Dynamic Crystallization of Cocoa Butter. I. Characterization of Simple Lipids in Rapid- and Slow-Nucleating Cocoa Butters and Their Seed Crystals

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ABSTRACT: Six cocoa butters with different crystallization induction times and their seed crystals were analyzed for simple lipid composition. The rapid-nucleating cocoa butter samples had higher concentrations of 1-palmitoyl-2-oleoyl-3-stearoylglycerol and 1,3-stearoyl-2-oleoylglycerol (SOS), and lower concentrations of the diunsaturated triacylglycerols, 1-palmitoyl-2,3-oleoylglycerol and 1-stearoyl-2,3-oleoylglycerol, as well as higher stearic acid concentrations within their diacylglycerol fractions when compared to the slow-nucleating samples. At the early stages of crystallization, under agitation conditions at 26.5°C, cocoa butters solidified into two fractions, high-melting and low-melting. The low-melting fractions were composed of polymorphs IV and V of cocoa butter, as indicated by the onset melting temperatures of the endotherms from differential scanning calorimetry. The high-melting fractions, which had wide melting ranges, had peak maxima of 38.5–52.2°C. Seed crystals isolated at the early stage of crystallization were characterized by high concentrations of complex lipids, saturated triacylglycerols, saturated fatty acid-rich diacylglycerols, and monoacylglycerols. The rapid-nucleating seed crystals had higher concentrations of SOS when compared to their respective cocoa butters. The slow-nucleating seed crystals did not exhibit this characteristic.
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KEY WORDS: Cocoa butter, dynamic crystallization, lipid composition, nucleation, seed crystal.

Cocoa butter, which contributes 30–40% by weight to finished chocolate, provides the desired texture, mouthfeel, and flavor release properties. Variations in cocoa butter composition can result in unacceptable physical properties, such as softness and susceptibility to fat bloom in the final product. Effects of triacylglycerol composition on hardness of the final product have been investigated (1,2). In addition to the effects on quality of the final product, compositional variations of cocoa butter may cause some difficulties during processing. Under the same crystallization conditions, different cocoa butters nucleate at different rates (3). It has been postulated that some minor components, such as glycolipids, phospholipids, and saturated triacylglycerols, may serve as seed crystals and promote the crystallization of cocoa butter (4). Seed crystals formed under static conditions contained high concentrations of glycolipids (11.1%), phospholipids (6.6%), and saturated triacylglycerols (StStSt) (67.7%). Cocoa butters containing higher concentrations of StStSt crystallized at faster rates than those containing lower concentrations of StStSt (5). In addition, Manning and Dimick (6) suggested that cocoa butter crystals with a high concentration of 1,3-distearoyl-2-oleoylglycerol (SOS) might act as seeds and induce crystallization. Addition of diacylglycerols to cocoa butter may promote or delay crystallization, depending upon enantiomeric forms and degree of unsaturation of the diacylglycerols (7). Diolein, both 1,2- and 1,3-forms, and 1,2-dipalmitin (at the concentration of 10%) delay cocoa butter crystallization, whereas 1,3-dipalmitin promotes solidification. Indigenous diacylglycerols could delay crystallization of cocoa butter (8). Cocoa butters that contain high concentrations of diacylglycerols and concomitantly contain high concentrations of free fatty acids crystallize at slow rates (9). The purpose of this study was to investigate the effects of the variations in the natural components, particularly simple lipids, on cocoa butter crystallization induction times.

EXPERIMENTAL PROCEDURES

Sample preparation. Commercial hydraulic prime-pressed cocoa butters from Malaysia, the Ivory Coast, Ghana, Ecuador, the Dominican Republic, and Brazil (Bahia) were investigated. Before the crystallization study and chemical analyses, molten cocoa butters were cleaned by centrifugation to remove the contaminating cocoa powder and nib particulates. In the cleaning process, samples were heated to 110°C for 2 h and centrifuged at 22,000 × *g* for 15 min in a Beckman J2-21 centrifuge with a JA-14 rotor (Beckman Instruments, Palo Alto, CA). Temperature of the molten cocoa butter at the end of the centrifugation was 45–50°C. After cleaning, samples were refrigerated at 4°C.

