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Fractionation of coconut oil *via* supercritical fluid extraction for production of cocoa butter substitute

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Abstract

Coconut oil (CNO) is a vegetable fat that can be applied as a cocoa butter substitute (CBS) due to its similar physical characteristics to cocoa butter. However, it must be fractionated or hydrogenated to be used as CBS. The aims of the present work was to fractionate CNO using supercritical fluid extraction (SFE), and determine the potential fraction which is suitable as CBS. CNO was fractionated by SFE at 48.3 MPa and 80°C into four different fractions, F1, F2, F3 and F4. Fraction 1 had the highest yield (48.9%) as compared to the other fractions. Fraction 4 had the lowest content of lauric acid, C12 (31.12%) and the highest amount of palmitic acid, C16 (16.43%); stearic acid, C18:0 (4.99%); and linoleic acid, C18:1 (17.44%). Fraction 4 also had the highest melting profile (25.24°C) and amount of solid fat content (state) closest to CB. Therefore, F4 was selected as a potential fraction for the application of CBS. This finding reveals that CNO can be fractionated by SFE and applied as CBS to help diversify the application of coconut products.

Introduction

Supercritical Fluid Extraction (SFE) (Figure 1) has been in practical use in producing superior and high quality extracted oil components. It is a rapid, selective and convenient method to separate and fractionate active compounds (Nik Norulaini *et al*., 2004; Reverchon and De Marco, 2006). Consequently, SFE has become an important extraction technique in the field of food applications because the extracts produced are free from solvents and thermally liable compounds of interest (Said *et al*., 2014). SFE has more advantages and is more environmentally friendly as compared to conventional methods because CO_2 is used as a solvent to extract and fractionate the elements. Organic solvents such as hexane and petroleum ether are widely used in lipid extraction and fractionation since these solvents result in the highest recovery of oil from the samples extracted. However, the use of these organic solvents leads to product contamination. This is due to solvent residues left in the extracted oil thus making it harmful to human health and the environment (Md Zaidul *et al*., 2006, Zaidul *et al*., 2007). In contrast, extracts obtained using SFE are sterilised, free from contaminants and remain in a chemically natural state. CO_2 is non-toxic, non-flammable, inexpensive and chemically stable (Abbas *et al*., 2008). In addition, SFE extracts the desired oil or elements in a shorter time as compared to conventional methods that require 8 to 24 h for complete extraction. Therefore, SFE is the more preferred method for extracting or fractionating fats and oil and meets consumers' demands for safe and natural fats and oils (Temelli, 2009).

SFE is not only used for extraction, but has also been widely applied in fractionation to modify milk fat and lauric fat. The modification of fat increases the melting point and solid fat content which is suitable for confectionery applications (Lonchampt and Hartel, 2004). Fatouh *et al*. (2007) fractionated

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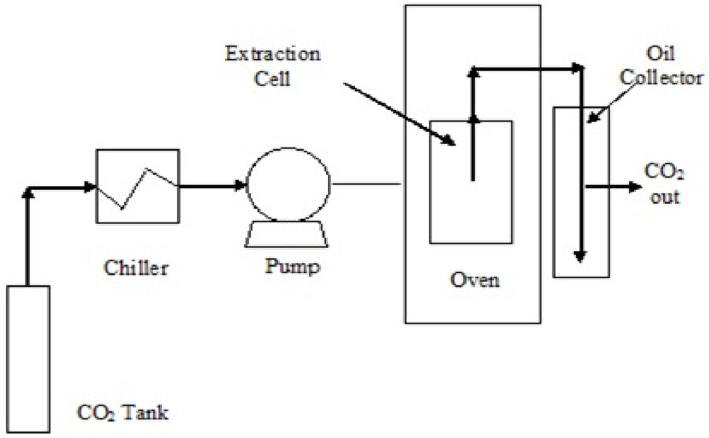

Figure 1. Schematic diagram of supercritical fluid extraction (SFE) used for the fractionation of CNO.

milk fat from buffalo butter oil with SFE at a pressure range from 10.9 MPa to 40.1 MPa and temperature from 50 to 70°C. They obtained two different fractions with different melting points; high and low melting fractions, which are useful for a wide variety of food applications. Nik Norulaini *et al*. (2004) reported that SFE fractionated oil based on fatty acid and triglyceride compounds. They had fractionated palm kernel oil by extracting the oil at different fractions; fraction 1 to fraction 8 from a pressure of 34.5 MPa to 48.3 MPa at 40°C. They determined that the earlier fractions were rich in short and medium chain triglycerides (C8 to C12) while later fractions were rich in long chain triglycerides (C16 to C18:2). They concluded that the later fractions had potential to be cocoa butter equivalent (CBE).

