EXTRACTION OF COCOA BUTTER BY SUPERCRITICAL CARBON DIOXIDE: OPTIMIZATION OF OPERATING CONDITIONS AND EFFECT OF PARTICLE SIZE

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ABSTRACT

The optimum operating conditions for the extraction of cocoa butter from cocoa liquor using supercritical carbon dioxide and the effect of sample particle size on cocoa butter extraction under optimized operating conditions were investigated. The optimization was conducted at 10–45 MPa and 35–75C, with extraction times of 1–12 h by response surface methodology. The effect of particle size was studied using cocoa liquor, ground cocoa nibs and crushed cocoa nibs with particle sizes of approximately 74 μm, 0.85–1 mm and 4–6 mm, respectively. The yield was analyzed for total fat content by gravimetric method and triacylglycerol (TAG) profile by high-performance liquid chromatography. The results showed higher yield of cocoa butter with higher values of pressure, temperature and extraction time. The optimum conditions for cocoa butter extraction were 45 MPa, 75C and 12 h. The smaller particle size produced a higher yield of cocoa butter. 1,3-Dipalmitoyl-2-oleoyl-glycerol (POP), 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS) and 1,3-distearoyl-2-oleoyl-glycerol (SOS) were the major TAGs present in the extracted cocoa butter, with POS being the highest (>30%) for all treatments studied.

PRACTICAL APPLICATIONS

The application of supercritical carbon dioxide extraction offers a pollution-free technology that will gain wide acceptance globally because of increasingly stringent environmental regulations. The results from the study

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would assist the industry in the production of cocoa butter that is safe for consumption and free from contaminants, in contrast to those produced by organic solvent and mechanical expression.

INTRODUCTION

Cocoa butter, which is a yellowish white fat, is derived from the cocoa bean. After removing the shell from the bean, the remaining nib contains about 55% cocoa butter. Unique to cocoa butter are its brittleness at room temperature and its quick and complete melting at body temperature. The special functional properties of cocoa butter make it indispensable in the food, cosmetic and pharmaceutical industries. In the food industry, cocoa butter is responsible for the smooth texture, mouthfeel, flavor release and gloss of chocolate products (Schilchter-Aronhime and Garti 1988; Liendo *et al.* 1997).

The customary methods of producing cocoa butter from cocoa liquor (finely ground cocoa nib) or cocoa bean at the present time are classified as hydraulic pressing, expeller pressing and solvent extraction with organic solvents. However, there is an increasing awareness of the health and safety hazards associated with the use of organic solvents, while pressing often introduces contaminants into the cocoa butter that must be removed later (Li and Hartland 1996). These have placed a new demand to develop clean and efficient technologies for obtaining cocoa butter.

Supercritical fluid extraction (SFE), mainly by supercritical carbon dioxide (SC-CO_2), has been studied as a potential alternative to the current extraction method in vegetable oil extraction. The application of SC-CO_2 extraction offers the advantages of rapid, nontoxic and contamination-free extraction, less destruction of thermally labile constituents, and prevention of any possible oxidation in the oxygen-free extraction condition (Lee *et al.* 2000; Mukhopadhyay 2000).

Attempts have also been made to produce cocoa butter by SFE technology. Roselius *et al.* (1975) reported the efficient extraction of cocoa butter from cocoa liquor and cocoa nibs with CO_2 in the pressure range of 25–35 MPa at a temperature between 45 and 60C. Nevertheless, McHugh and Krukonis (1986) found that less than 5% of the cocoa butter could be extracted from nibs, even if the extraction was carried out at 48.3 MPa for a period of 8 h at 40–60C. Venter *et al.* (2006) stated that the solubility of vegetable oil in SC-CO_2 is generally on the order of only 1–5 wt % under normal extraction conditions of 25–30 MPa and 40–60C. Rossi *et al.* (1989a) reported that the particle size of the sample could have a pronounced influence on the efficiency of the SC-CO_2 extraction. Rossi *et al.* (1989b) also examined the composition of cocoa butter extracted from various cocoa products by SC-CO_2.