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Dynamic crystallization. Crystallization studies were carried out under dynamic conditions at 50 rpm in a Brabender Viscocorder (C.W. Brabender Instrument, Inc., South Hackensack, NJ). Prior to the studies, samples were heated to 110°C in an oven for 2 h to destroy residual nuclei. Crystallization temperature was maintained at 26.5°C with a Brabender Pressure Circulator. The starting time (T_0) was the time when the temperature of the melts reached 26.5°C. Progress in solidification was monitored from the increase in absorbance value at 500 nm in a Gilford Spectrophotometer-250 (Gilford Instruments, Oberlin, OH). Vegetable oil was used as a blank. Approximately 1.5 mL of sample from the Brabender cup was transferred to the disposable polystyrene microcuvettes every five minutes. The narrow sides of the cuvettes were placed perpendicularly to the beam in the spectrophotometer.

Seed crystal isolation. Crystallizations were carried out at 26.5°C in a Brabender cup rotating at 50 rpm. The induction time in this study is the time from T_0 to the time of isolation when Δ absorbance was 0.02. The seed crystal isolation procedure was adapted from work by Davis and Dimick (4,10). Crystallized samples from a Brabender cup were centrifuged in a JA-14 rotor at 18,600 × *g* for 8 min in a Beckman J2-21 centrifuge. The liquid cocoa butter was discarded, and the crude seed crystals were rinsed with 10 mL ACS-grade acetone. The washed crystals were then dispersed in 3 mL of cocoa butter–acetone solution (5 g/100 mL). This solution was prepared from the cocoa butter of interest just before isolation and used instead of pure acetone to minimize the solubilization of sterols and glycolipids from the solid phase into the washing solvents. The dispersed seed crystals were transferred into conical centrifuge tubes, which were immersed in a 26.5°C water bath. Samples were subsequently centrifuged at 2,000 × *g* for 2 min in a table-top centrifuge, and the resulting liquid fractions were discarded. The resulting seeds were washed with 2 mL cocoa butter–acetone solution and were centrifuged in the same manner as the first wash. The seed crystals obtained after the second washing were dried under a nitrogen stream and were stored at −80°C until further analyses.

Thermal analysis. Thermal analysis was conducted on a Perkin-Elmer differential scanning calorimeter (DSC-4) equipped with an Intracontroller I Freon refrigeration unit (Perkin-Elmer Corp., Norwalk, CT). Gallium (99.999% purity) was used as the standard for instrument calibration. Two to five milligrams of cocoa butter and their seed crystals were placed in differential scanning calorimetry (DSC) aluminum sample pans and heated from 0 to 100°C at the rate of 20°C/min. The onset melting temperatures were determined by a Perkin-Elmer Thermal Analysis Data System modem 3600.

Chemical analysis. Samples were analyzed in three replicates with duplication for each replicate. Each replicate was a combination of seed crystals from two isolations. Major lipid classes—simple lipids, glycolipids, and phospholipids—were separated by silicic acid column chromatography. Sil-R silicic acid, 100+ mesh (Sigma Chemical Co., St. Louis, MO), was prewashed with ACS-grade methanol, acetone, and chloroform. Approximately 5 g cocoa butter, dissolved in chloroform, was applied to the column. Simple lipids were eluted with 700 mL chloroform; glycolipids were subsequently eluted with 500 mL acetone, and the phospholipids were washed from the column with 700 mL methanol. The lipid solutions were dried under a nitrogen stream and quantitated by gravimetric analysis. Efficiencies of the column separations were monitored by two-step development thin-layer chromatography (TLC).

Simple lipids of cocoa butter and their seed crystals were analyzed by high-temperature capillary gas chromatography (CGC). The method was adapted from the work by Myher and Kuksis (11). Samples were subjected to trimethylsilyl derivatization with *bis*(trimethylsilyl) trifluoroacetamide + trimethylchlorosilane, 99:1 (Supelco, Inc., Bellefonte, PA). A Hewlett-Packard 5890A gas chromatograph (Hewlett-Packard Co., Avondale, PA) with a fused-silica Supelco SPB-5, 8 M × 0.32 mm, 0.1 μm film thickness, was used in the analyses. Helium was the carrier gas at 10 psig. The temperature program was 40–230°C at the rate of 20°C/min, to 280°C at 10°C/min and to 340°C at 5°C/min. A flame-ionization detector (FID) was maintained at 350°C. Peaks were identified by comparison of the retention times with those of standards. Lipid standards—mono-, di-, and tripalmitin; mono-, di-, and triolein; and mono-, di-, and tristearin—were obtained from Sigma Chemical Co. Free fatty acid and plant sterol standards were obtained from Supelco Inc. Simple lipid classes were quantitated as percent areas calculated by a Hewlett-Packard Integrator model HP 3996A.