Similar to palm kernel oil, coconut oil (CNO) is a vegetable oil that contains a multi-component mixture of triglycerides containing 50% lauric acid and 35% of C16 to C18:2 (Canapi *et al*., 2005) which can be fractionated for confectionery products. Coconut oil has hardness, mouth feel and flavour release similar to CB which means it can be applied as CBS. According to Shukla (2005), CNO is hydrogenated or fractionated to produce CBS for application in chocolate formulation (Shukla, 2005). Previously, there was a report on the fractionation of palm kernel oil using SFE to produce cocoa butter replacer (CBR) (Md Zaidul *et al*., 2006). They successfully reduced the amount of lauric acid from 60.09% to 28.11% in the fractionation of palm kernel oil. They concluded that the fraction with the lowest amount of lauric acid had the potential for the production of CBR. However, research on fractionation of coconut oil by SFE to produce CBS has not been reported. Therefore, the aims of the present work was to fractionate CNO by SFE, and to determine the potential fraction for application as CBS.

Materials and methods

Raw materials

The raw material used in the present work was dried copra. The copra was selected based on the characteristics of being the same variety, maturity index, diameter and area of cultivation. The variety of coconut used was MATAG (*Cocos nucifera* L.) of maturity index no 2 (12 to 14 months cultivation) with diameters of 10 cm to 20 cm. The coconuts were cultivated in Tanjung Karang, Malaysia. Commercial cocoa butter (CB) was used as a sample reference (control), and obtained from Barry Callebaut (Port Klang, Malaysia), which was natural and deodorised with 52% fat content.

Chemicals

The chemicals used for fatty acids analysis were hexane, sodium methoxide and sodium chloride; they were of analytical grade and purchased from Merck, Germany. The mix standards of methyl esters (C8 to C22) were obtained from Supelco, USA.

Sample preparation

The copra was cut using commercial knife and the juice removed from the freshly opened copra. The flesh was then grated by an electric grinder (Phillips, Malaysia) into grated coconut. The grated coconut was sun-dried from 50% to 3% moisture content in accordance with Che Yunus *et al*. (2014) in order to protect the bioactive compound. The drying process was conducted during the hot climate season between March to April 2015, and the temperature was controlled between 30 to 34°C. Then, the dried grated coconut was ground, sieved into powder of sizes ranging between 0.5 to 1.0 mm, and kept in an air-tight container to keep out humidity prior to extraction.

Soxhlet extraction

The Soxhlet extraction of CNO was carried out in order to obtain the CNO extracts. This CNO was regarded as 'unfractionated CNO' and compared with CNO fractions obtained after the fractionation process. Approximately 5 g desiccated coconut was weighed into the thimble. Then, 150 mL hexane was added and refluxed for 6 h using the Soxhlet apparatus. The extracts were filtered and concentrated under reduced pressure until dried (AOACS, Method 945.16, 1998). The extracts were compared with the CNO fractions in terms of fatty acids composition, melting profile and solid fat content.

Fractionation of coconut oil (CNO)

The fractionation of CNO was carried out to obtain different fractions with different properties for potential use as CBS. The fractionation was performed with SFE where the system consisted of an Intelligent HPLC Pump (Model PU-1580 Jasco Corporation, Tokyo, Japan). A back pressure regulator (Model BP- 1580-81, Jasco Corporation, Tokyo, Japan) was used to control the extraction pressure. The fractionation was carried out following the method described by Md Zaidul *et al*. (2006) with some modification. Approximately 20 g dried grated coconut was loaded into a 50 mL extraction vessel. Liquid carbon dioxide (purity of 99.9%) was pumped into the heated extraction cell. The extraction was performed at 48.3 MPa and 80°C based on the preliminary study at an average CO_2 flow rate of 6.0 mL/min. The fractionation was performed for 40 min. The CNO was fractionated into four different fractions. The CNO fractions were collected in four different collecting flasks at the end of every 10 min namely F1, F2, F3 and F4. The fractions obtained were characterised based on the fatty acids, melting profiles and solid fat contents, and compared with unfractionated CNO and commercial CB.

