Crystallinity properties and crystallization behavior of chocolate fat blends

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Abstract Cocoa butter (CB) provides unique crystallization characteristics to chocolates and confectionary products; hence, it is an important value-add product. However, other alternative fats that minimally affect the crystallization behaviour of chocolates and confectionary products are now being increasingly used. This study analyzed the crystallization behaviour of CB, cocoa butter substitutes (CBSs), and their blends. Blends were prepared using CBS concentrations: 5, 10, 15, 20 and 37.5%. CB, CBS, and their blends were evaluated by following analysis: solid fat content, isothermal analysis, polarized light microscopic, thermal behaviour, X-ray diffraction and consistency. Crystallization analysis showed an incompatibility between the 2 fats, with a reduction in the crystallinity and increase in liquid content in all the blends. Eutectic crystallization at 20 °C was only observed for the blend containing 20% CBSs. This was considered as a positive result because previous studies have indicated that CBS concentration in CB blends should not be more than 5%.

Keywords Cocoa butter · Cocoa butter substitute · Compatibility · Eutectic crystallization

Introduction

Cocoa butter (CB) is a common and essential ingredient of chocolate and other confectionary products. It is solid at room temperature (~ 25 °C) and liquid at body temperature (~ 37 °C). CB mainly contains palmitic (P), stearic (St), and oleic (O) acids. CB can crystallize into several polymorphic forms, including the γ , α , β' , and β forms. Its special crystallization properties, such as polymorphism and consistency, and its consequent sensorial properties are attributed to its triacylglycerol (TAG) composition, as CB is almost exclusively composed of symmetric TAGs (SUS/saturated–unsaturated–saturated) such as POST, POP, and StOST (Schenk and Peschar 2004; Jahurul et al. 2013). These characteristics make CB one of the most valuable tropical fats. However, its use is hampered by the difficult cultivation and low productivity of cocoa bean and by pest attacks on these plants. Moreover, the price of CB has increased greatly in recent years due higher demand (Afoakwa 2010).

Supply shortages, poor CB quality of individual crops, economic rewards and certain technological improvements have encouraged the chocolate and confectionery industries to seek alternative fats with the physical and sensory properties of CB that are cheaper to obtain (Sonwai et al. 2014), such as cocoa butter substitutes (CBS) from natural sources (Lonchamp and Hartel 2004; Jahurul et al. 2013). CBSs are fats derived from palm kernel, coconut or babassu oils. They exhibit high contents of lauric (La) and myristic (M) acids, resulting in short-chain TAGs. The physical properties of CBSs are similar to those of CB, but due to the chemical incompatibility between the TAGs of these fats, mixtures of these fats can develop eutectic effects (Jahurul et al. 2013). Eutectic interaction is observed in many fat mixtures and defines one of the

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criteria for the degree of compatibility of fats. This type of interaction tends to occur when fats differ in their molecular volume, shape, or polymorph. A mixture with a eutectic effect will have a lower solid fat content (SFC) than either of the two pure fats, showing that the two fats are not compatible with each other (Bigalli 1988). The compatibility of CBSs with CB is quite low (less than 5%). This low compatibility is consistently reported to result in negative effects in chocolates, such as excessive softening and acceleration of fat bloom development (Williams et al. 1997; Lonchamp and Hartel 2004).

Compatibility analyses have been performed between many fats and oils, including Malaysian CB, CBS and milk fat (Sabariah et al. 1998a); Malaysian CB, CBE and milk fat (Sabariah et al. 1998b); palm oil, sunflower oil and palm kernel oil (Noor Lida et al. 2002); CB and soybean oil and CB and canola oil (Perez-Martinez et al. 2007); and CB and CBE (Bootello et al. 2012). However, little is known about the mechanisms underlying the crystallization behaviour of alternative fats that are rich in the lauric fatty acids in CB, and countless studies on this topic have shown that incompatibility of these fats has a negative effect. Along these lines, the aim of the present study was to evaluate the crystallization behaviour of CB, CBSs, and blends of these fats to better elucidate the characteristics of these blends and determine the concentration of CBS that can be mixed with CB with a minimal effect on the crystallization characteristics of CB for futures applications.