Triacylglycerols were separated by high-performance liquid chromatography (HPLC) on a 25-cm Adsorbosphere C-18 reverse-phase column (Alltech Associates, Avondale, PA). The HPLC system was equipped with a Waters pump model 510, a U6K injector, and a Waters differential refractometer detector (model 410; Waters, Milford, MA). The solvent system was acetonitrile/chloroform (6.5:3.5, vol/vol), pumped at the rate of 0.4 mL/min. Quantitations were based on the comparisons of peak areas to the standard curves of 1,3-palmitoyl-2-oleoylglycerol (POP), 1-palmitoyl-2-oleoyl-3-stearoylglycerol (POS), SOS, SSS, and 1-stearoyl-2,3-oleoylglycerol (SOO) (Matreya, Inc., Pleasant Gap, PA). Peak areas were calculated by Waters Data Module model 745 and were identified by using internal standards and by comparison with the works by Shukla *et al.* (12) and Davis and Dimick (4).

Minor simple lipids—free fatty acids, mono- and diacylglycerols, and sterols—were quantitated by high-temperature CGC in the same manner as total simple lipid profiles. Before derivatization, triacylglycerols were removed by solid-phase extraction (SPE) to prevent overloading of the CGC column. The SPE method was modified from the method used by Kaluzny *et al.* (13). Five milligrams of the simple lipid fractions were dispersed in 0.5 mL hexane and applied to 500-mg Bond Elut aminopropyl columns (Analytichem International, Harbor City, CA). The columns were placed on a Supelco

Visiprep Solid Phase Extraction Vacuum Manifold. Lipid classes were eluted at 10 kPa. The efficiency of separation by SPE was monitored by TLC and CGC. TLC was used to detect polar lipid contamination, and CGC was used to monitor the purity and the recovery of minor simple lipids. Quantitation was based on external standard curves of the particular lipid classes.

Fatty acid distribution of the free fatty acid, diacylglycerol, and polar lipid fractions were analyzed separately as fatty acid methyl esters (FAME). Lipid classes were purified by TLC. Ten milligrams of cocoa butter or seed crystal material were dissolved in 2% (vol/vol) methanol in chloroform and loaded onto the preparative silica gel G Uniplates™ (Analtech Inc., Newark, DE). Approximately 1 mL of the FAME/hexane solution was injected directly onto a Supelco Nukol fused-silica capillary column (15 m × 0.53 mm i.d., 0.5-mm film thickness), installed in a Hewlett-Packard 5890A gas chromatograph. Helium was used as the carrier gas at 5 psig. The temperature program was isothermal at 140°C for 4 min and to 180°C at 20°C/min. The FID was maintained at 190°C, and the FAME were quantitated as area percent calculated by a Hewlett-Packard Integrator model HP 3996A. Peak identification was based on the retention times in comparison to those of the standards, Nu-Chek-Prep 20-A and Supelco GLC-10.

Statistical analyses. Data were analyzed by using the Statistical Analysis System (SAS). Analysis of variance was done with the General Linear Model. Multiple comparison with the least significant difference was applied to separate the means at $\alpha = 0.05$. Linear regression of the average nucleation times and the average compositional concentrations were calculated with the Cricket Graph program (Cricket Software, Malvern, PA).

RESULTS AND DISCUSSION

Crystallization behavior. The crystallization studies were carried out under mild agitation at 26.5°C. These conditions induce cocoa butter to crystallize in polymorphic forms IV and V and to transform from form IV to form V. The samples nucleated at different rates. Cocoa butters from Malaysia and the Ivory Coast exhibited rapid nucleation rates with induction times of 78 and 80 min, respectively (Table 1). Cocoa butters from the Dominican Republic and Bahia had distinctively slower nucleation rates with induction times of 277 and 300 min, respectively. Ghanaian and Ecuadorian samples had intermediate induction times of 95 and 117 min, which were closer to those of the rapid-nucleating samples.

TABLE 1
Crystallization Induction Times of Cocoa Butters Under Dynamic Conditions at 26.5°C

Cocoa butter source	Induction time (min)[a]
Malaysia	78 ± 10 A
Ivory Coast	80 ± 13 A
Ghana	95 ± 14 AB
Ecuador	117 ± 18 B
Dominican Republic	277 ± 44 C
Brazil (Bahia)	300 ± 51 C

[a]Mean ± standard deviation (n = 7). Means within the same column with the same letter are not significantly different at $\alpha = 0.05$.

