Effects of polar cosolvents on cocoa butter extraction using supercritical carbon dioxide


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Abstract
Cocoa butter was successfully extracted from cocoa liquor by supercritical carbon dioxide (SC–CO₂) at 35 MPa, 60 °C and 2 mL/min with 5%, 15% and 25% cosolvents. The extraction yield of triglycerides (TG) and fatty acid (FA) compositions were significantly influenced by the concentration of polar cosolvents. The SC–CO₂ extraction efficiency was increased with cosolvent significantly. Ethanol was found to be the best cosolvent for cocoa butter extraction using SC–CO₂, followed by isopropanol and acetone. The triglycerides of 1,3-dipalmitinyl-2-oleoylglycerol (POP), 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS) and 1,3-dioleoyl-2-stearoyl-glycerol (SOS) were contained in the extracted cocoa butter with POS being the major component. Where palmitic, stearic and oleic were the main fatty acids in the cocoa butter samples, with stearic being the highest component. The lower molecular weight (MW) of TGs and FAs showed the higher selectivity compared to the high MW of TGs and FAs. Thus, they were fractionated during the first stage of SC–CO₂ process.

Industrial relevance: The cocoa butter was successfully extracted from cocoa liquor by SC–CO₂ at 35 MPa, 60 °C and 2 mL/min using different concentrations of polar cosolvents (ethanol, isopropanol and acetone). The extraction yield was significantly (p < 0.05) influenced by the concentration of polar cosolvents. Similarly, polar cosolvent concentration had significant (p < 0.05) effects on the TG and FA compositions. Ethanol was found to be the most efficient polar cosolvent for cocoa butter extraction compared to isopropanol and acetone. POS (42.2–45.9%) being the major triglycerides component, followed by SOS (27.6–31.4%) and POP (20.3–22.7%). Palmitic, stearic and oleic acids were the main fatty acids in the extracted cocoa butter, with stearic being the highest (34.9–37.8%), followed by oleic (30.3–31.8%) and palmitic (28.3–30.0%) acids, respectively. The choice of modifiers becomes a great challenge and ethanol was shown to be the best polar cosolvent, and it enhanced the solubility during the cocoa butter extraction by SC–CO₂. This method can be feasibly implemented in the cocoa industry for the production of high quality cocoa butter.

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1. Introduction

Cocoa butter is highly demanded by food, cosmetic and pharmaceutical industries. Mechanical expression and solvent extraction with hexane are generally employed for obtaining cocoa butter. However, there is an increasing concern of the health and safety hazards associated with the use of organic solvents, while expression by hydraulic method often introduces contaminants into the cocoa butter that must be removed later. Greater concern over the disposal of such toxic organic solvents and their effect on the environment has led to a move towards cleaner extraction methods such as supercritical fluid extraction (SFE). Supercritical fluid extraction, mainly by supercritical carbon dioxide (SC–CO₂) is a potential alternative to the customary methods of producing cocoa butter. It offers the advantages of rapid, nontoxic, environmental-friendly, contamination-free and easily manipulated conditions (Li & Hartland, 1996).

The efficiency of SFE process mainly depends on the solvent capacity of supercritical carbon dioxide. This can easily be modified by varying pressure and temperature (Dauksas, Venskutonis, & Sitvik, 1998; Francisco & Dey, 2003; Reverchon & Camillis, 1991). Due to the poor solvent capacity of supercritical CO₂, the addition of small quantity of organic solvent as a cosolvent or entrainer has also been recommended to increase the solubility of the analyte or possibly to increase the separation of coextractives (Dobbs, Wong, Lahiere, & Johnston, 1987; King, 1993a, b; King & France, 1998; Francisco & Dey, 2003; Reverchon & Camillis, 1991).
of nonpolar carbon dioxide is to use another solvent with higher polarity. However, there are several practical limitations. Supercritical ammonia would be very attractive from the view point of solvent strength, but it is difficult to pump since it tends to dissolve pump seal. Furthermore, it is also a chemically reactive solvent, thus making it too dangerous for routine use (Hawthorne, 1990). Other solvents like methanol and isopropanol may also be excellent supercritical solvents, but their high critical temperatures make it difficult to attain the supercritical states. Other cosolvents such as acetone and isopropanol have also been used as the second polar cosolvent for commercial deblocking processes. Hence, a polar modifier is usually added to supercritical carbon dioxide to remove the highly polar organic compounds. The modifier enhances the solubility of SC-CO₂, thereby increasing the extraction efficiency. Moreover, the modifier interacts with the analyte/matrix complex to promote rapid desorption into the supercritical fluid (Luque de Castro & García-Ayuso, 1998).