Materials and methods

Experimental design

CB and CBS were acquired in Brazil. The samples were melted, weighed, and mixed via shaking. Temperature was controlled to ensure complete miscibility of the fats. Concentrations were evaluated on the basis of the fat content in the chocolate (~32%), of which added CB accounted for 12% and the CB naturally present in cocoa liquor for 20%. Samples A–E exhibited 5, 10, 15, 20, and 37.5% CBS, respectively. The initial concentration of CBS was set at 5% because this concentration is commonly used in various products. A sample containing 37.5% CBS simulated the replacement of all of the added pure CB (12% of the chocolate).

Methods

Fatty acid composition

The fatty acid composition of CB and CBSs was determined using an Agilent 6850 Series gas chromatography

(GC) system (Agilent Technologies, Santa Clara, CA, USA) equipped with a capillary column, following esterification according to a method described by Hartman and Lago (1973). Fatty acid methyl esters were separated using AOCS method Ce 2-66 (AOCS 2009), employing a 60-m DB-23 capillary column (50% cyanopropyl methylpolysiloxane; Agilent Technologies) with an internal diameter of 0.25 mm, coated with a 0.25- μ m film. Chromatography was performed under the following conditions: heating at 110 °C for 5 min in an oven, followed by heating to 215 °C at a rate of 5 °C/min and holding at 215 °C for 24 min. The detector temperature was 280 °C, the injector temperature was 250 °C, helium was used as the carrier gas, the split ratio was 1:50, and the injection volume was 1.0 μ L. The quantitative composition was determined via area normalization and was expressed as the mass percentage. Values are expressed as a mean from 3 injections. The fatty acid composition of the blends was calculated proportional to that of CB and CBSs based on their tested concentrations.

Triacylglycerol composition

The TAG compositions of CB and CBS were determined in triplicate by performing capillary GC with an Agilent 6850 Series GC system equipped with a DB-17 HT capillary column (50% phenyl-methylpolysiloxane; length, 15 m; internal diameter, 0.25 mm; Agilent Technologies) coated with a 0.15- μ m film. Chromatography was performed using the following conditions: the initial column temperature was set at 250 °C, the column was then heated to 350 °C at a rate of 5 °C/min, the split injection ratio was 1:100, helium was used as the stripping gas (flow rate, 1.0 mL/min), the injector temperature was 360 °C, the detector temperature was 375 °C, the injection volume was 1.0 μ L, and the sample concentration in tetrahydrofuran was 10 mg/mL. TAGs were identified by comparing retention times with standard samples and were quantified based on their relative peak areas (Filho et al. 1995). The TAG composition of the blends was calculated proportional to that of CB and CBSs based on their tested concentrations.

Solid fat content (SFC)

SFC was determined using a nuclear magnetic resonance (NMR) spectrometer (Bruker pc120 Minispec; Silberstreifen, Rheinstetten, Germany) and TCON 2000 high-precision dry baths (0–70 °C; Duratech, Carmel, USA), according to AOCS method Cd 16b-93 (AOCS 2009), to stabilizing confectionery fats. The samples were first tempered as follows: they were melted at 100 °C for 15 min, chilled at 0 °C for 90 min, and then held at 26 °C for 40 h and at 0 °C for 90 min in a high-precision dry

bath. The samples were subsequently held at each measurement temperature (10, 15, 20, 25, 30, 35 and 40 °C) for 60 min prior to SFC measurements. The results are expressed as the mean of 3 measurements. A compatibility diagram was plotted using the SFC versus the CBS concentration in a blend at each temperature (Quast et al. 2013).

Isothermal analysis

The samples were melted at 100 °C for 15 min and then maintained at 70 °C for 1 h in a high-precision dry bath (TCO 2000, Duratech, Carmel, IN, USA) before measurements were taken. The increase in the solid fat content due to the crystallization time was monitored automatically every minute for 180 min at 17.5 °C using a Bruker pc120 Minispec NMR spectrometer. Crystallization kinetics were determined based on the induction period (t_{SFC}), maximum SFC (SFC_{max}), and relative time required to obtain 50% SFC_{max} ($t_{1/2}$) (Campos 2005). The results are expressed as the mean of 3 different measurements. The original Avrami equation was used to model the crystallization process (Eq. 1).