Yield of coconut oil fractions (F-CNO)

The yield of each CNO fraction was calculated using the following equation:

$$\text{Yield (\%)} = \frac{\text{Weight of flask (after)} - \text{Weight of flask (before)}}{\text{Weight of sample}} \times 100\%$$

Fatty acids composition of coconut oil fractions (F-CNO)

The fatty acid composition was determined by a gas chromatography system equipped with flame ionisation detector (7683 B Series, Agilent Technologies, USA) and a polar capillary column (DB5-Agilent Technologies, USA, length 60 m × internal diameter 0.25 mm, radius 0.25 μm). The CNO fractions were first esterified into methyl esters. About 100 μL CNO fractions was esterified with 1 mL hexane, and the mixture was vortexed to dissolve the oil. Then, 1 μL sodium methoxide was added and vortexed again for 5 to 10 s. The mixture was then allowed to separate into a methyl ester layer for a few minutes. About 1 μL methyl ester layer was injected into the gas chromatography system. The oven temperature was set to 190°C, and the detector and injector temperature held constant at 250°C. The carrier gas flow rate was 1 mL/min with a split ratio of 1:100. The total run time was 40 min. The peaks were identified based on the retention time of standards of methyl ester (C8 to C22), and the results were reported as relative percentages of fatty acids (Md Zaidul *et al*., 2006).

Melting behaviour of coconut oil fractions (F-CNO)

A melting behaviour test was performed to determine the melting temperatures (Tonset, Tpeak, Tendset) of CNO fractions, (F1, F2, F3, F4), unfractionated CNO and commercial CB. The melting behaviour was evaluated using a differential scanning calorimeter (Pyris DSC Series 6, Perkin Elmer Corporation, and Wellesley, USA). The instrument was calibrated using metallic Indium prior to use. About 3 to 5 mg of each oil samples, F-CNO, unfractionated CNO and CB were pipetted separately into a 40 μL aluminium pan and sealed. An empty sealed pan was used as a reference. For melting characteristics, the samples were stabilised according to the IUPAC method by keeping the samples at 0°C for 90 min, 26°C for 40 h followed by 0°C for 90 min prior to introduction into the DSC cell. Then, the melting profile of the oil was measured at a heating rate of 20°C/min from -80°C to 80°C. The onset temperature (Tonset), end temperature (Tend) and enthalpy of melting (H) were determined by DSC thermogram obtained using the TA universal analysis software.

Solid fat content (SFC) of coconut oil fractions (F-CNO)

The solid fat content was determined to measure the remaining solid in the oil fractions at specific temperature and compared with unfractionated

CNO and commercial CB. SFC of the oil samples were measured with active pulsed nuclear magnetic resonance (pNMR) and Bruker Minispec pc 20 (Bruker, Karlsruhe, Germany) as described by Kadivar *et al*. (2016). About 5 mL of the oil samples were placed in NMR tubes (three replicates) and submitted to the tempering treatments of the IUPAC 2.150 serial tempering method. SFC was determined in the range of 10 to 40°C at 5°C intervals following 60 min incubations at each interval.

Statistical analysis

All the analysis was performed in triplicate. The data obtained were statistically analysed using Minitab software version 16 (USA). One-way ANOVA was used to evaluate the significant differences between each data. The significant differences were determined with Tukey's test based on a 95% significance level.

Results and discussion

Yield of coconut oil fractions (F-CNO)

Figure 2 shows the yield of the F-CNO collected into four different fractions at 48.3 MPa and 80ºC. The yield obtained significantly decreased ($p < 0.05$) in each fraction from F1 to F4. The highest yields of 48.9% was obtained in F1 followed by 34.3% in F2 then 8.30% in F3 and 2.60% in F4. The decrease in yield of each fraction is believed to be due to the amount of short, medium and long chain fatty acids present in the CNO. According to Gopala *et al.* (2010), CNO contains about 70% short and medium chain fatty acids and 30% long chain fatty acids. These short and medium chain fatty acids have low molecular weight making them more soluble in CO_2 as compared to long chain fatty acids. Therefore, most of these short and medium chain fatty acids were extracted in the earlier fractions, thus contributing to the highest yield in F1. The remaining 30% of long chain fatty acids were extracted at the later fraction, (F4) thus giving lower yield.