Ethanol is commonly used as cosolvent or modifier for the extraction of natural products because the toxicity of ethanol to the human body is low (Catchpole, Grey, & Noermark, 1998; Temelli, 1992; Walsh, Greenfield, Ikonomou, & Donohue, 1989; Walsh & Ikonomou, 1987). Furthermore, it can be easily removed from the food matrix although there is a limited information regarding the effect of ethanol and other cosolvents on the SC-CO₂ extraction of cocoa butter. Thus, the objective of this study was to investigate the effect of type and concentration of polar cosolvents (ethanol, isopropanol and acetone) on the extraction efficiency and selectivity, and TG and FA profiles of cocoa butter extracted by using SC-CO₂. The overall quality of extracted cocoa butter was determined by the qualitative and quantitative analyses of TG profile and FA composition. The SC-CO₂ extraction process was carried out at different concentrations (5, 15 and 25%, mol%) of cosolvent.

2. Materials and methods

2.1. Materials

Cocoa liquor samples (size of diameter = ~0.074 mm) were purchased from K.I. Kepong Sdn. Bhd., Port Klang Malaysia. Liquid CO₂ with a purity of 99.9% was obtained from Malaysian Oxygen (MOX), Petaling Jaya, Selangor, Malaysia. Chromatography grade solvents (i.e. petroleum ether, methanol and acetonitrile) and cosolvents (i.e. ethanol, acetone, isopropanol) and standard of triglycerides, fatty acid methyl esters were supplied by Sigma Aldrich Sdn. Bhd. (Petaling Jaya, Malaysia), Chemolab Sdn. Bhd. (Kuala Lumpur, Malaysia) and Fisher Scientific Sdn. Bhd (Shah Alam, Malaysia), respectively.

2.2. Supercritical fluid extraction (SFE) method

The SFE apparatus consisted of 2 intelligent high performance liquid chromatography (HPLC) pump (model PU-1580, Jasco Corporation, Tokyo, Japan), i.e. CO₂ pump and cosolvent pump. The CO₂ pump was fitted with a cooling jacket to deliver CO₂ and cosolvent pump to deliver cosolvents (as modifier/entrainer). In order to cool the pump head of CO₂ pump, ethylene glycol-deionized water mixture (50:50, v/v) was circulated through the cooling jacket using a low temperature bath circulator (model 631D, Tech–Lab Manufacturing Sdn. Bhd., Petaling Jaya, Malaysia) which can deliver coolant down to ~20 °C. A 10 g of sample was loaded into a 50 mL extraction vessel (model EV-3, Jasco Corporation, Tokyo, Japan) that was placed in column oven (model CO-1560, Jasco Corporation, Tokyo, Japan).

The column oven was used to maintain the extraction temperature. Both CO₂ and cosolvent pump were connected to extraction vessel which was placed inside the column oven to maintain the extraction flow rate of fluid. A back pressure regulator (BPR) (model BP-1580-81, Jasco Corporation, Tokyo, Japan) was used to control the extraction pressure. The extraction was carried out at 35 MPa pressure, 60 °C extraction temperature and 2 mL/min flow rate using the mixture of cosolvent and carbon dioxide. Ethanol, isopropanol and acetone were used as polar modifiers at the cosolvent/CO₂ concentration ratios of 5, 15 and 25% (mol%). A 100 mL of blue cup bottle was used as a collection vessel.

The concentrations of cosolvent were determined based on the actual volume of cosolvent delivered into a known volume of liquid carbon dioxide. In this approach, the actual molar percentage of cosolvent to carbon dioxide would change as the density of carbon dioxide varied as the pressure changed. The molar percentage of cosolvent in carbon dioxide was calculated by the following equation:

\[
\text{mol\% cosolvent in CO}_2 = \frac{\text{mol}\% \text{cosolvent in g mixture}}{\text{MW cosolvent}} \cdot \frac{\text{MW CO}_2}{V_{\text{g mixture}}}
\]

where \(V\) is the volume, \(p\) is the density at 35 MPa and 60 °C, and \(MW\) is the molecular weight.

2.3. Determination of yield

The initial weight of the 100 mL test tubes used for collecting the yield of the extracted cocoa butter was measured gravimetrically using 3 digital balance (Mettler-Toledo (M) Sdn Bhd, Selangor, Malaysia). After SFE extraction, the test tubes containing the extracted fat were transferred into a desiccator and held at room temperature until the constant weight was obtained. The residue of cosolvents was evaporated from the extracted fat using a rotary evaporator (model WB/VR 2000, Heidolph, Schwabach, Germany) under vacuum at temperature of 70 °C; the extracted fat was then placed in an oven (model ULM 500, Memmert, Schwabach, Germany) at 45 °C for 30 min and later transferred into the desiccator for drying. Yield was calculated by dividing the percentage of cocoa butter in the samples with 53% factor amount of cocoa butter in cocoa nibs, determined by a Soxhlet method using petroleum ether according to AOAC (AOAC, 1998). The expression of yield was calculated by the following equation:

\[
\text{Yield (wt\% fraction)} = \frac{\text{weight of fat}}{\text{weight of sample}} \times 100\%.
\]

