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Physical properties of pre-crystallized mixtures of cocoa butter and cupuassu fat

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RESUMEN

Propiedades físicas de mezclas pre-cristalizadas de manteca de cacao y grasa de cupuassu.

Se han determinado las características físicas de mezclas binarias pre-cristalizadas de manteca de cacao (mezcla de Bahía + Indonesia) con 5, 10, 15, 20, 25 y 30 % (m/m) de grasa de cupuassu. La pre-cristalización se ha efectuado a escala de laboratorio utilizando un reactor de vidrio con camisa (700mL) con agitación. Las muestras fueron analizadas mediante calorimetría diferencial de barrido, difracción de rayos-X, contenido en grasa sólida y tensión de ruptura. Los valores de tensión de ruptura de las mezclas pre-cristalizadas disminuyeron con la incorporación de grasa de cupuassu, siendo este comportamiento observado también para el punto de fusión. Después de la pre-cristalización, manteca de cacao y grasa de cupuassu confirmaron la presencia de polimorfismo β , por medio de análisis de difracción de rayos-X. La adición de hasta 30% de grasa de cupuassu en la manteca de cacao no afecta de modo significativo en las propiedades físicas cuando son comparadas con la manteca de cacao pura.

PALABRAS CLAVE: *Atemperado o pre cristalización – Contenido de gordura sólida – Difracción de rayos-X – Grasa de cupuassu – Manteca de cacao – Mezcla binaria de grasas – Tensión de ruptura.*

SUMMARY

Physical properties of pre-crystallized mixtures of cocoa butter and cupuassu fat.

The physical characteristics of pre-crystallized binary mixtures of cocoa butter (Bahía + Indonesian blend) and 5, 10, 15, 20, 25 and 30% (w/w) cupuassu fat were determined. Pre-crystallization was carried out using a lab-scale agitated jacket vessel reactor (700 mL). Samples were submitted to differential scanning calorimetry and X-Ray diffraction. The solid fat content and rupture force were also quantified. The snap values of the crystallized mixture decreased with an increase in the amount of alternative fat. A similar trend was observed with respect to the melting point values. The cocoa butter and cupuassu fat X-ray diffraction patterns confirmed the predominant formation of the β polymorph. The addition of up to 30% cupuassu fat did not significantly affect the values of the physical properties when compared to pure cocoa butter.

KEY-WORDS: *Binary mixture of fats – Cocoa butter – Cupuassu fat – Snap test – Solid fat content – Tempering – X-ray diffraction.*

1. INTRODUCTION

Cocoa butter, milk fat and alternative fats constitute the continuous phase in chocolate production and are therefore responsible for the dispersion of the other constituents. Cocoa butter is responsible for the functional attributes in chocolate products, such as hardness at room temperature and pleasant mouth feeling due to melting at body temperature. It contains three main fatty acids: palmitic (P), stearic (S) and oleic (O) acids. Practically all oleic (unsaturated) acid is esterified at the *sn*-2 position of the glycerol molecule so that more than 75% of the total triacylglycerols (TAGs) are 1,3-dipalmitoyl-2-oleoylglycerol (POP), 1-palmitoyl-2-oleoyl-3-stearoylglycerol (POS) and 1,3-distearoyl-2-oleoylglycerol (SOS) (Minifie, 1989).

Triacylglycerols display a complex polymorphic behavior, strongly influenced by momentum, heat and mass transfer during crystallization. Polymorphic crystallization is primarily determined by the rate of nucleation, being governed by thermodynamic and kinetic factors. Depending on the cooling rate and agitation level, triacylglycerols appear in various crystal lattices: α (*hexagonal sub-cell*), β' (*orthorhombic sub-cell*) and β (*triclinic sub-cell*). The three polymorphs are based on subcell structures which define cross-sectional packing modes of the zigzag aliphatic chain. Each polymorph has a unique melting point and crystal structural properties (Piska *et al.*, 2005; Martínez *et al.*, 2007).

During the manufacturing of chocolate, a proper pre-crystallization or tempering protocol is essential before molding and cooling in order to enhance the stability of the product. The tempering process induces the formation of beta nuclei crystals which are thermodynamically more stable. Properly tempered chocolates have more adequate melting properties and retard the appearance of fat bloom, a grayish white film on the surface of the chocolate (Wille and Lutton, 1966; Maleky and Marangoni, 2008).