$$\text{SFC} = \text{SFC}_{\text{max}}(1 - e^{-kt^n}), \quad (1)$$

where, $\text{SFC}(t)$ is the percentage of SFC as a function of time; SFC_{max} is the asymptotic value of SFC; k is the Avrami constant (min^{-n}), which considers both nucleation and the rate of crystal growth; and n is the Avrami exponent, which indicates the mechanism of crystal growth. The equation was non-linearized to obtain the values of k and n . $t_{1/2}$ expresses the magnitude of values in accordance with Eq. 2:

$$t_{1/2} = \left(\frac{0.693}{k} \right)^{1/n} \quad (2)$$

Polarized light microscopy

Crystal morphology was evaluated using a polarized light microscope (Model BX 51; Olympus, San Jose, USA) connected to a digital video camera (Media Cybernetics, Bethesda, USA). The samples were melted at 100 °C, and approximately 1 drop of each sample was placed on a preheated glass slide using a capillary tube and then covered with a coverslip. The samples were stabilized according to AOCS method Cd 16b-93 (AOCS 2009) for special fats (60 °C/5 min, 0 °C/30 min and 26 °C/40 h) and subsequently analysed.

Thermal behaviour

Thermal behaviour was analyzed using differential scanning calorimetry (DSC) (Model Q2000; TA Instruments,

New Castle, DE, USA) according to AOCS method Cj 1-94 (AOCS 2009). The conditions for crystallization were as follows: initial temperature of 80 °C for 5 min, cooling to −40 °C at a cooling rate of 2 °C/min, and holding at −40 °C for 5 min. Approximately 10 mg of the melted sample was weighed, and an empty sealed aluminium pan was employed as a reference. The equipment was calibrated using indium. The following process data were calculated using the onset temperature (T_{ons}), peak temperature (T_{p}), enthalpy (ΔH), and end temperature (T_{e}).

X-diffraction and crystallinity analysis

X-ray diffraction analyses of the samples were performed according to AOCS method Cj 2-95 (AOCS 2009) by using Philips PW 1710 diffractometer (PANalytical, Almelo, the Netherlands) with Bragg–Brentano (θ : 2θ) geometry and $\text{CuK}\alpha$ radiation ($\lambda = 1.51418$ Å; tension, 40 kV; and current, 30 mA). The geometry used caused the X-ray beam diffracted by the sample to pass through a graphite monochromator crystal located just before the detector. All measurements were obtained on the short spacing at steps of 0.03 °C in 2θ and acquisition time of 2 s, scans from 15° to 30° (Schenk and Peschar 2004). Samples were also prepared for the analysis by using the tempering process for fat stabilization according to the AOCS method Cd 16b-93 (AOCS 2009). For crystallinity evaluation, using 1 s acquisition time at 0.03° steps along the 2θ range between 6° and 60°, long spacing at 25 °C. According to Ruland (1961), the weight fraction of a crystalline material (x_{cr}) can be described using a ratio of integrated intensities from diffraction peaks (I_{cr}), which represents the crystalline amount and from the total coherent scattering that considers thermal corrections. In the present study, these integrated intensities were obtained using Rietveld method by using TOPAS Software (Bruker Corporation). The I_{cr} value was acquired from diffraction peaks by using the structure of β -V Ivory Coast CB (van Mechelen et al. 2007). The experimental background intensity was described using a second-order Chebyshev polynomial function and by assuming that all the intensities were caused only by the sample, such that the experimental background intensity could be subtracted from the total observed intensity I . To represent the intensity contribution from an amorphous content (I_{am}), a pseudo-Voigt peak was inserted or fitted at $2\theta \approx 20.3^\circ$. Thus, the total observed intensity I could be written only in terms of I_{am} and I_{cr} . The crystallinity of the sample (x_{cr}) was assumed using the following equation (Eq. 3):

$$x_{\text{cr}} = \frac{I_{\text{cr}}}{I} = \frac{I_{\text{cr}}}{I_{\text{cr}} + I_{\text{am}}} \quad (3)$$

Consistency

Consistency was determined using a TA-XT Plus texture analyser (Stable Micro Systems, Surrey, UK). The samples were also stabilized according to AOCS method Cd 16b-93 (AOCS 2009). The test was performed at 25 °C. Probes consisting of 45° Plexiglas® cones with non-truncated tips were used. The penetration depth was 10 mm, the probe velocity was 2 mm/s, and the time was 5 s (Campos 2005). Compression force was expressed as gram force (gF). The maximum compression force was converted to the yield value (YV) according to Haighton (1959) using the following equation (Eq. 4):

$$YV = \frac{KxW}{p^{1.6}}, \quad (4)$$

where, YV is the yield value in gF/cm², and K is a constant that depends on the cone angle. For a 45° cone, K is 4.700, W is the compression force in gF, and p is the penetration depth in units of 0.1 mm. The results are expressed as the mean of 4 tempering experiments that were analyzed separately.