2.4. Determination of triglycerides

Triglycerides composition was determined by HPLC according to the AOCS method (AOCS, 1993). Ten percent cocoa butter solution was prepared using acetone as solvent. The solution was then filtered using TE 36 membrane filter (PTFE; 0.45 μm) (Millipore, Massachusetts, USA) before being filtered through the Sep-Pak Plus Silica cartridge (Waters, Virginia, USA) to discard any impurities. Determination of triglyceride was conducted using Waters (Virginia, USA) HPLC instrument, model 600 controller and model 410 RI detector, and C18 column (3.9 mm id × 300 mm length), with a column temperature of 30–35 °C, column pressure of 5–6 MPa, flow rate of mobile phase (acetone:acetonitrile 75:25 v/v) of 1 mL/min and injection volume of 10 μL. The value of triglycerides was expressed as a percentage. All analyses were done in three replications and duplicate injections.

2.5. Determination of fatty acid methyl esters (FAMES)

The fatty acid composition of fat mixtures was determined as fatty acid methyl esters (FAMES) using gas chromatography (AOCS, 1993). Restek Rtx-2330 column (Restek Corporation, Pennsylvania, USA)
(30 m; 0.25 mm; 0.2 μm) was used. FAMEs were prepared by dissolving 0.05 g sample into 0.8 mL petroleum ether (b.p. 40–60 °C) and 0.2 mL of 1 M sodium methoxide (30% methanol in sodium methoxide) was added. The mixture was then shaken gently using an autovortex (Stuart, Manchester, U.K.) for 30 s and kept for 5 min for it to form two layers. A volume of 0.5 μL of the upper layer was injected into an injector port of gas chromatography GC HP 5890A (Hewlett Packard, Wilmington, USA) with the temperature programme that started at 115 °C, a heating rate of 6 °C/min and a final temperature of 200 °C (AOCS, 1993). Analysis was carried out at 5, 10 and 15 h extraction time of cocoa butter with three replications.

2.6. Selectivity (α)

The effect of cosolvents on TG profile and fatty acid (FA) composition of cocoa butter extracted by SC-CO2 process was determined by estimating the selectivity (α) using the following equation:

$$\alpha = \frac{[X_1]_e}{[X_2]_e}$$

where (X1)ex and (X2)ex denote concentration of two different TGs or FAs in the extract phase (supercritical fluid); while (X1)s and (X2)s are the concentration of two different TGs or FAs in the solid phase (Soxhlet extraction) (Li & Hartland, 1992; Rossi, 1996; Rossi, Arnoldi, Salvioni, & Schiraldi, 1989; Scheider, Kautz, & Tuma, 2000). The selectivity (α) of TG was calculated by comparison of TG relative to 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. The cocoa butter was also extracted by a Soxhlet method (AOAC, 1998).

2.7. Statistical analysis

The data obtained from the measurements were subjected to analysis of variance (ANOVA) to determine the significant differences among all treatments using the SAS software Version 8 (TS M1, SAS Institute Inc., NC, USA). The experimental data were reported as the mean ± SD of independent trials. Significant differences among all treatments were determined by Duncan’s multiple test at significant level of p < 0.05.

3. Results and discussion

3.1. Effects on the yield

The effects of ethanol as a polar cosolvent on the efficiency of cocoa butter extraction using SC–CO2 are shown in Fig. 1. The results show that the concentration of ethanol had a significant (p < 0.05) effect on the extraction yield of cocoa butter extracted by SC–CO2 at 35 MPa, 60 °C and a flow rate of 2 mL/min. The extraction yield increased as the concentration of ethanol was increased. The addition of 25% ethanol (E3) resulted in 100% extraction efficiency after 16 h of extraction time; the efficiency decreased to 97.50% and 92.45% with the addition of 15% and 5% ethanol at the same extraction time. Asep, Jinap, Russly, Harcharan, and Nazimah (2008) reported that a supercritical fluid of cocoa butter extraction from cocoa liquor using SCF with pure CO2 for nearly 16 h and found a relatively high extraction yield of 83.25%, and prolonging the extraction time up to 28 h resulted in 100% extraction yield. This indicates that the yield of cocoa butter extracted increases with cosolvent concentration. Every concentration of ethanol used in the study produced a higher yield of cocoa butter compared to that of pure CO2 and significantly (p < 0.05) increased the SC–CO2 extraction efficiency. The hydroxyl group in the chemical structure of ethanol may form a hydrogen bond to enhance the solute solubility. Ethanol may also substantially increase the solubility of cocoa butter. Li and Hartland (1996) reported that the addition of ethanol as a cosolvent significantly (p < 0.05) enhanced the solubility of cocoa butter depending on the concentration of ethanol, thereby increasing the extraction efficiency of cocoa butter up to 25–33% w/w of ethanol where the extraction efficiency became constant. Cocero and Calvo (1996) reported that the solubility of sunflower oil in CO2 is enhanced at a rate of 5 g/kg CO2 for each percent (by weight) of ethanol added to SC–CO2 at 30 MPa and 42 °C. The ability to dissolve cocoa butter by using a sufficient amount of
ethanol as a cosolvent was found to be higher than that obtained by either \( \text{CO}_2 \) or ethanol alone. The cosolvent can facilitate selective separation of solutes with different polarities, hydrogen bonding and abilities for association or complexation ability (Dobbs et al., 1987). The solvent power of \( \text{SC}-\text{CO}_2 \) could be increased by the addition of a small amount of cosolvents, and the effect is dependent on the concentration of the cosolvent in the supercritical phase, which is determined by the phase behaviour of the mixture under operating conditions (Olimpio et al., 2005). Shi et al. (2009) also reported that an increase in the concentration of isopropanol would result in the decrease of the density of the supercritical fluid. Furthermore, the extraction selectivity of astaxanthin may also become smaller, as other components of the feed matrix could be easily extracted.