Cupuassu (*Theobroma grandiflorum* Schumann) is a native fruit of the Amazon region, botanically related to cacao (*T. cacao* L.). Among the *Theobroma* species, cupuassu has the largest fruit. The fresh seeds contain about 84% moisture and

the fat content is approximately 60% of the dry weight. Cupuassu has found applications in the food, pharmaceutical and chemical industries and the cupuassu fat can be used as an alternative fat substitute for cocoa in chocolate production (Gilabert-Escrivá *et al.*, 2002; Lannes *et al.*, 2003; Medeiros *et al.*, 2006). Just like cocoa, cupuassu seeds can be fermented and dried to obtain cupuassu liquor, which is used to elaborate a Brazilian product, “cupulate”, with has nutritional and sensorial characteristics that are very close to chocolate (Oliveira *et al.*, 2004).

Studies reported by Silva *et al.*, (2009), using differential scanning calorimetry, concluded that the polymorphic behavior of cupuassu fat is similar to that of cocoa butter with predominance to crystallization of the beta form.

The sensory attributes of some foods rich in lipids such as chocolates and margarines depend on the structure created by the crystal network. The physical properties of oils and fats such as cocoa butter have been studied using differential scanning calorimetry, solid fat content and X-ray diffraction (Lannes *et al.*, 2003, Czerniak *et al.*, 2005). The solid fat content is used to indicate the physical attributes of fats like its hardness (Himawan *et al.*, 2006). Fats with low solid fat content when used in chocolate manufacturing can result in soft products (Lannes *et al.*, 2003).

X-ray diffraction is a suitable technique to identify the polymorphism of oils and fats and to confirm thermal events during phase transitions (Keller *et al.*, 1996). X-ray diffraction studies have been used to identify phase transitions and the polymorphism of cocoa butter as reported by several authors (Loisel *et al.*, 1998, Marangoni and Narine, 2002; Marangoni and McGauley, 2003; Mazzanti *et al.*, 2003; MacMillan *et al.*, 2002, Schenk and Peschar, 2004; Maleky and Marangoni, 2008; Rousseau and Sonwai, 2008).

The objective of this study is to evaluate the physical properties of pre-crystallized mixtures of

cocoa butter and cupuassu fat by using the snap test, melting point, solid fat content curve and x-ray diffraction techniques.

2. MATERIALS AND METHODS

2.1. Material

Deodorized cocoa butter (mixture of Brazilian and Indonesian cocoa butter supplied by Barry Callebaut Brasil S/A, Bahia, Brazil).

Natural cupuassu fat from the Amazonian region supplied by Aboissa Óleos Vegetais, (São Paulo, Brazil). Before use, the natural cupuassu fat was neutralized to a final acidity of 0.04%.

The experiments were conducted using eight (8) different proportions (0, 5, 10, 15, 20, 25, 30 and 100%) of cupuassu fat and cocoa butter as presented in Table 1.

2.2. Methods

Triacylglycerol composition

The triacylglycerol composition was determined using an Agilent 6850 gas chromatograph. A 150m length, 0.25mm internal diameter DB 17 (50% phenyl methyl polysiloxane) column was used with the following column temperature program: initial temperature 250°C raised at the rate of 5.0°C/min to a final temperature of 350°C, where it was held for 20 min. The detector temperature was 375°C and the injector temperature was 360°C. The carrier gas was helium and the sample concentration was 20mg/mL diluted in tetrahydrofurane, with at least two repetitions for each mixture.

Pre-crystallization

Pre-crystallization was conducted in a lab scale glass jacketed vessel reactor (700mL) coupled to a stirring system. The fat mixture temperature was

Table 1
Denomination and proportion of the mixtures of cocoa butter and cupuassu fat, rupture tension of fat in bar samples after storage and melting points using a differential scanning calorimeter

Proportion (%)		Samples	Rupture tension kg/cm ²	Melting point (°C)
Cocoa butter	Cupuassu Fat			
100	0	CB100	2.48±0.15 ^{ab}	35.28±0.51
95	5	CUP5	2.60±0.30 ^a	35.36±0.28
90	10	CUP10	2.76±0.25 ^a	35.33±0.02
85	15	CUP15	2.69±0.15 ^a	34.94±0.03
80	20	CUP20	2.21±0.25 ^{bc}	34.75±0.05
75	25	CUP25	2.13±0.12 ^{bc}	34.56±0.10
70	30	CUP30	1.90±0.14 ^c	34.30±0.05
0	100	CUP100	1.08±0.06 ^d	34.62±0.10

a, b, c and d differ statistically according to Tukey test with 95% reliability. For rupture tension, nominal cross section area is 2.2cm².