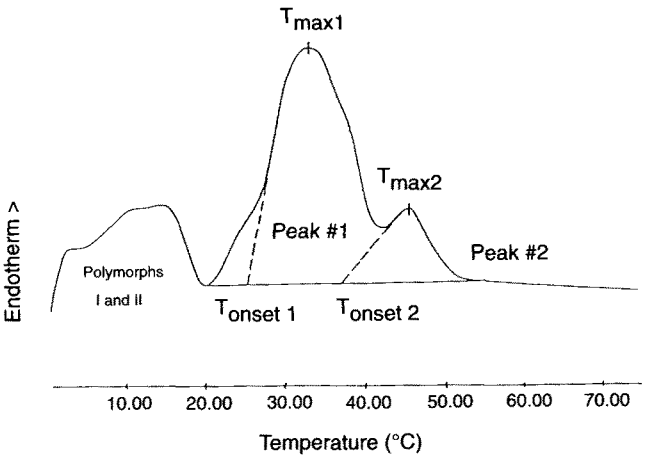

FIG. 1. Differential scanning calorimetry thermogram of cocoa butter seed crystals illustrates melt contamination (polymorph I & II), low-melting fraction (Peak #1), and high-melting fraction (Peak #2). T_{onset1} is the onset transistion temperature and T_{max1} is the peak temperature of Peak #1. T_{onset2} is the onset transition temperature and T_{max2} is the peak temperature of Peak #2.

Thermal analyses. During the early stages of crystal growth, cocoa butter crystallized into a low-melting fraction and into a small quantity of high-melting fraction. The thermogram in Figure 1 is an example of the DSC endotherms for the seed crystals. The low-melting fractions (Peak #1) had onset melting temperatures ranging from 25.4 to 29.2°C (Table 2). The high-melting fractions of seed crystals (Peak #2) had onset melting temperatures ranging from 35.6 to 45.5°C. The onset melting temperatures imply that the low-melting fraction of seed crystals was composed mostly of polymorphs IV. The endotherms of low-melting fractions were broad, which indicated the possibility of co-existing polymorph forms IV and V. The onset melting temperatures of the high-melting fraction of seed crystals isolated in this study were lower than the onset melting temperatures (55–72°C) of the seeds isolated under static conditions (10,14). Under static conditions, seed crystals develop slowly and favor fractional crystallization of the high-melting com-

TABLE 2
Melting Characteristics from DSC Thermograms of Cocoa Butter Seed Crystals from Different Countries

Seed crystal	Peak #1[a] T_{onset} 1 (°C)	Peak #2 T_{onset} 2 (°C)
Malaysia	25.4 ± 1.8	35.6 ± 5.5
Ivory Coast	29.2 ± 0.1	45.5[b]
Ghana	27.9 ± 3.6	44.4 ± 4.4
Ecuador	25.4 ± 1.0	37.4 ± 3.1
Dominican Republic	27.1 ± 4.2	36.5[b]
Brazil (Bahia)	27.7 ± 4.2	36.3 ± 4.0

[a]Refer to Figure 2 for the definitions of Peak #1, Peak #2, T_{onset} 1 and T_{onset} 2.
[b]Only single assay determined.

TABLE 3
Major Lipid Classes of Cocoa Butters and Their Seed Crystals

Sample	Lipid class (wt%)[a]		
	Simple lipid	Glycolipid	Phospholipid
Cocoa butter			
Malaysia	99.0 ± 0.7	0.8 ± 0.7	0.2 ± 0.1
Ivory Coast	99.5 ± 0.1	0.4 ± 0.0	0.1 ± 0.0
Ghana	99.4 ± 0.2	0.5 ± 0.1	0.1 ± 0.1
Ecuador	99.2 ± 0.1	0.5 ± 0.0	0.2 ± 0.0
Dominican Republic	99.7 ± 0.0	0.3 ± 0.0	<0.1
Brazil (Bahia)	99.7 ± 0.0	0.3 ± 0.0	<0.1
Seed crystals			
Malaysia	93.3 ± 2.7	1.9 ± 0.1	4.8 ± 2.8
Ivory Coast	95.9 ± 2.6	1.3 ± 0.0	2.8 ± 2.6
Ghana	95.2 ± 1.5	1.9 ± 0.1	1.9 ± 0.1
Ecuador	98.3 ± 0.9	1.3 ± 0.7	0.4 ± 0.2
Dominican Republic	95.9 ± 0.5	2.4 ± 0.4	1.8 ± 1.0
Brazil (Bahia)	97.2 ± 0.3	1.2 ± 0.1	1.6 ± 0.2

[a]Mean ± standard deviation (n = 3).

ponents, especially the saturated triacylglycerols from the cocoa butter melt. Under dynamic conditions, crystallization requires less supersaturation and occurs at a rapid rate. More components can crystallize during the early stages, resulting in lower melting temperatures of the high-melting crystals.