The nonpolar solvents tend to most efficiently dissolve nonpolar solutes such as hydrocarbons, while more polar solvents tend to most efficiently dissolve more polar solutes. The dipole moment of ethanol (2.91 Debye, D) was more polar than ethanol (1.69 D) and acetone (2.91 D). However, because acetone had a smaller molecular interaction with the solute through hydrogen bonding, the yield of cocoa butter extraction was much lower.

### 3.2. Effects on triacylglycerol profile and selectivity \((\alpha)\)

As shown in Tables 1 to 3, several TG profiles of cocoa butter extracted by \( \text{SC}-\text{CO}_2 \) at 35 MPa, 60 °C and 2 mL/min were significantly \((p < 0.05)\) affected by the cosolvent (i.e. ethanol, isopropanol and acetone) concentrations except in the effect of ethanol \((I)\) on POS \((E_1\), \(E_2\), \(E_3\), \(E_4\)), POP \((5\ h)\), POS \((10, 15\ h)\) and other TGs \((5, 10\ h)\), in the effect of isopropanol \((I)\) on POS \((E_1)\), other TGs \((E_2)\), POP \((5, 10\ h)\), POS \((5, 10\ h)\), and other TGs \((5, 10\ h)\), and in the effect of acetone \((AC)\) on POP \((AC_2)\), other TGs \((AC_3)\) and others TG \((10, 15\ h)\). The results demonstrated that cocoa butter extracted under different processing conditions mostly contained three main TGs namely POS, POP and SOS.

![Fig. 3. The yield of cocoa butter extracted from cocoa liquor using acetone as cosolvent in SC-\( \text{CO}_2 \) at 35 MPa, 60 °C and 2 mL/min as a function of acetone concentration and the extraction time.](image)

### Table 1

Triacylglycerols composition (area %) changes of cocoa butter extracted from cocoa liquor with ethanol as cosolvent in SC-\( \text{CO}_2 \) at 35 MPa, 60 °C and 2 mL/min with different ethanol concentration and extraction time.

<table>
<thead>
<tr>
<th>Sample</th>
<th>5 h extraction time</th>
<th>10 h extraction time</th>
<th>15 h extraction time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POP</td>
<td>POS</td>
<td>SOS</td>
</tr>
<tr>
<td>(E_1)</td>
<td>23.15(^{a})</td>
<td>43.48(^{b})</td>
<td>28.69(^{d})</td>
</tr>
<tr>
<td>(5%)</td>
<td>± 1.03</td>
<td>± 1.23</td>
<td>± 0.52</td>
</tr>
<tr>
<td>(E_2)</td>
<td>22.41(^{c})</td>
<td>44.81(^{b})</td>
<td>28.03(^{c})</td>
</tr>
<tr>
<td>(15%)</td>
<td>± 1.02</td>
<td>± 1.57</td>
<td>± 0.69</td>
</tr>
<tr>
<td>(E_3)</td>
<td>23.04(^{e})</td>
<td>44.98(^{a})</td>
<td>27.26(^{c})</td>
</tr>
<tr>
<td>(25%)</td>
<td>± 1.25</td>
<td>± 1.65</td>
<td>± 1.48</td>
</tr>
</tbody>
</table>

\(^{a}\) Means within a row with different letters are significantly different \((p < 0.05)\).

\(^{b}\) Means within a column with different letters are significantly different \((p < 0.05)\).

\(^{c}\) Means value ± standard deviation of three replications.

\(^{d}\) Cocoa butter extracted obtained from cocoa liquor by ethanol as cosolvent in SC-\( \text{CO}_2 \) at 35 MPa, 60 °C and 2 mL/min with concentration of ethanol 5% \((E_1)\), ethanol 15% \((E_2)\) and ethanol 25% \((E_3)\).