This result is similar to the finding of Rahman *et al*. (2001) who stated that the extraction of ground palm kernel with supercritical carbon dioxide (SC-CO_2) at 34.5 to 48.3 MPa and 60°C slowed down in the later fractions after 80% of the oil had been extracted due to the low solubility of longer chain fatty acids. Hassan *et al*. (2000) studied the fractions of palm kernel oil extracts with SC-CO_2 at 27.6 to 48.3 MPa and 40 to 80°C at 15 min intervals. They found that the yield of each fraction decreased from 21.88% in the first fraction to 7.7% in the fifth fraction.

Fatty acids composition of coconut oil fractions (F-CNO)

Table 1 shows the fatty acid compositions of CNO and the fractions (F1, F2, F3, F4) obtained at 48.3 MPa and 80°C. The major fatty acids found were caprylic acid (C8), capric acid (C10), lauric acid (C12), myristic acid (C14), palmitic acid (C16), stearic acid (C18), and oleic acid (C18:1). There was a significant difference ($p < 0.05$) between fatty acid composition in each fraction and unfractionated CNO. The amount of short chain fatty acids (C4 to C8) and medium chain fatty acids (C10 to C14) significantly decreased ($p < 0.05$) whereas long chain fatty acids (C16:0 to C18:2) significantly increased ($p < 0.05$) from F1 to F4. F1 had a high amount of short and medium chains with a low amount of long chain fatty acids. Concurrently, F4 has a high amount of long chains with a low amount of short and medium chain fatty acids.

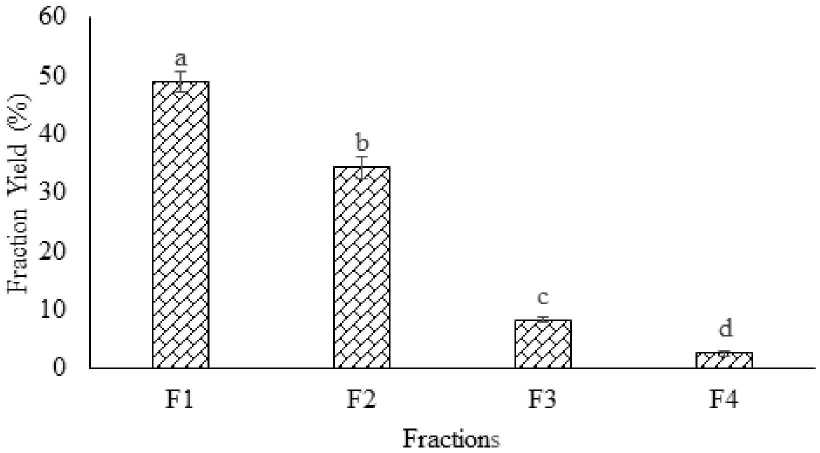

Figure 2. Yields of fractions 1 to 4 (F1 – F4) obtained from the fractionation of coconut oil (CNO). Different lowercase indicate significant difference (p < 0.05) in yields of different fractions.

F1 contained higher constituents of C8 to C14 due to the greater solubility of these fatty acids in CO_2 as compared to C16 to C18:2. Fatouh *et al*. (2007) stated that the fundamental of fractionation by SFE is based on the molecular weight of the fatty acid. According to Hassan *et al*. (2000), low molecular weight fatty acids have a greater solubility in CO_2, therefore they are easily dissolved in the supercritical phase, and hence would be first extracted during fractionation. Consequently, the high molecular weight fatty acids were extracted later due to their low solubility in CO_2. The C8 to C14 have low molecular weight fatty acids from 144.21 to 228.38 g/mol whereas C16 to C18:1 have molecular weight from 256.43 to 282.47 g/mol. This might be the reason why F1 contained high amounts of C8 to C12 whereas F4 had high amounts of long chain fatty acids, C16 to C18:1 (Table 1).