Statistics analysis

All statistical analyses were performed using Statistica 8.0 (Statsoft, USA). Analysis of variance and the Tukey test were used to determine significant differences between the means at a probability level of 5% ($p < 0.05$).

Results and discussion

Fatty acid and triacylglycerol composition

The concentrations of P, St, and O acids in CB were 25.7, 33.9, and 34.9%, respectively, confirming that these 3 fatty acids are the main components of CB (94.5% in total). The concentration of each fatty acid was within the limits indicated in the literature (Lipp et al. 2001; Masuchi et al. 2014). The main fatty acids in CBSs were La, M, P, and St acids, with concentrations of 52.5, 21.2, 9.6, and 10.2%, respectively (93.6% in total), which was consistent with the data reported in the literature (Sabariah et al. 1998a; Quast et al. 2013). Blends containing 5 and 10% CBSs (A and B) exhibited the same main fatty acid as CB; however, blends containing ≥15% CBSs also contained La and M acids among the main fatty acids.

CB mainly contained symmetrical TAGs such as POP (16.5%), POSt (40.3%), and StOSt (23.6%), accounting for 80.4% in total, a value in accord with the literature (Lipp et al. 2001). The CBSs mainly contained TAGs such as LaLaLa (26.4%), LaLaM (24.6%), LaLaP (15.9%), and

LaLaSt (9.25%), accounting for 76.15% in total. Different authors have reported different TAG composition of CBSs, with Quast et al. (2013) reporting LaLaLa, LaLaP, LaMP, and LaMSt as the main TAGs in CBSs, while Lipp et al. (2001) reported LaLaLa, LaLaM, and LaMM as the main TAGs. Because of the large differences in the TAG composition of the raw materials, the blends also showed large variations in the TAG composition depending on the amount of CB replaced. However, the TAG composition of the blends was always proportional to that of the CBSs added to the blends.

The TAG compositions of the fats and blends were examined according to the different groups of TAGs present. TAGs are divided into 4 main groups: trisaturated, or SSS; monounsaturated, or SUS; di-unsaturated, or SUU; and tri-unsaturated, or UUU. The CBSs contained 98.5% SSS TAGs, while CB contained 83.8% SUS TAGs. The concentration of SSS TAGs in the blends increased with an increase in the content of CBSs. TAGs are responsible for important properties, such as the melting profile and spreadability of the final product. These properties are dependent on the crystallization and polymorphic structures of TAGs; moreover, different TAGs confer different properties (Toro-Vazquez et al. 2002).

Solid fat content and compatibility

In the present study, an expressive difference was observed between the 2 types of fats (Fig. 1a). The CBSs showed a higher initial SFC (97.3%), which subsequently decreased rapidly, conferring unique melting characteristics on the CBSs. In contrast, CB exhibited a lower SFC at 10 °C (75.5%), but the decrease in the SFC was slower than that for CBSs. At 35 °C, the 2 fats displayed approximately the same SFC. The SFC of the CBSs determined in the present study was consistent with that reported in the literature (Sabariah et al. 1998b). This SFC of the CBSs could be attributed to their TAG composition because the melting point of the saturated TAGs of CBSs is lower than that of CB TAGs, which allows faster melting of the final product (Karabulut et al. 2004). For blends, the first relevant difference in SFC was observed for the blend containing 10% CBSs (sample B). The SFC of this blend was initially higher and then decreased rapidly compared with CB. This effect intensified with an increase in the CBS concentration in the blend. This blend proportion has also been reported to exhibit faster melting in the literature (Quast et al. 2013).