\(^{e}\) PLiO, PLiP, POP, SOO, and SOA where P = palmitic, O = oleic, S = stearic, Li = linoleic, A = arachidic.
Table 2
Triacylglycerols composition (area %)\(^a\) changes of cocoa butter extracted from cocoa liquor with isopropanol as cosolvent in SC-CO\(_2\) at 35 MPa, 60 °C and 2 mL/min with different isopropanol concentration and extraction time.

<table>
<thead>
<tr>
<th>Sample(^\pm)</th>
<th>5 h extraction time</th>
<th>10 h extraction time</th>
<th>15 h extraction time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POP</td>
<td>POS</td>
<td>SOS</td>
</tr>
<tr>
<td>I(_1) (5%)</td>
<td>21.71(^{Ab})</td>
<td>45.05(^{Ab})</td>
<td>29.01(^{Ba})</td>
</tr>
<tr>
<td></td>
<td>±1.07 ±1.19 ±0.50 ±0.26</td>
<td>±0.91 ±0.90 ±0.51 ±0.33</td>
<td>±1.92 ±1.01 ±0.62 ±0.34</td>
</tr>
<tr>
<td>I(_2) (15%)</td>
<td>21.07(^{Ab})</td>
<td>45.92(^{Ab})</td>
<td>29.12(^{Ba})</td>
</tr>
<tr>
<td></td>
<td>±1.06 ±1.52 ±0.67 ±0.75</td>
<td>±1.09 ±1.40 ±0.81 ±1.16</td>
<td>±1.80 ±1.89 ±1.19 ±0.82</td>
</tr>
<tr>
<td>I(_3) (25%)</td>
<td>21.15(^{Ab})</td>
<td>45.71(^{Ab})</td>
<td>28.84(^{Ba})</td>
</tr>
<tr>
<td></td>
<td>±1.30 ±1.39 ±1.43 ±0.59</td>
<td>±0.87 ±0.56 ±1.13 ±0.59</td>
<td>±0.93 ±0.84 ±1.08 ±0.27</td>
</tr>
</tbody>
</table>

\(^a\) Means within a row with different letters are significantly different (p < 0.05).

\(^b\) Means within a column with different letters are significantly different (p < 0.05).

\(^c\) Means value ± standard deviation of three replications.

\(^\pm\) Cocoa butter extracted obtained from cocoa liquor by ethanol as cosolvent in SC-CO\(_2\) at 35 MPa, 60 °C and 2 mL/min with concentration of isopropanol 5% (I\(_1\)), isopropanol 15% (I\(_2\)) and isopropanol 25% (I\(_3\)).

\(^\#\) LIo, PLiP, POO, SOO, and SOA where P = palmitic, O = oleic, S = stearic, Li = linoleic, A = arachidic.

Table 3
Triacylglycerols composition (area %)\(^a\) changes of cocoa butter extracted from cocoa liquor with acetone as cosolvent in SC-CO\(_2\) at 35 MPa, 60 °C and 2 mL/min with different acetone concentration and extraction time.

<table>
<thead>
<tr>
<th>Sample(^\pm)</th>
<th>5 h extraction time</th>
<th>10 h extraction time</th>
<th>15 h extraction time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POP</td>
<td>POS</td>
<td>SOS</td>
</tr>
<tr>
<td>AC(_1) (5%)</td>
<td>23.01(^{Ab})</td>
<td>44.33(^{Bb})</td>
<td>28.51(^{Cb})</td>
</tr>
<tr>
<td></td>
<td>±1.16 ±1.12 ±0.50 ±0.27</td>
<td>±1.94 ±1.03 ±0.66 ±0.42</td>
<td>±1.52 ±1.01 ±0.53 ±0.39</td>
</tr>
<tr>
<td>AC(_2) (15%)</td>
<td>22.37(^{Ab})</td>
<td>45.30(^{Ab})</td>
<td>27.96(^{Bc})</td>
</tr>
<tr>
<td></td>
<td>±1.15 ±1.43 ±0.68 ±0.81</td>
<td>±1.12 ±1.60 ±1.10 ±1.49</td>
<td>±1.42 ±1.89 ±1.03 ±0.95</td>
</tr>
<tr>
<td>AC(_3) (25%)</td>
<td>21.40(^{Bc})</td>
<td>44.82(^{Aa})</td>
<td>29.11(^{Aa})</td>
</tr>
<tr>
<td></td>
<td>±1.41 ±1.50 ±1.41 ±0.63</td>
<td>±0.90 ±1.09 ±1.54 ±0.76</td>
<td>±0.74 ±0.84 ±0.93 ±0.31</td>
</tr>
</tbody>
</table>

\(^a\) Means within a row with different letters are significantly different (p < 0.05).