controlled using two recirculating water baths. The mixture was initially stirred at 96rpm and heated to 40°C to remove any crystal remains, and then cooled at a rate of 2.0°C/min until its crystallization temperature of 24°C. The sample was kept at this temperature for 8 minutes and re-heated to 31°C to remove unstable crystals, and to complete the tempering cycle.

After pre-crystallization, the melted fat mixture was poured into molds (8.2 x 2.5 x 0.7cm) and cooled down to 12°C in an 8m long tunnel using convective forced air. The residence time of the product in the tunnel was 23 minutes. After cooling, the samples were demolded, wrapped manually with aluminum foil and transferred to a 24±0.5°C temperature controlled chamber, and kept for a period of 15 days to promote crystal network stabilization. After this period, the samples were submitted to rupture force, melting point and X-Ray diffraction determinations.

Rupture Tension

A Universal TA-XT2i texturometer (Stable Micro Systems, England) with an HDP/3PB – Three Point Bend Rig probe was used to perform the snap test on the bars. The determinations were performed according to the methodology described by Jorge *et al.*, (1999). The conditions used were: distance between bar supports: 6cm; pre-test velocity: 3mm/sec; test velocity: 1.7mm/sec; post-test velocity: 10mm/sec. The rupture force applied at the center of the bars, expressed in kg_f, was obtained from the force vs deformation graphs. Analyses were made in a 20±0.5°C controlled temperature room using ten (10) repetitions per sample formulations. To avoid the influence of possible variations in the thickness of the bars, the values of the force obtained in each test were divided by the cross section area of each bar and the rupture tension was expressed in kg_f/cm².

Melting Point

A differential scanning calorimeter – DSC-7 (Perkin Elmer, Germany), heat flow type, was used to determine the melting point using the methodology described by Bolliger *et al.*, (1998). About 6mg of solidified sample were sealed in small aluminium pans and heated at the rate of 2.0°C/min from 20°C to 40°C. Duplicate results were obtained.

Solid fat content (SFC)

Solid fat content (SFC) was determined following the Cd16b-93 Method described by AOCS (2002), using a Minispec mq20 (ND 1607, Bruker, Germany) nuclear magnetic resonance spectrometer. The fat samples were previously submitted to the following sequential heat treatment: heating to 100°C, maintained at 100°C for 15min; 5min at 60°C; 90±5min at 0°C; 40±0.5hr at 26°C and 90±5min

at 0°C. Solid fat content was measured at the following temperatures: 10°C, 20°C, 21.1°C, 25°C, 26.7°C, 30.0°C, 33.3°C and 35°C. Before each measurement, the sample was stabilized at the test temperature for 60min. Duplicate analyses were performed.

X-Ray diffraction

X-Ray diffraction analyses of the samples were made in a Philips (PW 1710, Holland) diffractometer, using the Bragg-Brentano (θ :2 θ) geometry with CuK α radiation, 40kV tension and 30mA current. In the geometry used, the beam of X-ray diffracted by the sample passes through a graphite monochromator crystal located just before the detector. All measurements were obtained at steps of 0.02°C in 2 θ with an acquisition time of 2sec. The analyses were performed on fat samples in their crystallized solid form at an average controlled temperature of 22°C. X-Ray diffraction pattern curves were obtained for samples of cocoa butter, cupuassu fat and select mixtures with 10, 20 and 30% of cupuassu fat contents.

3. RESULTS AND DISCUSSION

3.1. Triacylglycerol Composition

The triacylglycerol compositions of the samples of cocoa butter and cupuassu fat are listed in Table 2.