Lipid classes. Data from column chromatography revealed that all cocoa butter samples had consistent concentrations of approximately 99% simple lipids, 0.3–0.8% glycolipids, and <0.1–0.2% phospholipids (Table 3). Their seed crystals had 6–20-fold more complex lipids. Seed crystals contained 93.3–98.3% simple lipids, 1.2–2.4% glycolipids, and 0.4–4.8% phospholipids (Table 3). There was no correlation between the crystallization induction times and total simple lipid, glycolipid, and phospholipid contents of both cocoa butter and their seed crystals.

Simple lipids. Simple lipid fractions, including triacylglycerols and other minor components, i.e., free fatty acids, mono- and diacylglycerols, and sterols, were separated based on

TABLE 4
Triacylglycerols and Total Minor Simple Lipid Contents Within Simple Lipid Fractions of Cocoa Butters and Their Seed Crystals

Sample	Content (%)[a]	
	Triacylglycerol	Minor simple lipid
Cocoa butter		
Malaysia	98.0 ± 0.2	2.0 ± 0.2
Ivory Coast	97.7 ± 0.4	2.4 ± 0.1
Ghana	96.8 ± 0.4	3.2 ± 0.4
Ecuador	98.0 ± 0.2	2.1 ± 0.3
Dominican Republic	96.9 ± 0.6	3.1 ± 0.7
Brazil (Bahia)	97.6 ± 0.8	2.4 ± 0.6
Seed crystals		
Malaysia	97.6 ± 1.0	2.5 ± 1.0
Ivory Coast	92.7 ± 2.8	7.3 ± 2.8
Ghana	98.9 ± 0.3	1.1 ± 0.3
Ecuador	97.9 ± 1.3	2.0 ± 1.2
Dominican Republic	96.5 ± 0.7	3.6 ± 0.7
Brazil (Bahia)	96.0 ± 2.5	4.1 ± 2.6

[a]Mean ± standard deviation (n = 3).

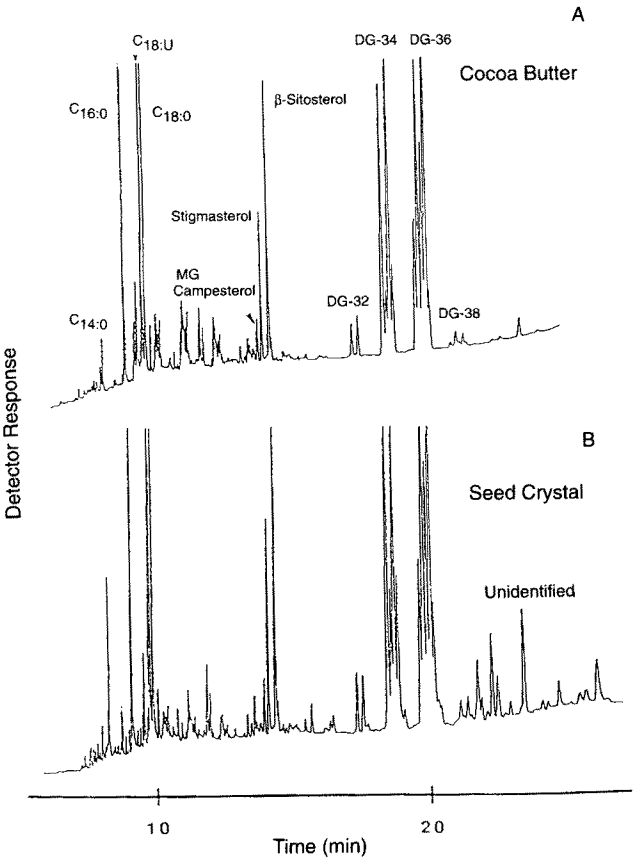

FIG. 2. Capillary gas chromatographic chromatograms of the minor simple lipids isolated from cocoa butter (A) and from seed crystals (B). MG, monoacylglycerol; DG, diacylglycerol.