The trend of fatty acids composition found in the present work is similar to Fattori *et al*. (1988), Md Zaidul *et al*. (2006) and Buyukbese *et al*. (2014). Fattori *et al*. (1988) fractionated canola seed extracts with SFE at 41.4 MPa and 60°C, and found that the later fractions were rich in long chain fatty acids, C22 and C24 fatty acids. Md Zaidul *et al*. (2006) also showed that the fractionation of palm kernel oil by SFE resulted in the final fraction containing C8 to C14 and high amounts of C16 to C18:1. Buyukbese *et al*. (2014) also reported that the first fraction obtained during fractionation of milk fat was enriched with short chain fatty acids.

Table 1 also shows the fatty acids composition present in the CB. The major fatty acids found were 23.43% C16, 30.96% C18:0 and 34.23% C18:1. According to Naik and Kumar, (2014), the presence of C16 to C18:1 in the CB is important for the melting properties of the finished chocolate products. Among the fractions obtained, C16 to C18:1 composition in F4 was more than three times higher than F1 from 3.09 to 16.43%, 1.13 to 4.99% and 2.01 to 17.44%, respectively. Hence, this fraction can be useful for confectionary fats as CBS due to the high amount of long chain fatty acids as compared to the other fractions.

In contrast, short and medium chain fatty acids were not found in the CB when compared to unfractionated CNO and the fractions (F1, F2, F3, F4). A high amount of lauric acid (31.12 to 53.06%) was found in the CNO and in each fraction. According to Shukla (2005), high amounts of lauric acid do not constitute a good protection barrier when exposed to moisture. When lauric acid is hydrolysed, it releases free fatty acids which leads to the creation of an unpleasant soapy flavour (Minifie, 1989; Shellhammer and Krotcha, 1997; Shukla, 2005; Tanabe and Hofberger, 2005). The present work significantly shows ($p < 0.05$) that the lowest amount of C12 (lauric acid) was obtained in F4 with a 20% reduction from 53.06% (F1) to 31.12%. Therefore, F4 was the best fraction to be applied as CBS as compared to unfractionated CNO and other fractions due to its lower lauric acid content (31.12%).

Table 1. Fatty acid composition of unfractionated coconut oil (CNO), coconut oil fractions (F1 – F4) and cocoa butter (CB).

Type of fatty acid	Fatty acids composition (%)					
	CNO	F1	F2	F3	F4	CB
C8	5.35 ± 0.29^{c}	11.11 ± 0.11^{a}	10.28 ± 0.36^{b}	5.03 ± 0.12^{c}	3.42 ± 0.01^{d}	ND
C10	6.48 ± 0.07^{b}	7.87 ± 0.01^{a}	6.48 ± 0.27^{b}	4.60 ± 0.08^{c}	3.28 ± 0.05^{c}	ND
C12	44.75 ± 0.04^{b}	53.06 ± 0.04^{a}	44.72 ± 0.08^{b}	36.76 ± 0.06^{c}	31.12 ± 0.07^{d}	ND
C14	20.10 ± 0.03^{b}	21.71 ± 0.05^{a}	20.95 ± 0.04^{b}	20.79 ± 0.09^{b}	19.10 ± 0.10^{c}	ND
C16	8.88 ± 0.06^{e}	3.09 ± 0.02^{f}	6.99 ± 0.12^{d}	12.97 ± 0.06^{c}	16.43 ± 0.04^{b}	23.43 ± 0.06^{a}
C18:0	3.16 ± 0.06^{c}	1.13 ± 0.03^{e}	2.99 ± 0.05^{d}	4.13 ± 0.04^{b}	4.99 ± 0.07^{b}	30.96 ± 0.36^{a}
C18:1	8.44 ± 0.04^{e}	2.01 ± 0.04^{f}	7.93 ± 0.06^{d}	14.36 ± 0.04^{c}	17.44 ± 0.05^{b}	34.23 ± 0.11^{a}
C18:2	2.15 ± 0.02^{d}	0.02 ± 0.01^{f}	0.66 ± 0.00^{e}	2.36 ± 0.08^{c}	4.23 ± 0.01^{b}	5.83 ± 0.05^{a}
Saturated fatty acids	88.72	97.97	92.41	84.28	78.34	54.39
Unsaturated fatty acids	10.59	2.03	8.59	16.72	21.67	40.06

Data are mean of triplicates ($n = 3$) ± standard deviations. Different lowercase in a row indicate significant difference ($p < 0.05$) in fatty acid composition between samples. C8:0: caprylic acid; C10:0: capric acid; C12:0: lauric acid; C14:0: myristic acid;

C16:0: palmitic acid; C16:1: palmitoleic acid; C18:0: stearic acid; C18:1: oleic acid; C18:2: linoleic acid.