\(^b\) Means within a column with different letters are significantly different (p < 0.05).

\(^c\) Means value ± standard deviation of three replications.

\(^\pm\) Cocoa butter extracted obtained obtained from cocoa liquor by acetone as cosolvent in SC-CO\(_2\) at 35 MPa, 60 °C and 2 mL/min with concentration of acetone 5% (AC\(_1\)), acetone 15% (AC\(_2\)) and acetone 25% (AC\(_3\)).

\(^\#\) PLiO, PLiP, POO, SOO, and SOA where P = palmitic, O = oleic, S = stearic, Li = linoleic, A = arachidic.

The results also show that POS, SOS and POP were the major TGS extracted in decreasing order (Tables 1, 2 and 3); for ethanol, POS (43.48–45.85%), SOS (27.26–30.60%) and POP (20.19–23.15%); for isopropanol POS (40.07–45.83%), SOS (27.70–31.85%) and POP (20.14–
The results also clearly indicated that the TG profile was significantly ($p < 0.05$) influenced by the cosolvent concentration and extraction time (Tables 1, 2 and 3). For instance, the percentage of POP decreased with an increase in the concentration of cosolvent and extraction time. Conversely, the percentages of POS and SOS increased as the cosolvent concentration and extraction time were increased. These observations could be explained by the fact that POP was more soluble than POS and SOS. Hence, it was differentiated by the first stage of SC–CO$_2$ extraction process, while POS and SOS were eluted in the second stage of SC–CO$_2$ extraction.

The separation of TGs during SC–CO$_2$ extraction was clearly shown by the selectivity ($\alpha$) in Figs. 4, 5 and 6, where the selectivity ($\alpha$) variation of TGs could be seen as a function of polar cosolvent concentration and extraction time. The selectivity of POP increased with the addition of 5% ethanol, followed by 15% and 25% ethanol (Fig. 4). However, the concentration of ethanol had significant $(p = 0.05)$ negative effects on the selectivity of SOS, in which the selectivity of SOS decreased with increasing concentration of ethanol. The highest selectivity of SOS was obtained by using 5% ethanol (Fig. 4). Furthermore, the selectivity of POP inconsistently changed with an increase in the isopropanol concentration. A significant change $(p < 0.05)$ in the selectivity of POP and SOS was achieved by the addition of 5% and 15% isopropanol, respectively, while the addition of 25% isopropanol did not produce a significant change $(p > 0.05)$ in the selectivity of SOS (Fig. 5). Similarly, the selectivity of POP was inconsistently affected as the acetone concentration was increased (Fig. 6). The addition of 5% acetone produced a significant change $(p < 0.05)$ in the selectivity of POP, whereas that of 15% or 25% isopropanol did cause a significant change $(p < 0.05)$ for POP.

The selectivity of TG was significantly $(p < 0.05)$ affected by extraction time and cosolvent concentration. This finding could be due to the change of cocoa butter extracted from cocoa liquor by isopropanol as cosolvent in SC–CO$_2$ at 35 MPa, 60 °C, whereas others TG are PLiO, PLiP, POO, SOO and SOA.

22.03%); and for acetone, POS (42.94–45.95%), SOS (27.73–31.81%) and POP (20.34–23.01%). The proposed TG profiles of cocoa butter were similar to the typical TG composition reported by Rossi et al. (1989).

### Table 4

<table>
<thead>
<tr>
<th>Sample$^a$</th>
<th>5 h extraction time</th>
<th>10 h extraction time</th>
<th>15 h extraction time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C16:0</td>
<td>C18:0</td>
<td>C18:1</td>
</tr>
<tr>
<td>E1 (5%)</td>
<td>29.90$^a$</td>
<td>34.95$^a$</td>
<td>31.44$^a$</td>
</tr>
<tr>
<td>E2 (15%)</td>
<td>29.35$^a$</td>
<td>35.04$^a$</td>
<td>31.72$^a$</td>
</tr>
<tr>
<td>E3 (25%)</td>
<td>29.09$^a$</td>
<td>35.58$^a$</td>
<td>31.33$^a$</td>
</tr>
</tbody>
</table>

$^a$ Means within a row with different letters are significantly different $(p < 0.05)$.

$^b$ Means within a column with different letters are significantly different $(p < 0.05)$.

$^c$ Means value ± standard deviation of three replications.

$^d$ Cocoa butter extracted from cocoa liquor obtained by ethanol as cosolvent in SC–CO$_2$ at 35 MPa, 60 °C and 2 mL/min of flow rate with different ethanol concentration and extraction time.