carbon number and on the difference between saturated and unsaturated acyl chains (Fig. 2). Simple lipid fractions of the cocoa butters were composed of 96.8–98.0% triacylglycerols and 2.0–3.2% minor simple lipids (Table 4). In this study, there was no indication that variations in total amounts of minor simple lipids, at the indigenous concentrations of 2–3%, caused variations in the crystallization induction times of cocoa butters. However, the fact that seed crystals contained higher concentrations of minor simple lipids (1.1–7.3%) than their original cocoa butters indicated that some minor simple lipids preferentially crystallized during the early stage of crystallization.

Minor simple lipids. Minor simple lipid fractions of cocoa butter were composed mainly of free fatty acids (36.4–52.0%), and diacylglycerols (40.4–57.4%) (Table 5). There were also small quantities of sterols (3.8–6.4%) and monoacylglycerols (1.1–1.4%). Major sterols in cocoa butter minor simple lipid fractions were β-sitosterol (2.0–3.8%), stigmasterol (1.1–1.8%), and campesterol (0.5–0.8%). Monoacylglycerol contents were consistent among different cocoa butters, with low concentrations amounting to 1.1–1.4% of minor simple lipid fractions, or 0.03–0.04% of the cocoa butters. Because of their low concentrations, monoacylglycerols may not have much effect on crystallization of cocoa butter. The simple lipid fractions of the seed crystals, except for

TABLE 5
Lipid Class Distributions Within Minor Simple Lipid Fractions of Cocoa Butters and Their Seed Crystals

Sample	Content (%)[a]			
	Fatty acid	Monoacyl-glycerol	Sterol	Diacyl-glycerol
Cocoa butter				
Malaysia	52.0 A[a]	1.3 A	6.4 A	40.4 D
Ivory Coast	46.2 BC	1.2 A	5.7 B	46.8 C
Ghana	44.5 BC	1.4 A	3.6 E	50.4 BC
Ecuador	42.1 C	1.1 A	5.0 C	52.0 B
Dominican Republic	47.3 B	1.4 A	3.8 DE	47.5 BC
Brazil (Bahia)	36.4 D	1.4 A	4.4 CD	57.8 A
Seed crystals				
Malaysia	49.4 A	2.3 B	6.8 A	41.6 B
Ivory Coast	55.8 A	2.1 B	5.9 AB	36.2 B
Ghana	54.2 A	1.5 B	3.4 D	41.0 B
Ecuador	53.1 A	6.7 A	5.7 B	35.6 B
Dominican Republic	49.3 A	2.4 B	3.7 CD	44.6 B
Brazil (Bahia)	34.2 B	2.4 B	4.7 BC	58.7 A

[a]Means of cocoa butters (n = 3) within the same column for each category with the same letter are not significantly different at $\alpha = 0.05$.

those of the Ecuadorian sample, were similar to those of their respective cocoa butters (Table 5). The gas chromatographic chromatograms of most seed crystals showed the presence of unidentified compounds that had molecular weights close to C_{38} diacylglycerols (Fig. 2B).

Free fatty acids. There was little variation in free fatty acid composition among different cocoa butters. The major free fatty acids were, as expected, palmitic acid (29.3–32.3%), oleic acid (29.2–31.6%), and stearic acid (24.5–27.8%). Saturated fatty acids rapidly crystallized at the early stages. Free fatty acids of seed crystals were composed mainly of palmitic acid (32.5–34.9%) and stearic acid (35.3–39.5%). Seed crystals were significantly higher in stearic acid (35.3–41.5%) and myristic acid (1.2–3.1%), but were lower in oleic acid content (15.2–19.5%) than their respective cocoa butters.

Diacylglycerols. Diacylglycerols of cocoa butter contained mainly oleic acid (37.7–42.3%), stearic acid (25.8–29.7%), and palmitic acid (22.0–23.4%). The rapid-nucleating cocoa butters from Malaysia, the Ivory Coast, and Ghana contained significantly higher concentration of stearic acid (28.0–29.7%) in the diacylglycerol fractions as compared to the slower-nucleating samples from Ecuador, the Dominican Republic, and Bahia (25.5–25.9%). It is possible that the stearate in diacylglycerols could facilitate the chain arrangement of SOS, which was also crystallized at a rapid rate in the early stage of crystallization. Diacylglycerols with saturated acyl chains crystallized at more rapid rates when compared with those with unsaturated chains. Seed crystals contained significantly higher saturated fatty acids and lower oleic acid in their diacylglycerol fractions than their respective cocoa butters. The major fatty acid in seed crystals was stearic acid, whereas oleic acid was the major fatty acid in cocoa butters.