The studies on cocoa butter extraction by SC-CO_2 are lacking by far. Moreover, none have optimized the operating conditions, which may be the key to expanding the application of SFE on cocoa butter extraction. Pressure, temperature and extraction time are the important operating parameters of SFE. These variables influence the physicochemical properties such as density, diffusivity and viscosity of a supercritical fluid that later on determine the dissolving power of the fluid and the yield. Therefore, this work was carried out to determine the optimum conditions in terms of pressure, temperature and extraction time for SC-CO_2 extraction of cocoa butter from cocoa liquor (particle size of approximately 74 μm). In practice, the cocoa grinders perform cocoa butter extraction from predetermined cocoa products, i.e., cocoa liquor, cocoa nibs and crushed cocoa nibs, which have different particle sizes. Therefore, the optimized operating conditions obtained in the study were used to determine the effect of particle size on extraction using cocoa liquor (particle size of approximately 74 μm), ground cocoa nibs (particle size of 0.85–1 mm) and crushed cocoa nibs (particle size of 4–6 mm). Furthermore, particle size does not interact with pressure, temperature and extraction time in influencing the physicochemical properties of supercritical fluid that would determine the yield. The quantity and quality of the extracted cocoa butter in terms of total fat content (%) and triacylglycerol (TAG) profile were determined.

MATERIALS AND METHODS

Materials

Cocoa beans and cocoa liquor (particle size of approximately 74 μm) with a minimum of 52% fat content were obtained from KL-Kepong Cocoa Products Sdn. Bhd. (Selangor, Malaysia). The CO_2 was supplied by Malaysian Oxygen Gases Bhd. (Selangor, Malaysia). The chemicals and standards used in the study were obtained from Fisher Scientific (Loughborough, Leics, U.K.) and Sigma Chemical Co. (St. Louis, MO), which were of analytical and high-performance liquid chromatography (HPLC) grade.

SFE Apparatus and Procedure

Extraction of cocoa butter was performed by SC-CO_2 extraction. The SFE apparatus consisted of an intelligent HPLC pump (Model PU-1580, Jasco Corp., Tokyo, Japan) fitted with a cooling jacket to deliver CO_2. In order to cool the pump head, 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., Selangor, Malaysia), which can deliver coolant down to –20C. A 10-g sample was loaded into a

50-mL extraction vessel (Model EV-3, Jasco Corp.) that was placed in a column oven (Model CO-1560, Jasco Corp.). The column oven was used to maintain the extraction temperature. A back pressure regulator (Model BP-1580-81, Jasco Corp.) was used to control the extraction pressure. A 10-mL test tube was used as a collection vessel. The sample was extracted at a pressure of 10–45 MPa, temperature of 35–75C, extraction time of 1–12 h and flow rate of 2 mL/min.

Total Fat Content Analysis

The yield of cocoa butter was measured as percentage of total fat content (*TFC*). The test tube, which was used as an extracted fat collector in the SFE, was weighed before extraction. After extraction, the test tube containing fat that had been collected was cooled to room temperature in a desiccator before it was weighed again to calculate the weight of extracted fat from the sample.

$$TFC\,(\%) = \frac{Weight\ of\ \ fat\,(g)}{Weight\ of\ \ sample\,(g)} \times 100 \tag{1}$$

TAG Analysis

The TAG profile of cocoa butter extracted with SC-CO_2 was determined by HPLC using the modified method of the American Oil Chemists' Society (1989). The sample was diluted in acetone to make up to 10% sample solution. The prepared solution was then filtered using a Whatman membrane filter (0.45 mm, 13 mm, Maidstone, U.K.) before it was filtered through the Sep-Pak Plus Silica Cartridge (Waters, Milford, MA) to discard any impurities. Determination of TAG profile was conducted using an HPLC system (Waters) consisting of a Waters Model 600 Controller and a Waters Model 410 differential refractometer. The column used was a Waters HPLC column (3.9 mm i.d. × 300-mm length) with C-18 packing. The column was maintained at 30–35C and at a pressure of 50–60 bar. The mobile phase used was acetone/ acetonitrile (75:25, v/v) with a flow rate of 1 mL/min, and the injection volume was 10 mL. TAG peaks were identified based on the retention time of TAG standards and the chromatographic data of Chaiseri and Dimick (1989). All TAG analyses were carried out in triplicate, and the values were expressed as percentage areas.