The SFC of CB between 25 and 35 °C is an industrial parameter for evaluating the quality of CB. Above 35 °C, SFC can be identified by the presence of a waxy feeling that is detected when the product is consumed. The presence of ≥50% SFC in chocolates or chocolate products at 25 °C provides adequate snap and good melting properties

Fig. 1 **a** Solid fat content and **b** compatibility diagram of the cocoa butter (CB), cocoa butter substitute (CBS), and their blends. Sample A (5% of CBS), sample B (10% of CBS), sample C (15% of CBS), sample D (20% of CBS) and sample E (37.5% of CBS)

to the chocolate at room temperature, particularly in tropical countries (Ribeiro et al. 2012). Our results for CB are in accord with both of these parameters, with a 50% SFC being observed at 25 °C and 0.2% at 35 °C, while the CBS exhibits an 88.5% SFC at 25 °C but 2% at 35 °C, which might result in a waxy feeling in the chocolate produced with this pure CBS. Even though both fats exhibited an SFC >50% at 25 °C, the blends did not show the same characteristic, which increases the difficulty of producing and storing chocolates produced using these blends, particularly when higher concentrations of CBSs are used. However, the melting properties of the products, particularly mouth feel at 35 °C, were in accordance with industrial requirements. The SFC of the blends was more similar to the CB SFC than pure CBS. This similarity was also observed for blends of cocoa butter replacers (CBRs), which is reported as a positive result when the objective of the blend is to replace CB (Jahurul et al. 2014b).

SFC normally decreases when the proportion of modified fats derived from lauric fats is low because the co-crystallization between the short- and medium-chain SSS TAGs of CBSs and the long-chain SUS TAGs of CB is quite weak, and the 2 fats present different crystalline polymorphic forms (Lonchamp and Hartel 2004).

SFC can also be used to study the compatibility of fats by determining the changes in the solid percentage associated with different proportions of fat (Noor Lida et al. 2002). If 2 fats are perfectly compatible and exhibit similar melting behaviour, the lines of constant SFC lie along straight horizontal lines. Two fats can also be compatible in phase behaviour but exhibit different melting behaviours (Williams et al. 1997). The eutectic behaviour of the 2 fats can be more clearly observed when the curves are redrawn (Fig. 1b), with the SFC being plotted against the percentage of 1 fat in the mixture at different temperatures (Quast

et al. 2013). The binary diagram of mixtures containing CBSs and CB showed that at 20, 25, and 30 °C, the SFC of the blends decreased with an increase in the concentration of CBSs. This effect occurred with a higher intensity for the blend containing 20% CBS at 20 °C, indicating that the incompatibility between CBSs and CB increased from this concentration onwards and at this temperature. Because of the incompatibility between the 2 fats, eutectic crystallization occurred, and a decrease of the SFC of the blend with 20% CBS was observed (Williams et al. 1997). Quast et al. (2013) also reported such higher incompatibility for these CBS concentrations, but at 25 °C. These results are positive because only 5% CBS is currently commonly used to replace the CB in chocolates and chocolate products when allowed under the law (Lonchamp and Hartel 2004).

Isothermal crystallization

Figure 2a shows the isothermal crystallization of CB, CBS and blends of the two fats at 17.5 °C. CBS presented faster isothermal crystallization in just 1 step. However, CB and all of the blends exhibited isothermal crystallization in 2 steps. According to Dewettinck et al. (2004), 2-step crystallization depends on the temperature used and the composition of each fat. It occurs as follows: first, the fat content increases without an appreciable induction time. This value then remains almost constant for some time and increases greatly thereafter. At this time, a *plateau* is reached. The existence of a *plateau* during isothermal crystallization represents the polymorphic transition of the sample from less- to more-stable polymorphs. Although all of the blends showed a profile similar to CB, some changes were observed after increasing the concentration of CBS, such as acceleration of the first step of crystallization, a delay in the beginning of the second step of crystallization

Fig. 2 **a** Isothermal crystallization obtained at 17.5 °C in NMR and **b** Crystallization curves in DSC. CB (cocoa butter); CBS (cocoa butter substitute); A (5% of CBS); B (10% of CBS); C (15% of CBS); D (20% of CBS) and E (37.5% of CBS)

Table 1 Isothermal crystallization of cocoa butter (CB), cocoa butter substitute (CBS), and their blends