Triacylglycerols. Triacylglycerols had more effect on the crystallization induction time of cocoa butter than the other components. Induction times of the samples correlated with the concentrations of 1-palmitoyl-2,3-oleoylglycerol (POO) + SOO ($R^2 = 0.86$) and POS + SOS ($R^2 = 0.73$) in cocoa butters. Samples that contained higher POO and SOO (Table 6), and concomitantly lower POS and SOS concentrations, had longer induction times. Malaysian and the Ivory Coast samples, the rapid-nucleating cocoa butters, contained significantly higher POS and SOS than those of the Dominican Republic and Bahian samples, the slow-nucleating cocoa butters. POO and SOO could act as competitors in the crystallization of the *sn*-2-oleodisaturated triacylglycerols. The extra oleate chains of POO and SOO retard the crystallization of cocoa butter by interfering with the molecular packing of the *sn*-2-oleodisaturated triacylglycerols. The concentrations of POO, SOO, POS, and SOS, besides influencing nucleation time, also affect the hardness characteristics of cocoa butter (2). As a result, soft cocoa butter is usually associated with a slow crystallization rate and hard butter shows rapid crystallization.

The triacylglycerol composition of the seed crystals showed similar trends (Table 7). There were correlations between nucleation time and concentrations of SOO ($R^2 = 0.85$), POO ($R^2 = 0.90$), and SOS ($R^2 = 0.74$) in seed crystals. Rapid-nucleating seeds contained higher concentrations of SOS and lower POO and SOO than the slow-nucleating seed crystals. In fact, there were significantly higher concentrations of SOS in the rapid-nucleating seed crystals than their respective cocoa butters, whereas the slow-nucleating seed crystals were similar in SOS concentration when compared to their respective cocoa butters. It could be that slow-nucleating cocoa butters originally had lower concentrations of

TABLE 6
Triacylglycerol Composition of Cocoa Butters from Different Countries

Cocoa butter	Triacylglycerol (wt%)[a]													
	PLO	PLP	OOO	POO	PLS	POP	SOO	SLS	POS	PPS	SOS	PSS	SOA	SSS
Malaysia	0.4 C	1.1 B	0.1 B	1.1 E	2.6 C	12.6 B	1.8 C	1.6 A	46.9 A	0.7 A	29.8 A	0.4 A	0.9 A	0.2 A
Ivory Coast	0.7 ABC	1.7 A	0.4 AB	1.8 D	3.7 BC	15.0 A	2.3 B	1.7 A	46.3 A	0.7 A	24.0 B	0.5 A	0.8 A	0.4 A
Ghana	1.0 A	1.8 A	0.8 A	2.0 D	3.6 BC	14.5 A	2.8 AB	2.0 A	42.8 BC	0.8 A	26.3 C	0.6 A	1.0 A	0.2 A
Ecuador	0.5 BC	1.6 A	0.7 A	2.7 C	3.1 AB	14.1 A	3.3 A	1.6 A	45.4 AB	0.8 A	24.8 C	0.4 A	0.8 A	0.3 A
Dominican Republic	0.7 AB	1.8 A	0.6 A	3.8 B	4.2 A	14.6 A	4.4 A	1.8 A	42.8 BC	0.7 A	22.8 D	0.5 A	1.0 A	0.4 A
Brazil (Bahia)	0.9 A	1.7 A	0.7 A	5.8 A	3.9 A	13.9 A	6.7 A	2.1 A	40.2 C	0.6 A	21.7 E	0.5 A	0.9 A	0.6 A

[a]Means within the same column with the same uppercase letter are not significantly different at $\alpha = 0.05$; P = palmitate, O = oleate, S = stearate, L = linoleate, A = arachidonate.