### Table 5

<table>
<thead>
<tr>
<th>Sample$^a$</th>
<th>5 h extraction time</th>
<th>10 h extraction time</th>
<th>15 h extraction time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C16:0</td>
<td>C18:0</td>
<td>C18:1</td>
</tr>
<tr>
<td>I1 (5%)</td>
<td>29.60$^a$</td>
<td>35.07$^a$</td>
<td>31.53$^a$</td>
</tr>
<tr>
<td>I2 (15%)</td>
<td>28.32$^a$</td>
<td>36.40$^a$</td>
<td>31.05$^a$</td>
</tr>
<tr>
<td>I3 (25%)</td>
<td>28.51$^a$</td>
<td>37.90$^a$</td>
<td>30.14$^a$</td>
</tr>
</tbody>
</table>

$^a$ Means within a row with different letters are significantly different $(p < 0.05)$.

$^b$ Means within a column with different letters are significantly different $(p < 0.05)$.

$^c$ Means value ± standard deviation of three replications.

$^d$ Cocoa butter extracted obtained from cocoa liquor by isopropanol as cosolvent in SC–CO$_2$ at 35 MPa, 60 °C and 2 mL/min with concentration of isopropanol 5% (I1) isopropanol 15% (I2) and isopropanol 25% (I3).

$^e$ C12:0, C14:0 and C18:3.
to the difference between the solubility of the three main TGs (i.e. POP, SOS and POS) (Asep et al., 2008; Schneider et al., 2000; Soares, Gamarra, Paviani, Gonçalves, & Cabral, 2007). In this case, the amount of short-chain TGs decreased, whereas that of the long-chain TGs increased. This observation was also reported in a previous study (Arul, Boudevreau, Makhlouf, Tardif, & Sahasrabudhe, 1987). The results indicate that the variation of POP composition was significantly (p < 0.05) higher when the extraction process was performed using 5% ethanol (Fig. 4), followed by 15% isopropanol (Fig. 5) and 5% acetone (Fig. 6). In fact, the addition of a low concentration of polar cosolvents resulted in the highest selectivity and excessive concentration resulted in less selectivity for POP.

The same trend was observed for SOS. The highest variation of SOS selectivity was obtained by using 15% ethanol, followed by 5% and 25% ethanol (Fig. 4). However, the addition of 5% or 25% polar cosolvent did not significantly (p > 0.05) induce a change in the SOS selectivity. Opposite patterns in the selectivity of POP and SOS were observed. In fact, POP was more quickly extracted at the beginning of the extraction process whereas SOS was more quickly extracted at the end. Soares et al. (2007) reported that the solubility of the supercritical carbon dioxide varied as a function of the solubility of the three main TGs (i.e. POP, SOS and POS) extracted from cocoa liquor by acetone as cosolvent in SC-CO2 at 35 MPa, 60 °C and 2 mL/min with concentration of acetone 5% (AC5), acetone 15% (AC15) and acetone 25% (AC25).

Furthermore, the results show that C16:0 (34.61–37.92%), followed by C18:1 (29.71–31.98%) and C18:0 (28.71–30.38%), was found to be the major fatty acid, as acetone was added as a polar cosolvent.

As shown in Tables 4, 5 and 6, C16:0 and C18:0 profiles decreased as the cosolvent concentration and the extraction time were increased. On the other hand, C18:1 and C18:2 increased with an increase in the cosolvent concentration and prolonged extraction time. These results indicate that during extraction, more soluble fatty acids, C16:0 and C18:0, came out in the first stage of extraction using SC-CO2, followed by less soluble fatty acids C18:1, and C18:2, which came out in the second stage.

### 3.3. Effects on fatty acid methyl ester profile and selectivity (α)

The results obtained from the GC analysis of fatty acid composition are shown in Tables 4, 5 and 6. The results showed that C16:0, C18:0 and C18:1 were found to be the three main fatty acids (FA) in the cocoa butter. In general, the concentration of ethanol and acetone were varied and showed significant (p < 0.05) effects on the fatty acid profile, except in the effect of ethanol (E) on C18:0 (E2, E3), other FA (15 h), and in the effect of acetone (AC) on C16:0 (AC5), C18:0 (AC2, AC3), C16:0 (5, 10, 15 h), C18:0 (5, 10, 15 h), C18:1 (5, 10 h) and other FA (10, 15 h). On the other hand, the concentration of isopropanol was also varied and showed a significant (p < 0.05) effect on the fatty acid profile, except for C16:0 (I1), C18:0 (I2, I3), C18:1 (I1, I2), C18:2 (I1, I2), other FA (I1, I2, I3, I15 h), C18:2 (5, 10, 15 h) and other FA (10 h). As shown in Tables 4 to 6, C18:0 (34.61–37.92%) followed by C18:1 (31.01–31.82%) and C16:0 (28.03–29.90%) were found to be the three major fatty acids as ethanol was used as a polar cosolvent. The results also show that C18:0 (35.07–38.38%), C18:1 (30.03–31.53%) and C16:0 (28.03–29.59%) were identified as the major FA when isopropanol was used as a cosolvent.