Samples	Avrami parameters					
	t_{sfc} (min)	SFC_{max} (%)	k (min^{-1})	n	$t_{1/2}$	R^2
CB	16 ± 0.47^a	63.50 ± 0.15^a	5.14×10^{-6a}	2.63 ± 0.08^a	88.30 ± 1.16^a	0.9927
CBS	8 ± 0.47^b	86.58 ± 0.05^b	0.000769^b	2.30 ± 0.16^b	19.25 ± 0.17^b	0.9953
A	14 ± 0.00^c	55.15 ± 0.15^c	1.20×10^{-6a}	2.97 ± 0.03^a	87.12 ± 0.45^a	0.9922
B	12 ± 0.47^d	48.37 ± 0.22^d	1.38×10^{-6a}	2.91 ± 0.01^a	91.50 ± 0.44^a	0.9909
C	11 ± 0.47^d	42.52 ± 0.48^e	1.50×10^{-6a}	2.80 ± 0.05^a	105.59 ± 1.95^d	0.9889
D	10 ± 0.47^d	36.02 ± 1.22^f	1.78×10^{-7a}	3.12 ± 0.15^a	130.10 ± 3.96^e	0.9791
E	8 ± 0.47^b	24.78 ± 0.18^g	3.16×10^{-3c}	1.63 ± 0.04^c	27.04 ± 0.21^f	0.9824

^a Values are shown as means $\pm$ SD of three replications. Means ($n = 3$) with different letters in the same column are significantly different ($p < 0.05$). ^b Samples A (5% of CBS), B (10% of CBS), C (15% of CBS), D (20% of CBS) and E (37.5% of CBS). ^c Parameters t_{sfc} = induction time; SFC_{max} = solid fat content maximum; k = crystallization rate; n = crystal morphology; $t_{1/2}$ = half time crystallization

for blends containing CBS concentrations up to 20%, and a reduction in SFC_{max} .

The delay in the second step of crystallization was not observed for the blend containing 37.5% CBS. For this blend, the first step of crystallization was shorter, similar to that observed for CBS, but with a reduced SFC (Wang et al. 2011).

The profiles shown in Fig. 2a are numerically represented for t_{sfc} , SFC_{max} , and $t_{1/2}$ in Table 1. The t_{sfc} and SFC_{max} significantly decreased as the concentration of CBS increased. This difference was also observed for blends containing 5% CBS. A previous study found that a CB to which 5% fully hydrogenated palm kernel oil (CBS raw material) was added did not show a significant difference in t_{sfc} with respect to pure CB. However, SFC_{max} and the beginning of the second step of crystallization showed similar changes to the results obtained for sample A (Ribeiro et al. 2013).

CB and CBS exhibited $t_{1/2}$ values of 88.3 and 19.25 min, respectively. In the blends, this parameter showed a significant difference when the proportion of CBS was $\geq 15\%$ (105.6 min), and the value continued to increase until 130.1 min (sample D). However, for the blend containing 37.5% CBSs (sample E), $t_{1/2}$ decreased greatly to 27.04 min. According to Ribeiro et al. (2013), $t_{1/2}$ is an effective indicator for quantifying changes in the crystallization rate, and an increase in the $t_{1/2}$ value indicates a decrease in crystallization. A significant difference between $t_{1/2}$ values was observed only for CBS proportions greater than 15%, which indicated the possibility of using 5 and 10% CBS in chocolates without bloom formation.

Thermal behaviour

Analysis of the crystallization curve (Fig. 2b) and the data it represents Table 2 showed a large difference between the

Table 2 Thermal crystallization behaviour CB/CBS: initial temperature (T_o), peak temperature (T_p), end temperature (T_e), crystallization enthalpy (ΔH) and height peak (H)

Samples	Crystallization behaviour			
	T_o (°C)	T_p (°C)	ΔH (J/g)	T_e (°C)
CB	16.53	14.04	80.10	−25.75
CBS	19.66	18.80	118.2	4.88
A	15.53	13.5	80.38	−21.41
B	15.18	13.13	83.66	−24.72
C	14.8	12.64	84.25	−19.07
D	13.98	11.86	87.17	−23.72
E	11.93	6.54	87.66	−12.92

crystallization profiles of CB and CBS. CB displayed small and large peaks, with some sub-peaks. The DSC curves of some oils and fats containing heterogeneous groups of TAGs showed 4 distinct exothermic peaks (high-, medium-, and low-temperature peaks), which correspond to the 4 groups of TAGs (SSS, SUS, SUU, and UUU). However, these peaks are not always well defined, which depends on the proportions of TAGs in oils or fats (Tan and Che Man 2000). CB initially presented a small sub-peak representing the crystallization of SSS TAGs, a second sub-peak (large part of the peak) representing the crystallization of SUS TAGs, and a final small sub-peak representing the crystallization of UUU and SUU TAGs. These sub-peaks cannot be referred to as peaks because they do not return to the baseline. However, differences in crystallization were visible and were attributed to the TAG composition and crystallization. The CBSs showed only a single specific peak because they were mainly composed of SSS TAGs (96%). Rapid crystallization of CBSs is sometimes considered to be a great advantage, mainly for products such as coatings (Wainwright 1996).