Experimental Design for Optimization of Operating Conditions

The cocoa liquor with particle size of approximately 74 µm was used to study the optimization of operating conditions. The optimization was carried

TABLE 1.
CODED AND ACTUAL LEVELS OF INDEPENDENT VARIABLES IN THE OPTIMIZATION OF COCOA BUTTER EXTRACTION BY SUPERCRITICAL CARBON DIOXIDE

Independent variables	Levels		
	–1	0	1
Pressure (MPa)	10.0	27.5	45.0
Temperature (C)	35	55	75
Extraction time (h)	1.0	6.5	12.0

out using the response surface methodology (RSM). Pressure (*P*), temperature (*T*) and extraction time (*t*), which are important operating parameters involved in cocoa butter extraction using SC-CO_2, were the independent variables. Three levels of each of the three variables were chosen and coded as –1 (lowest), 0 (middle) and 1 (highest). Table 1 shows the corresponding coded and uncoded (actual) levels of the variables. The ECHIP Software Version 6.3 (Echip Inc., Hockessin, DE) was used to provide experimental designs and make statistical evaluations and a regression model. Altogether, 15 combinations (19 experiments including five replicates of the centerpoint, each signed the coded value 0) were used in random order according to a central composite design (Giovanni 1983; Wheeler *et al.* 1993). For each of the experiments done, the total fat content (%) and TAG profile of cocoa butter were determined.

The model was analyzed to fit the following second-order equation:

$$Y = C_0 + C_1P + C_2T + C_3t + C_4PT + C_5Pt + C_6Tt + C_7P^2 + C_8T^2 + C_9t^2 \qquad (2)$$

where Y is the response function, C_is are constant regression coefficients, and T, P and t are independent variables.

The analysis of variance (ANOVA) table was generated, and the effect and regression coefficients of individual linear, interaction and quadratic terms were determined. The significances of all terms were judged statistically by computing the F value at a probability (P) of 0.001, 0.01 or 0.05. The regression coefficients were then used to a make statistical calculation to generate contour plots from the regression model.

Particle Size Study

Cocoa liquor, ground cocoa nibs and crushed cocoa nibs were used to investigate the effect of sample particle size. The cocoa liquor with a particle size of approximately 74 μm was used without pretreatment. The cocoa beans were crushed, the shell removed and then sieved to obtain a particle size ranging from 4 to 6 mm, and were ground and sieved to obtain a particle size ranging from 0.85 to 1 mm. The effect of the particle size of the sample on cocoa butter extraction was studied under optimized operating conditions and a flow rate of 2 mL/min. The extracted cocoa butter was analyzed in terms of total fat content (%) and TAG profile.

Statistical Analysis

Data in five replicates for the particle size study were analyzed by ANOVA for a completely randomized design using the Statistical Analysis System software package version 6.12 (SAS Institute, Cary, NC) (SAS 1989). The Duncan multiple-range test was used to determine significant differences between means at the 5% level of probability.

RESULTS AND DISCUSSION

Optimization of Operating Conditions

The design of the experiments in the uncoded (actual) and coded levels of variables for SC-CO_2 extraction together with the experimental data for response are presented in Table 2. The percentage of total fat content extracted by SC-CO_2 was greatly influenced by pressure, temperature and extraction time (Table 2). Increment in pressure, temperature and extraction time up to 45 MPa, 75C and 12 h gave the highest value of total fat content (41.28%). The lowest value of total fat content (1.53%) was observed at 10 MPa, 35C and 1 h.