TABLE 7
Triacylglycerol Composition of Cocoa Butter Seed Crystals from Different Countries

Cocoa butter	Triacylglycerol (wt%)[a]													
	PLO	PLP	OOO	POO	PLS	POP	SOO	SLS	POS	PPS	SOS	PSS	SOA	SSS
Malaysia	0.2B	1.0C	0.1C	0.9E	2.0 D	110B	1.1E	1.1C	40.4A	1.5A	34.4A	3.5A	1.0AB	2.0A
Ivory Coast	0.6A	1.3B	0.2CB	1.4D	2.7 BC	13.4A	1.9D	1.5B	42.6A	1.3A	27.7B	2.8A	0.6B	1.8A
Ghana	0.9A	1.6A	0.5AB	2.0C	3.0 AB	13.3A	2.6C	1.8A	39.4A	1.4A	28.7B	2.1A	1.2A	1.4A
Ecuador	0.7A	1.2B	0.2CB	1.9C	2.4 C	12.8A	2.5C	1.4B	40.1A	1.5A	29.0B	3.3A	1.0AB	2.0A
Dominican Republic	0.7A	1.4AB	0.7A	3.2B	3.3 A	13.6A	4.1B	1.6B	41.8A	1.3A	23.8C	2.4A	0.8AB	1.6A
Brazil (Bahia)	0.8A	1.3B	0.7AB	4.3A	2.9 B	12.4A	5.5A	1.4B	38.9A	1.5A	23.3C	3.6A	0.9AB	2.1A

[a]Means within the same column with the same uppercase letter are not significantly different at $\alpha = 0.05$. Abbreviations as in Table 6.

SOS and had more POO + SOO that interfere with the crystallization of SOS than the rapid-nucleating ones. The POS contents of seed crystals, unlike those of cocoa butters, were consistent among different seed crystals. Davis and Dimick (10) proposed that the indigenous saturated triacylglycerols, 1,2-palmitoyl-3-stearoylglycerol (PPS), PSS, and 1,2,3-stearoylglycerol (SSS), in cocoa butter have the potential to serve as seeds for crystallization due to their low solubility in cocoa butter melt at the study temperature. However, in this study, the concentrations of saturated fatty acids were consistent among different samples; thus, they did not cause the variations in the induction times. All seed crystals were significantly higher in saturated triacylglycerols, PSS and SSS, than their original cocoa butters. The distributions of the saturated triacylglycerols were similar among the different seed crystals. Saturated triacylglycerol concentrations of seed crystals, formed under dynamic conditions, were 4.9–7.2%. The concentrations were much less than the 57.7% of seed crystals formed under static conditions (10). The high-melting fractions from the DSC thermogram could be the crystals that contain high concentrations of saturated triacylglycerols and SOS. The onset melting temperatures of seed crystals, which ranged from 35.6–44.4°C (Table 2), are higher than the melting temperatures of polymorph V and VI of cocoa butter and are within the range of the β-forms of *sn*-2-oleodisaturated triacylglycerols. The melting temperatures of the β-forms of SOS range from 41 to 43°C (15). Peaks #2 were broad enough to cover the melting temperatures of the α-forms of the saturated triacylglycerols. The α-forms of PPS, PSS, and SSS had melting temperatures of 47.4, 50.6, and 54.0°C, respectively (16). The onset melting temperatures of most seed crystals are slightly lower than the melting temperatures of pure saturated triacylglycerols due to the co-crystallization among different saturated triacylglycerols and with the other components.

Complex lipids. Fatty acid distribution of the complex lipids, which included both glycolipids and phospholipids, were not different among cocoa butter samples. The major fatty acids—stearic, oleic, and palmitic—were 22.9–31.0%, 23.6–28.7%, and 24.8–26.8%, respectively. Complex lipids of seed crystals, in contrast to the other lipid classes, were richer in unsaturated fatty acids than those in the original cocoa butters. Complex lipids of seed crystals, except for those of Ghanaian and Ecuadorian samples, had higher oleic and linoleic acid concentrations and lower stearic acid concentrations than the respective cocoa butters. During dynamic crystallization of the molten cocoa butter at 26.5°C, the molecular arrangement of complex lipids could be more dependent on the polar head groups than the degree of saturation of the acyl chains.

This study shows that during early stages of crystallization under dynamic conditions cocoa butter crystallizes into two fractions, high-melting and low-melting. During the early stages of crystallization, the saturated components, such as saturated triacylglcyerols, free stearic acid, stearic and palmitic-rich diacylglycerols, occurred at faster rates than the unsaturated ones. In addition to the saturated components, SOS preferentially crystallized under study conditions. Variation in the triacylglycerol composition was best correlated with the crystallization induction times of cocoa butter. Rapid-nucleating cocoa butters contained slightly more stearic acid-rich diacylglycerols. Slow-nucleating cocoa butters had opposite characteristics.

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