Melting behaviour of coconut oil fractions (F-CNO)

The melting behaviour of CNO and its fractions are shown in Table 2. The Tonset of the F-CNO significantly increased ($p < 0.05$) from 13 to 25°C for F1 to F4, respectively. The Tendset of the F-CNO also significantly increased ($p < 0.05$) from 26 to 34°C for F1 to F4, respectively. These results indicated that the different fractions had different melting profiles. According to Fatouh *et al*. (2007), the differences in the melting profiles between the fractions were believed to be due to the different types and amount of fatty acids present in the fractions. Fatouh *et al*. (2007) reported that the melting point of a later fraction was much higher than the earlier fraction during fractionation of butter oil by SFE due to the variation of fatty acids present. They observed that the fraction with a high amount of short and medium chain fatty acids had a low melting profile while the fraction containing a high amount of long chain fatty acids had a higher melting profile. This was due to short and medium chain fatty acids having lower melting points as compared to long chain fatty acids.

Table 2. Melting behaviour of unfractionated coconut oil, coconut oil fractions and cocoa butter.

Sample	Melting behaviour		
	T_{Onset} (°C)	T_{Peak} (°C)	T_{Endset} (°C)
CNO	13.64 ± 0.98^{d}	24.08 ± 0.96^{d}	27.03 ± 0.37^{d}
Fraction 1	13.46 ± 0.59^{d}	23.88 ± 0.70^{d}	26.92 ± 0.29^{e}
Fraction 2	15.72 ± 0.47^{c}	25.14 ± 0.35^{c}	27.83 ± 0.85^{d}
Fraction 3	15.63 ± 0.02^{c}	26.63 ± 0.35^{b}	30.07 ± 0.38^{c}
Fraction 4	25.24 ± 0.13^{b}	29.61 ± 0.36^{a}	33.64 ± 0.47^{b}
CB	27.48 ± 0.28^{a}	29.96 ± 0.78^{a}	37.13 ± 0.59^{a}

Data are mean of triplicates ($n = 3$) ± standard deviations. Different lowercase in a column indicate significant difference ($p < 0.05$) in melting behaviour of samples. CNO: coconut oil (unfractionated); F1 to F4: fraction 1 to 4; CB: cocoa butter.

A similar result was found in the present work where the change in melting profile was consistent with the fatty acids composition found in F1-F4 (Table 1). According to Marikkar *et al*. (2013) and De Man (1990), the change of melting profile is determined by the change in fatty acid composition. This is because the thermodynamic parameter of oils and fats are closely related to their fatty acid constituents (Che Man *et al*., 1999). According to the data presented in Table 1, F1 contained a greater amount of short and medium chains, leading to the lower Tonset and Tendset (13.46°C and 26.92°C, respectively) than F4 which contained a high amount of long chain fatty acids with high Tonset and Tendset (25.24°C and 33.64°C, respectively). Buyukbese *et al*. (2014) fractionated milk fat into six different fractions, and observed the increasing melting temperature from F1 to F6 due to the different fatty acids composition in each fraction.

According to Pantzaris and Ahmad (2001), CNO has a rapid-melt down behaviour which is well appreciated for its application in confectionery products. They also opined that CNO components which have sharp melting points are characterised by a cool, non-greasy sensation on the palate, making them useful as specialty fats. CNO components are beneficially used for the formulation of toffees, chocolate-type coatings, biscuits sprays, non-dairy ice cream and filling creamers (Pantzaris and Basiron, 2002). According to Lipp *et al*. (2001), the CBS should have a sharp melting point above the room temperature (24°C). From Table 2, it can be observed that F4 had the closest melting point (27.48°C) to CB as compared to the other fractions. Hence, this fraction is probably more suitable for CBS application.