The regression coefficients for the response of total fat content (%) and its corresponding coefficients of determination (R^2) with statistical analysis are summarized in Table 3. The results showed that the linear effect of pressure, temperature and extraction time, the interaction effect of pressure and temperature, pressure and extraction time, and temperature and extraction time, and the quadratic effect of pressure and extraction time contributed significantly to the extraction efficiency of cocoa butter using SFE, with an R^2 of 0.985. The R^2 of more than 0.75 is statistically considered accurate for developing a model or equation (Henika 1982). The probability value of the regression model was less than 0.000.

In order to describe how the test variables affect the response, contour plots for response were generated by the RSM model using the ECHIP software. Figure 1 shows the predicted total fat content (%) extracted by SC-CO_2 as a function of pressure and temperature at a constant extraction time of 1, 6.5 and 12 h, respectively. The contour plots demonstrate that total fat content (%)

TABLE 2.
DESIGN OF EXPERIMENTS IN THE ACTUAL AND CODED LEVELS OF VARIABLES WITH TOTAL FAT CONTENT (%) EXTRACTED FROM COCOA LIQUOR BY SUPERCRITICAL CARBON DIOXIDE

Trial no.	Pressure (MPa)	Temperature (C)	Extraction time (h)	Total fat content (%)
11	45* (1)†	75 (1)	1 (−1)	8.25
6	27.5 (0)	55 (0)	12 (1)	17.00
4	27.5 (0)	75 (1)	6.5 (0)	18.18
2	45 (1)	55 (0)	6.5 (0)	25.78
7	45 (1)	75 (1)	12 (1)	41.28
1	10 (−1)	55 (0)	6.5 (0)	8.04
12	10 (−1)	75 (1)	1 (−1)	2.57
3	27.5 (0)	35 (−1)	6.5 (0)	11.50
14	10 (−1)	35 (−1)	1 (−1)	1.53
15	27.5 (0)	55 (0)	6.5 (0)	13.89
8	10 (−1)	75 (1)	12 (1)	12.88
13	45 (1)	35 (−1)	1 (−1)	4.90
9	45 (1)	35 (−1)	12 (1)	24.52
15	27.5 (0)	55 (0)	6.5 (0)	13.92
5	27.5 (0)	55 (0)	1 (−1)	3.40
15	27.5 (0)	55 (0)	6.5 (0)	13.81
15	27.5 (0)	55 (0)	6.5 (0)	13.95
10	10 (−1)	35 (−1)	12 (1)	7.65
15	27.5 (0)	55 (0)	6.5 (0)	13.78

* Actual level of variables.
† Coded level of variables.

increased with increasing pressure, temperature and extraction time. This may be caused by the increase in density and, subsequently, the dissolving power of the SC-CO_2, which increase the solubility of cocoa butter at higher pressure and temperature. This is in agreement with the findings of Rizvi *et al.* (1986), who stated that the solubility of a given solute tends to increase with the operating pressure and temperature. Meanwhile, as expected, the total fat content (%) increased as the extraction time increased.

Optimum conditions of pressure, temperature and extraction time were determined by the single response optimization type maximize technique (Wheeler *et al.* 1993) from the ECHIP software version 6.3 (Echip Inc.). The result provided by the statistical program showed that the optimum conditions for cocoa butter extraction by SC-CO_2 are at a pressure of 45 MPa, temperature of 75C and extraction time of 12 h, with a predicted total fat content of 40.25% (low limit of 35.34% and high limit of 45.16%).