### Table 6

<table>
<thead>
<tr>
<th>Sample</th>
<th>5 h extraction time</th>
<th>10 h extraction time</th>
<th>15 h extraction time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C16:0</td>
<td>C18:0</td>
<td>C18:1</td>
</tr>
<tr>
<td>AC1</td>
<td>29.79Ab</td>
<td>36.50Ab</td>
<td>29.98Ac</td>
</tr>
<tr>
<td>(5%)</td>
<td>±1.01</td>
<td>±1.01</td>
<td>±0.31</td>
</tr>
<tr>
<td>AC2</td>
<td>26.90Ab</td>
<td>34.65Ab</td>
<td>31.98Ab</td>
</tr>
<tr>
<td>(15%)</td>
<td>±1.02</td>
<td>±1.29</td>
<td>±0.41</td>
</tr>
<tr>
<td>AC3</td>
<td>29.36Ab</td>
<td>36.41Ab</td>
<td>31.12Ab</td>
</tr>
<tr>
<td>(25%)</td>
<td>±1.25</td>
<td>±1.36</td>
<td>±0.87</td>
</tr>
</tbody>
</table>

Means within a row and within a column with different letters are significantly different (p < 0.05). Means within a column with different letters are significantly different (p < 0.05). Means value ± standard deviation of three replications.

### Fig. 7

Selectivity (α) of fatty acid methyl esters as function of ethanol concentration as cosolvent and extraction time on cocoa butter extraction using SC-CO2 at 35 MPa and 60 °C.
Our study clearly shows that in the extraction of cocoa butter using SC–CO₂ at 35 MPa 60 °C, the polar cosolvent’s concentration and extraction time show significant effects (p < 0.05) on the selectivity (α). In general a 25% polar cosolvent concentration showed the largest increase in C16:0 selectivity (α), followed by 15 and 5%, respectively. The effects of ethanol, isopropanol and acetone concentrations on the selectivity for different FAs are shown in Figs. 7, 8 and 9, respectively. There was a large increase in the selectivity of C16:0 with an increase in extraction time and the addition of 25%, followed by 15% ethanol. However, C18:1, C18:2 and other FAs showed a small variation in the selectivity with the addition of ethanol for all cosolvent concentrations. It is clear that C16:0 was separated more rapidly compared to C18:1, C18:2 and other FAs during extraction (Fig. 7a–b). For isopropanol, a larger increase in the selectivity of C16:0 was observed with the addition of 25% ethanol, followed by 15% ethanol. Similarly, other FAs showed large changes in the selectivity with the addition of 25% isopropanol [Fig. 8]. However, unsaturated FAs (C18:1, C18:2) showed a small change in selectivity. As with ethanol, the saturated C16:0 was also separated more rapidly compared to the unsaturated fatty acids, C18:1 and C18:2, during the SC–CO₂ extraction of cocoa butter using isopropanol. These results are consistent with those of other studies on SC–CO₂ fractionation of crude palm oil (Markom, Singh, & Hasan, 2001; Zaidul, Norulaini, Mohd Omar, & Smith, 2007a, 2007b), in which the lower-molecular-weight compounds such as saturated FAs were separated first, followed by the higher-MW compounds such as unsaturated FAs. The same effect of acetone concentration on the selectivity of fatty acids (Fig. 9) also showed the same trend when isopropanol was used as a cosolvent.

4. Conclusion

The cocoa butter was successfully extracted from cocoa liquor by SC–CO₂ at 35 MPa, 60 °C and 2 ml/min using different concentrations of polar cosolvents (ethanol, isopropanol and acetone). The extraction yield was significantly (p < 0.05) influenced by the concentration of polar cosolvents. Similarly, polar cosolvent concentration had significant (p < 0.05) effects on the TG and FA compositions. Ethanol was found to be the most efficient polar cosolvent for cocoa butter extraction compared to isopropanol and acetone. POS (42.2–45.9%) being the major triglycerides component, followed by SOS (27.6–31.4%) and POP (20.3–22.7). Palmitic, stearic and oleic acids were the main fatty acids in the extracted cocoa butter, with stearic being the highest (34.9–37.8%), followed by oleic (30.3–31.8%) and palmitic (28.3–30.0%) acids, respectively. In terms of the selectivity, the lower-molecular-weight of TGs and FAs showed higher selectivity compared to the higher-molecular-weight TGs and FAs; therefore, POS was the major triglyceride at the beginning of extraction, while POP, followed by SOS, were the major triglycerides at the end of the extraction process. However, the FA composition was found to be more selective when a high concentration of polar cosolvent (25%) was used. The choice of modifiers becomes a great challenge and ethanol was shown to be the best polar cosolvent, and it enhanced the solubility during the cocoa butter extraction by SC–CO₂. This method can be feasibly implemented in the cocoa industry for the production of high quality cocoa butter.
Acknowledgements

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References