Solid fat content of F-CNO

The SFC profiles of unfractionated CNO and its fractions are shown in Figure 3. The SFC of the F-CNO was significantly different ($p < 0.05$) among all fractions. According to Zarringhalami *et al*. (2012), these variations in the SFC were believed to be due to the different fatty acid compositions in the oil samples, which is similarly reported in the present work. This could indicate that the differences found between the chemical compositions of the fractions determine their physical properties. Observations show that the SFC of the fractions decreased in the following order: F4 > F3 > F2 > F1 at any temperatures. This might be due to F4 containing a high amount of long chain fatty acids as compared to F3, F2 and F1, therefore the SFC values in F4 were much higher as compared to the other fractions (Table 1).

The data showed that the SFC values decreased significantly as the temperature increased from 0 to 40°C. This trend was similar in all fractions. Similar results had been previously reported by Zarringhalami *et al*. (2012) and Karabulut *et al*. (2004). They reported that the SFC values of interesterified oil blend samples decreased as measured temperature increased. Figure 3 shows that the SFC of the CB rapidly decreased from 20°C to 30°C. In addition, at 40ºC, the CB completely melted and the value of SFC was 0%. According to Lipp *et al*. (2001), this character is important in chocolate products where the fat should almost completely melt at body temperature (37°C). The SFC profiles also indicated that the unfractionated CNO and all CNO fractions completely melted up to 40°C, similar to CB.

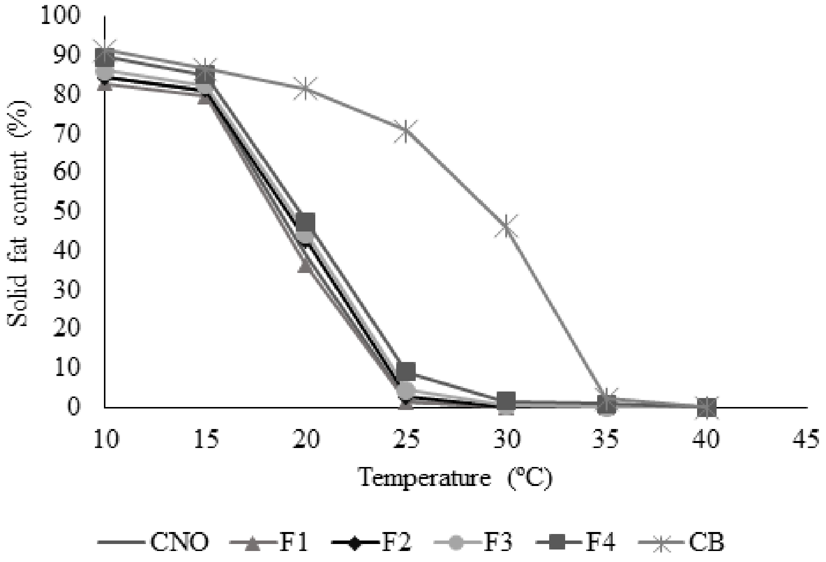

Figure 3. Solid fat content (%) of unfractionated coconut oil (CNO), fractions 1 to 4 (F1 – 4), and cocoa butter (CB).

Therefore, all the fractions obtained have potential as CBS for chocolate production. However, since F4 yielded the lowest lauric acid content, the highest amount of long chain fatty acids, the nearest melting point to CB and completely melted at 40°C, this fraction was selected as a potential fraction for CBS.

Conclusion

Fractionation of CNO at 48.3 MPa and 80°C contributed to different fractions with different properties. In the present work, the short and medium chain fatty acids content significantly decreased ($p < 0.05$) from F1 to F4. F4 had significantly the lowest ($p < 0.05$) lauric acid whereas the long chain fatty acids significantly increased ($p < 0.05$) from F1 to F4. F4 had significantly the highest ($p < 0.05$) palmitic acid (16.43%), stearic acid (4.99%), oleic acid (17.44%) and linoleic acids (4.23%). Fractionation with SFE successfully reduced 20% of lauric acids and increased by three times the amount of palmitic, stearic, oleic and linoleic acids than the unfractionated CNO. The melting profile of the F-CNO significantly decreased ($p < 0.05$) from F1 to F4. F4 had the highest initial and end melting point of 25.24°C and 33.64°C, respectively. All fractions had 0% solid fat content in the range of 35 to 40°C. F4 was found to possess properties closest to CB. Therefore, F4 was selected as a potential CNO fraction to be applied as CBS.

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