However, the practical limits of each variable are governed by different factors. Temperature must be kept below a level where the material begins to

TABLE 3.
REGRESSION COEFFICIENTS, R^2 AND P VALUES FOR THE RESPONSE OF TOTAL FAT CONTENT (%) EXTRACTED BY SUPERCRITICAL CARBON DIOXIDE

Coefficient	Term	Value
C_0	Constant	14.0782
	Linear	
C_1	P	0.411771***
C_2	T	0.1653***
C_3	t	1.50327***
	Interaction	
C_4	$P \times T$	0.00494286*
C_5	$P \times t$	0.047039***
C_6	$T \times t$	0.02**
	Quadratic	
C_7	P^2	0.00839655*
C_8	T^2	0.00125361
C_9	t^2	–0.136812**
R^2		0.985
P		0.0000***

* Significant at 0.05 level; ** Significant at 0.01 level; *** Significant at 0.001 level.

P, pressure; T, temperature; t, extraction time.

suffer thermal degradation. Cocoa butter is comprised principally of TAGs (94–96 wt %), although minor components include di- and monoacylglycerols, free fatty acids, glycolipids, phospholipids and other complex lipids (Lehrian and Keeney 1980). SFE using CO_2 provides the oxygen-free condition for its extraction. In general, very high temperatures of heating are required for the thermal nonoxidative decomposition of lipids. No significant decomposition of TAGs and methyl esters of fatty acids can be detected when heated at a temperature below 200C, in the absence of oxygen (Nawar 1996). On the other hand, pressure and extraction time are more related to economic considerations, as construction and operating costs increase with higher system pressure and longer extraction time.

Response surfaces can also be visualized as three-dimensional contour plots by presenting the response as a function of two factors and keeping the others constant. As illustrated in Fig. 2, the response surface showed a strong degree of curvature, where the optimum can be readily determined (the point marked by the arrow). The figure also shows that pressure had a major influence on the yield of total fat content as compared with temperature, which indicates the greater importance of this variable.

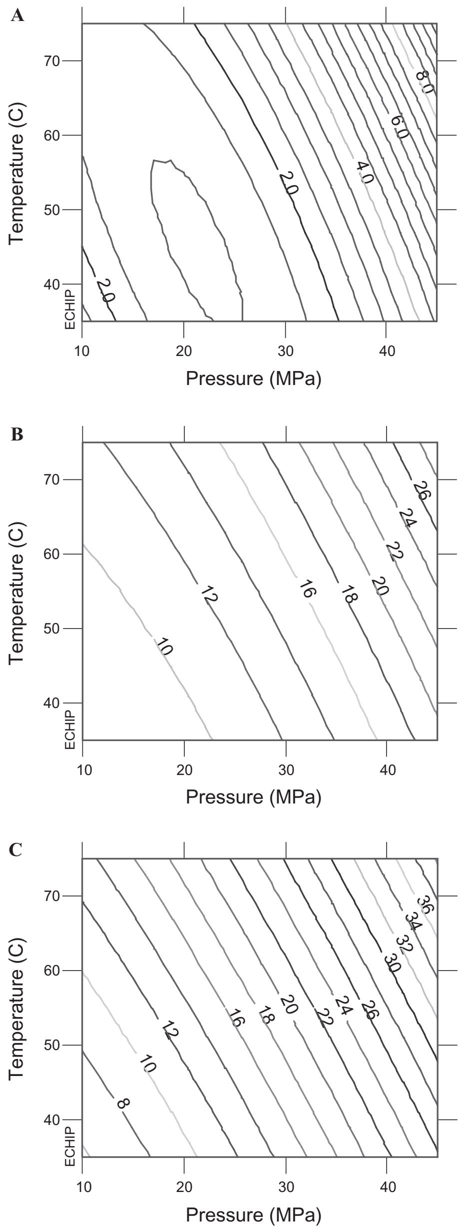

FIG. 1. CONTOUR PLOTS FOR TOTAL FAT CONTENT (%) AS A FUNCTION OF PRESSURE AND TEMPERATURE AT AN EXTRACTION TIME OF (A) 1 h, (B) 6.5 h AND (C) 12 h