Analysis of the blends indicated that displacement of the curve characteristic of CB at low temperatures led to a greater delay in the beginning of the second sub-peak (SUS TAGs). This delay resulted in a clear division of crystallization between SSS and SUS TAGs, which was proportional to the amount of CBS added. The concentration of these SSS TAGs was quite low in CB (~2%) and high in CBS (~96%). This division occurs because although SSS TAGs crystallize first, they do not act as seeds for the crystallization of SUS TAGs due to the presence of different polymorphic forms (i.e., double-chain instead of triple-chain lengths of the β form of SUS) (Smith 2001). These results were validated by data presented in Table 2 for 3 temperatures (T_{ons} , T_p and T_e), where the lower temperatures decreased with the addition of CBSs. The same peak division and delay in the crystallization of the second peak were observed in a CBR produced with mango fat and palm stearin (Jahurul et al. 2014a).

The second important observation about the crystallization profile was the increase in crystallization enthalpy (ΔH). This was expected because pure CBS presents a high ΔH . Ribeiro et al. (2013) studied CB with 5% fully hydrogenated palm kernel oil and observed that ΔH significantly decreased, and the dislocation of the peak was not clear.

The triglyceride and fatty acid compositions of the blends could be responsible for increasing or decreasing crystallization onset and offset temperatures. The dilution and solubilization of TAGs and fatty acids in blends could be another reason for the different crystallization onset and offset temperatures (Jahurul et al. 2014a).

Microstructure

Crystallization characteristics include solid state polymorphism, the size and shape of crystals, and the spatial distribution of the network mass (Campos et al. 2002). Figure 3 shows microscopic images of CB, CBS and blends of the two fats after stabilization via the AOCS method. The characteristics of large clusters from cocoa butter make it impossible to quantify the crystallized area and diameter of the crystals, but even without measurements, differences between the samples are clearly visible. CB was characterized by large clusters of large crystals, with no crystals being observed between the clusters, while CBS showed uniform crystallization with many small crystals that formed a compact mass, which is a characteristic of SSS TAGs (Himawan et al. 2006).

Images of the blends after stabilization showed 2 main changes. The first change was in the density of the crystals in the clusters. The addition of CBS to CB decreased the number of crystals in the clusters until no clusters were formed (sample E). The second change was in the crystal network between the clusters. CB showed almost no crystals between the clusters but exhibited small nuclei that joined to form clusters. In contrast, the addition of CBS resulted in an increase in the amount of the crystals tending to form the same compact CBS mass.

When the microstructure was examined, it was possible to visualize eutectic crystallization, as the 2 main changes showed that CB and CBS could not co-crystallize together. CBS crystals crystallized separately and did not form characteristic clusters with CB; similarly, CB crystals did not crystallize in the characteristic fat network of CBS. Increasing the content of CBS decreased the number of crystals formed, as observed in samples A, B, C, D, and E.

X-ray diffraction and degree of crystallinity

X-ray diffraction was performed after tempering, and the results are given in Fig. 4. For short spacing, the main

Fig. 3 Microstructure of cocoa butter (CB), cocoa butter substitutes (CBS) and blends with CB/CBS, **a** (5%), **b** (10%), **c** (15%), **d** (20%) **e** (37.5%), after stabilization (the *scale bar* for all images is 100 μm)

D-spacing peaks occurred at 4.15 Å for the α polymorphic form; strong peaks occurred at 4.2 and 3.8 Å for the β' polymorphic form; and strong peak occurred at 4.6 Å, followed by 4 small peaks between 3.6 and 3.9 Å, for the β

polymorphic form. For a mixture of β and β' , the predominant polymorphic form exhibited a higher intensity in the main D-spacing (D'Souza et al. 1990) (i.e., 4.2 Å for β' and 4.6 Å for the β polymorphic form).

