. At a pressure of 35 MPa, the solubility of FA with larger MW is higher because higher pressure provides greater solvating power and enhances the interaction between CO_2 solvents with cocoa butter.

On the other hand, based on the solubility of FA in $scCO_2$, it was found that at lower pressures (20 MPa), the short chain fatty acid constituents (C16) were extracted with higher selectivity than the long chain fatty acid constituents (C18:0 and C18:1). At higher pressures (35 MPa), the C16:0 started to reduce, and the C18:0 and C18:1 constituents increased. This indicates that more of the shorter chain fatty acid (C16:0) was extracted than the long chain fatty acid constituents (C18:0 and C18:1) at lower pressures (20 Mpa), whereas more of the longer chain fatty acids (C18:0 and C18:1) were extracted than the 16:0 at higher pressures. Stahl et al. (1988) reported that the solubility of fatty acids in $scCO_2$ extraction depends on the length of the hydrocarbon chain and the presence of functional groups. Moreover, it is also depends on the effect of the extraction parameters, pressure and temperature (Stahl et al. 1988). This behavior is similar to the findings of previous works investigating the extraction and fractionation of fatty acids from palm kernel oil (PKO) in $scCO_2$, as reported by Norulaini et al. (2004) and Zaidul et al. (2006, 2007a, b).

Conclusion

The effects of different flow rate, pressures and temperatures on cocoa butter extraction using scCO2 were investigated. The results indicated that the extraction yield is significantly ($P < 0.05$) influenced by the variables and the yields was increased with flow rate, pressure, and temperature where the optimum values were 2 mLMin^{-1}, 35 Mpa and 60 °C, respectively. The solubility of FAs and TGs were also increased with pressure and temperature. TG and FA compositions were found to be similar to those of cocoa butter obtained by conventional methods. In terms of the selectivity, the lower molecular weight TGs and FAs showed higher selectivity compared to higher molecular weight TGs and FAs. This indicates that POP was more easily extracted at the beginning of the extraction process, while the amount of POS, followed by that of SOS, was found to be higher at the end of the extraction process. In contrast, C16:0 and C18:0 were separated more easily than C18:1, and C18:2 during extraction. Thus, they were fractionated during the first stage of the $scCO_2$ extraction process.

Table 3 Fatty acids composition (area %)$^{\alpha}$ changes of cocoa butter extracted by supercritical carbon dioxide ($scCO_2$) for effect of flow rate (f) with different pressures at 50 °C

Sample $^{\beta}$	5 h Extraction Time					10 h Extraction Time					15 h Extraction Time				
	C16:0	**C18:0**	**C18:1**	**C18:2**	**Others$^{\gamma}$**	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**Others$^{\gamma}$**	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**Others$^{\gamma}$**
f1	29.08	36.51	30.72	2.40	1.29	28.64	36.57	31.15	2.37	1.27	26.49	38.60	31.54	2.19	1.18
(0.5 mL min^{-1}; 20 MPa)	±0.35	±0.64	±0.64	±0.11	±0.07	±0.41	±0.61	±0.79	±0.23	±0.08	±0.27	±0.86	±0.47	±0.04	±0.10
f2	28.55	36.66	31.38	2.29	1.13	28.33	36.68	31.63	2.25	1.11	26.01	38.87	32.01	2.09	1.03
(1.0 mL min^{-1}; 20 MPa)	±0.43	±0.62	±1.20	±0.29	±0.26	±0.48	±0.56	±1.40	±0.58	±0.28	±0.71	±0.78	±0.81	±0.09	±0.23
f3	28.32	36.84	31.55	2.14	1.15	28.14	36.75	31.87	2.11	1.13	26.12	38.77	32.12	1.95	1.05
(2.0 mL min^{-1}; 20 MPa)	±0.90	±1.14	±0.72	±0.95	±0.98	±0.96	±0.98	±0.80	±1.79	±0.80	±1.17	±0.42	±1.54	±0.24	±0.36
f4	28.11	36.98	31.86	1.97	1.08	28.05	36.84	32.11	1.94	1.06	25.94	38.92	32.36	1.80	0.98
(4.0 mL min^{-1}; 20 MPa)	±1.31	±1.40	±0.54	±0.51	±0.92	±1.16	±1.15	±0.57	±0.92	±0.72	±1.39	±0.34	±0.88	±0.24	±0.47
f5	31.33	33.11	31.05	2.83	1.68	31.18	32.74	31.60	2.81	1.67	30.09	33.44	32.15	2.71	1.61
(0.5 mL min^{-1}; 35 MPa)	±1.17	±1.80	±0.71	±1.23	±1.12	±0.98	±1.40	±0.71	±2.10	±0.83	±1.32	±0.80	±1.09	±0.24	±0.54
f6	30.14	34.37	31.43	2.57	1.49	29.99	33.99	31.98	2.56	1.48	28.94	34.63	32.53	2.47	1.43
(1.0 mL min^{-1}; 35 MPa)	±1.19	±1.89	±1.53	±0.96	±0.87	±0.95	±1.39	±0.86	±1.56	±0.61	±0.85	±0.63	±0.52	±0.12	±0.23
f7	29.82	34.67	31.51	2.49	1.50	29.67	34.30	32.06	2.48	1.50	28.63	34.93	32.61	2.39	1.44
(2.0 mL min^{-1}; 35 MPa)	±1.06	±1.25	±1.22	±1.30	±0.92	±0.80	±0.87	±0.65	±2.00	±0.61	±0.67	±0.64	±0.91	±0.16	±0.24
f8	29.43	34.84	31.74	2.49	1.50	29.28	34.46	32.28	2.48	1.50	28.26	35.08	32.83	2.39	1.44
(4.0 mL min^{-1}; 35 MPa)	±0.94	±0.82	±0.98	±1.75	±0.96	±0.68	±0.55	±0.50	±2.56	±0.61	±0.53	±0.66	±1.12	±0.21	±0.26