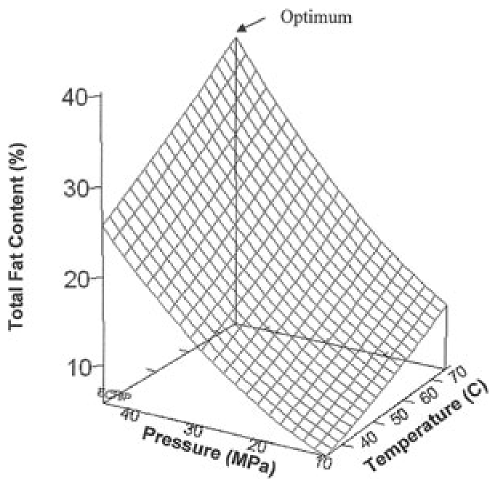

FIG. 2. THREE-DIMENSIONAL CONTOUR PLOT SHOWING THE RESPONSE SURFACE OF TOTAL FAT CONTENT (%) AS A FUNCTION OF PRESSURE AND TEMPERATURE AT AN EXTRACTION TIME OF 12 h

Effect of Particle Size

The rate of removal of a solute from a matrix using SFE is a function of its solubility in the fluid media and the rate of mass transport of the solute out of the sample matrix (King and France 1992). The physical morphology of the sample matrix can have a profound effect on the yield obtained by SC-CO_2 extraction (King and France 1992).

The different particle sizes of cocoa samples were found to significantly influence the total fat content (%) extracted by SC-CO_2 under optimum operating conditions. The highest yield was obtained from cocoa liquor with a particle size of approximately 74 µm, with a total fat content of 40.38% (SD ± 1.07), followed by ground cocoa nibs (particle size of 0.85–1 mm), with a total fat content of 29.33% (SD ± 1.02), and crushed cocoa nibs (particle size of 4–6 mm), with a total fat content of 14.07% (SD ± 0.91), and which were significantly different ($P < 0.05$) among each other.

It is apparent that the smaller the particle size of the sample, the higher the total fat content will be extracted by SC-CO_2. This effect may be a result of the shorter internal diffusional path lengths over which the extracted solutes must travel to reach the bulk fluid phase. Studies have shown that the geometric size of the matrix particles can influence the speed and completeness with which an SFE can be conducted (King and France 1992). Our results confirm the value reported by Rossi *et al.* (1989a), who suggested that the low extraction yields observed from the nibs (particle size ranging from 2 to 4 mm) were caused by the lipid-bearing cells remaining intact in the nib, and concluded that the extraction of cocoa butter appears to be more of a leaching phenomenon.

TAG Profiles of Cocoa Butter Extracted by SC-CO_2

The effects of various operating conditions and the sample matrix with different particle sizes on the TAG profiles of cocoa butter extracted using SC-CO_2 are given in Table 4. The TAG profiles of cocoa butter extracted using

TABLE 4.
TRIACYLGLYCEROL PROFILES (AREA %) OF COCOA BUTTER EXTRACTED AT VARIOUS OPERATING CONDITIONS AND DIFFERENT SAMPLE MATRICES USING SUPERCRITICAL CARBON DIOXIDE