Fig. 4 X-ray diffraction patterns of cocoa butter (CB), cocoa butter substitute (CBS), A (5% of CBS), B (10% of CBS), C (15% of CBS), D (20% of CBS) and E (37.5% of CBS) crystallized at 25 °C for 40 h, and degree of crystallinity calculated

Analysis of polymorphic profiles showed that CB crystallized in the β polymorphic form and that CBS crystallized in the β' polymorphic form. These results are in accord with those of previous studies for CB (Marty and Marangoni 2009) and CBS (Jin et al. 2008) and are similar to those expected by D'Souza et al. (1990) based on the TAG composition because symmetric SUS TAGs are more stable in the β form and SSS TAGs in the β' form.

Analysis of the blends showed that the polymorphic crystallization characteristics of CB were maintained when up to 20% CBS was added (sample D). The sample with 37.5% CBS showed a mixture of β and β' polymorphs, with a predominance of the β form because the peak at 4.6 Å was more expressive. Some other studies have also shown polymorphism in these blends. One previous study (Sabariah et al. 1998a) indicated that the β' polymorph was clearly observable in these blends, and the amount of this polymorph increased with an increase in the concentration of CBSs in the blends. However, Quast et al. (2013) did not find the β' polymorph, even in blends containing 30% CBSs, possibly because of the tempering performed before the analysis.

For tempered fats, the formation of small amounts of the β' polymorph may occur because of eutectic crystallization. This effect could be attributed to the increase in the concentration of CBSs in the blends, which increased the liquid phase in the X-ray profile because CBSs are present in this phase (Fig. 4). The degree of crystallinity confirmed

this increase in the liquid phase. CB exhibits a natural crystallinity of 63.5%. However, the addition of CBSs decreased this percentage greatly, which was clearly also caused by eutectic crystallization because the two fats did not co-crystallize together, and almost all of the added CBSs were in the liquid state in the CB blends. Immiscibility of metastable phases occurs because of differences in carbon chain lengths of 4 or 6 carbons, as observed in mixtures of La and P acids or La and St acids (Himawan et al. 2006). A liquid phase is formed because of incompatibility and eutectic crystallization and can be visualized because SFC decreases with an increase in the amount of liquid content compared with that in the original raw materials (Smith 2001). This results corroborates with the results founds for all crystallization analysis before, and confirm the increase in amorphous phase resulted from eutectic crystallization.

Consistency

The consistency of plastic fats is strongly affected by the thermal and mechanical properties of the crystal network formed by the fat (Campos et al. 2002). Consistency is represented by YV in gF/cm²; CB and CBS showed YVs of 20,119.74 and 42,264.93 gF/cm², respectively. It was expected that the blends would exhibit an intermediate consistency value compared with the 2 fats; however, this was not observed. Blends containing CB and CBSs were softer than both of the raw materials; the YVs for samples A–E were 15,208.71, 10,979.17, 4116.85, 1227.42, and 1337.29 gF/cm², respectively. Statistically significant differences were obtained for blends containing $\leq 20\%$ CBSs (sample D). Studies have been performed on blends containing 5–30% CBSs, and significant softening has been observed for blends containing $\leq 25\%$ CBS (Quast et al. 2013). Extreme softness could be attributed to eutectic crystallization because of the incompatibility between the 2 fats (Himawan et al. 2006).

Conclusion

CB and CBS blends showed significant incompatibility in all analyses. These results were attributed to the reduction of SFC and crystallinity and the consequent increase in the liquid phase, which will result in a softening product, as observed for all of the blends subjected to texture analysis. However, the incompatibility between the 2 fats should not be always regarded as a negative result because a softened product is required in some cases, as in milk chocolate. At present, a maximum CBS concentration of 5% is used in chocolates and chocolate products, in accordance with the law. In the European Union, for example, 5% vegetable fat

is allowed in final chocolate products in some member States. In Brazil, there is no such limit as long as the final chocolate product contains 25% cocoa solids. The present study clearly showed that 5% CBSs resulted in few changes in the crystallization behaviour of CB, and blends with 10% did not cause significant changes in the crystallization process. Moreover, only the blends containing 20% CBSs showed a eutectic effect according to the compatibility diagram.

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