$^{\alpha}$ Means value ± standard deviation of three replications

$^{\beta}$ Cocoa butter extracted obtained by $scCO_2$ at 50 °C with diferent flow rate and pressure

$^{\gamma}$ C12:0, C14:0 and C18:3

Table 4 Fatty acids composition (area %)[α] changes of cocoa butter extracted by supercritical carbon dioxide ($scCO_2$) for effect of flow rate (f) with different pressures at 60 °C

Sample [β]	5 h Extraction Time					10 h Extraction Time					15 h Extraction Time				
	C16:0	**C18:0**	**C18:1**	**C18:2**	**Others[γ]**	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**Others[γ]**	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**Others[γ]**
f1	29.67	35.94	30.62	2.45	1.32	29.23	36.19	30.87	2.41	1.30	27.03	38.41	31.12	2.23	1.20
(0.5 mL min^{-1}; 20 MPa)	±0.24	±0.63	±0.57	±0.12	±0.05	±0.36	±0.51	±0.74	±0.09	±0.03	±0.32	±0.99	±0.24	±0.05	±0.02
f2	29.13	36.07	31.32	2.34	1.15	28.69	36.31	31.57	2.30	1.13	26.54	38.47	31.82	2.13	1.05
(1.0 mL min^{-1}; 20 MPa)	±0.30	±0.61	±1.07	±0.32	±0.16	±0.42	±0.47	±1.31	±0.23	±0.11	±0.84	±0.90	±0.41	±0.12	±0.05
f3	28.96	36.10	31.62	2.16	1.16	28.52	36.34	31.87	2.13	1.14	26.38	38.47	32.12	1.97	1.06
(2.0 mL min^{-1}; 20 MPa)	±0.74	±1.12	±0.64	±1.03	±0.63	±1.00	±0.82	±0.75	±0.70	±0.42	±1.38	±0.48	±1.20	±0.43	±0.08
f4	28.76	36.30	31.86	1.99	1.09	28.33	36.53	32.11	1.96	1.07	26.20	38.63	32.36	1.81	0.99
(4.0 mL min^{-1}; 20 MPa)	±1.07	±1.38	±0.48	±0.56	±0.59	±1.38	±0.96	±0.53	±0.36	±0.37	±1.65	±0.39	±0.69	±0.43	±0.11
f5	31.49	32.90	31.08	2.84	1.69	31.33	32.53	31.63	2.83	1.68	30.24	33.23	32.18	2.73	1.62
(0.5 mL min^{-1}; 35 MPa)	±1.06	±1.77	±0.63	±1.64	±1.69	±1.29	±1.17	±0.67	±1.00	±1.01	±1.56	±0.92	±1.50	±1.02	±0.12
f6	30.14	34.30	31.50	2.57	1.49	29.99	33.93	32.05	2.56	1.48	28.94	34.57	32.60	2.47	1.43
(1.0 mL min^{-1}; 35 MPa)	±1.30	±1.86	±1.36	±1.28	±1.32	±1.51	±1.16	±1.37	±0.74	±0.75	±1.01	±1.23	±0.72	±0.49	±0.05
f7	29.52	34.85	31.67	2.47	1.49	29.38	34.47	32.22	2.45	1.48	28.35	35.08	32.77	2.37	1.43
(2.0 mL min^{-1}; 35 MPa)	±1.16	±1.23	±1.09	±1.73	±1.38	±1.28	±0.73	±1.04	±0.95	±0.75	±0.79	±1.26	±1.25	±0.66	±0.05
f8	29.14	34.99	31.96	2.44	1.47	28.99	34.60	32.51	2.43	1.47	27.98	35.20	33.06	2.34	1.41
(4.0 mL min^{-1}; 35 MPa)	±0.54	±0.45	±0.36	±0.02	±0.08	±0.26	±0.36	±0.25	±0.02	±0.01	±0.26	±0.34	±0.26	±0.02	±0.01

[α] Means value ± standard deviation of three replications

[β] Cocoa butter extracted obtained by $scCO_2$ at 60 °C with diferent flow rate and pressure

[γ] C12:0, C14:0 and C18:3

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