Pressure (MPa)	Temperature (C)	Extraction time (hour)	Triacylglycerol profile (area %)*						
			PLO	PLP	POO	POP	POS	SOS	SOA
45	75	1	0.39	0.79	3.59	21.18	44.10	29.32	0.63
27.5	55	12	0.41	1.59	6.65	22.03	36.62	31.64	1.06
27.5	75	6.5	0.60	1.75	2.17	21.20	43.97	29.66	0.65
45	55	6.5	0.88	1.71	2.47	21.94	43.96	27.79	1.25
45	75	12	0.60	1.55	2.17	21.20	43.97	29.66	0.85
10	55	6.5	0.61	1.71	2.20	21.41	42.25	30.97	0.85
10	75	1	0.31	1.60	6.77	22.37	41.01	27.20	0.74
27.5	35	6.5	0.74	2.32	7.65	27.51	30.91	29.59	1.28
10	35	1	0.59	1.68	2.17	21.34	42.78	30.82	0.62
27.5	55	6.5	0.76	1.56	3.69	23.44	40.70	28.84	1.01
10	75	12	0.47	1.11	3.42	23.94	43.62	26.60	0.84
45	35	1	0.81	1.29	4.34	24.11	37.06	31.55	0.84
45	35	12	0.45	1.00	3.88	22.68	40.19	30.92	0.88
27.5	55	6.5	1.03	1.32	4.96	22.87	37.59	31.09	1.14
27.5	55	1	0.43	0.83	4.57	23.41	38.45	31.74	0.57
27.5	55	6.5	0.47	1.67	4.90	22.61	37.42	31.33	1.60
27.5	55	6.5	0.97	1.35	4.06	19.92	41.96	30.61	1.13
10	35	12	0.88	1.45	3.58	21.69	41.35	29.84	1.21
27.5	55	6.5	0.81	1.53	5.98	22.64	36.98	31.14	0.92

Sample matrix	Particle size	Triacylglycerol profile (area %)*						
		PLO	PLP	POO	POP	POS	SOS	SOA
Cocoa liquor	~74 µm	0.77	1.21	4.16	20.25	41.08	31.51	1.02
Ground cocoa nibs	0.85–1 mm	0.65	1.38	4.87	22.59	38.82	30.87	0.82
Crushed cocoa nibs	4–6 mm	0.80	1.32	4.25	21.89	39.65	31.22	0.87

* Mean of triplicates.

P, palmitic acid (C16:0); S, stearic acid (C18:0); O, oleic acid (C18:1); L, linoleic acid (C18:2); A, arachidic acid (C20:0).

$SC\text{-}CO_2$ contain monounsaturated TAGs, i.e., POP, POS, SOS, SOA and PLP, and di-unsaturated TAGs, i.e., POO and PLO. The results demonstrated that POP (C50), POS (C52) and SOS (C54) were the major TAGs in the extracted cocoa butter, whereby POS showed the highest concentration of more than 30% for all treatments. These findings were in good agreement with the typical TAG composition (area %) of cocoa butter obtained by Chaiseri and Dimick (1989). Rossi *et al.* (1989b) reported the composition of cocoa butter obtained by $SC\text{-}CO_2$ extraction of various cocoa products. They observed that the TAG composition of cocoa butter extracted by $SC\text{-}CO_2$ at 40 MPa and 50–60C were within the range required for this product.

Our results depicted the apparent lack of selectivity of $SC\text{-}CO_2$ for any of the TAGs present in cocoa butter. This is because of the narrow range in carbon number (C50–C56) of its TAG. The cocoa butter extracted with $SC\text{-}CO_2$ was found to be unfractionated, and is therefore of the same quality as that produced by the customary method. This is supported by the findings of Azevedo *et al.* (2003), who stated that the little fractionation capacity of $SC\text{-}CO_2$ is attributed to the similar TAG compositions in the extracted fat of Cupuaçu (*Theobroma grandiflorum*).

CONCLUSIONS

The study found that increment in pressure, temperature and extraction time caused an increase in the total fat content extracted from cocoa liquor. Pressure had a stronger effect on the yield of total fat content compared with temperature. The optimum conditions for cocoa butter extraction using $SC\text{-}CO_2$ were 45 MPa, 75C and 12 h. Particle size had a significant effect ($P < 0.05$) on $SC\text{-}CO_2$ extraction of cocoa butter, and the efficiency decreased with increasing particle size. 1,3-Dipalmitoyl-2-oleoyl-glycerol (POP), 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS) and 1,3-distearoyl-2-oleoyl-glycerol (SOS) were the major TAGs in the extracted cocoa butter, whereby POS was the highest component for all treatments studied. There was no high selectivity of $SC\text{-}CO_2$ for any of the TAGs present in the cocoa butter. The TAG profiles of the extracted cocoa butter were in agreement with the TAG compositions of typical cocoa butter.

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