The values in Table 2 show that the triacylglycerols present in larger proportions in cocoa butter are POS, SOS and POP, representing 78.2% of the total triacylglycerols. These triacylglycerols are responsible for the well defined crystallization behavior of cocoa butter. Cupuassu fat did not contain POP or PliP, but high contents of SOS and also 2.13% of POP + PliP and SOO + OOO (57.8%). On the other hand, cupuassu fat showed triacylglycerols of the SOA and OOA types, with amounts of 11.87% and 10.20% respectively, which were not identified in cocoa butter. Cupuassu fat presents a high content of symmetrical triacylglycerol of the SUS type (saturated, unsaturated, saturated), which can provide crystallization patterns similar to that of cocoa butter, while the SOO, OOA and OOO triacylglycerols may be responsible for its softness (Gilabert-Escrivá *et al.*, 2002).

3.2. Rupture Tension

Snap tests results of the evaluated samples are shown in Table 1.

The values obtained indicate that sample mixtures containing up to 25% cupuassu fat present snap values that are statistically similar to those of 100% cocoa butter which show a rupture tension of 2.48kg_f/cm².

The similarity in snap values confirms that with proper pre-crystallization conditioning, these mixtures can be used in the manufacturing of

Table 2
Triacylglycerol composition of cocoa butter and cupuassu fat

Number of Carbons	Triacylglycerol	% of area on chromatogram	
		Cocoa butter	Cupuassu fat
C50	POP	15.80±0.16	—
	PlIP	4.63±0.30	—
	POP+PlIP	—	2.13±0.40
C52	POS	40.12±0.45	—
	POO	8.61±1.44	7.52±0.56
	PSS+POS	—	10.46±0.66
C54	SOS	22.30±0.70	27.54±3.86
	SOO	8.54±0.44	—
	SOO+OOO	—	30.28±3.73
C56	SOA	—	11.87±0.52
	OOA	—	10.20±0.11

--- Not found; P – Palmitic, O – Oleic, li -Linoleic, S – Stearic, A - Arachidic

chocolates. The lowest value for rupture tension was observed with the samples containing only cupuassu fat. The decreasing trend of the mechanical resistance observed with the sample containing increasing proportions of cupuassu fat can be attributed to differences between cocoa butter and cupuassu fat fatty acids and triacylglycerol profiles, especially in relation to SOO, OOO and SOA. The snap resistances of chocolates is related to the tempering process and to the ingredients used in the formulation. Thus, in the manufacturing of chocolates using alternative fats such as cupuassu fat, the crystallization parameters should be adjusted for each composition in order to ensure adequate hardness of the final product. Cohen *et al.*, (2004) used 17% cupuassu liquor and 5% cupuassu fat added to 17% cocoa liquor and 5% cocoa butter in their milk chocolate formulation. Using a pre-crystallization temperature of 29.5°C and a tempering time of 7.5min, they produced chocolate bars with rupture tension of the order of 1.96kg/cm², a value which is similar to the rupture tension of the sample with 30% cupuassu fat shown in Table 1.

3.3. Melting Point

The melting point obtained by differential scanning calorimetry of 100% cocoa butter, 100% cupuassu fat and of the sample mixtures are presented in Table 1.

The value of the melting point of cocoa butter obtained in this study is close to the values obtained by other researchers (Undurraga *et al.*, 2001; Dhonsi and Stapley 2005; Himawan *et al.*, 2006). The mixtures CUP5 and CUP10 showed melting points which are very close to that of 100% cocoa butter. A slight lowering trend for the melting point of CUP15 (15% of cupuassu fat) to CUP30 (30% cupuassu fat) can be observed, with the lowest value of 34.3°C corresponding to CUP30. The difference between the melting point of cupuassu fat and cocoa butter, only 0.66°C, is, in fact, very small. According to Ali *et al.*, (2001), the melting

of cocoa butter occurs in a narrow zone between 27°C and 33°C and is essentially complete at 35°C. Studies done by Wille and Lutten (1966), Chapman (1971) and Keller *et al.*, (1996), showed that the beta polymorphic form in cocoa butter has melting points varying from 29°C to 35°C. Based on these data, it can be assumed that the crystallization procedure used for cocoa butter, cupuassu fat and the sample mixtures in this study was sufficient to induce the beta crystalline form.

3.4. Solid Fat Content (SFC)

Solid fat content (SFC) determined for the fats and their mixtures are shown in Figure 1.

The solid fat content at 25°C reflects the hardness of the fat. The higher this value, the harder the fat is at this temperature. Figure 1 shows that 100% cupuassu fat has the lowest SFC. For mixtures of up to 20% cupuassu fat in cocoa butter, the behavior of the curves is close to that

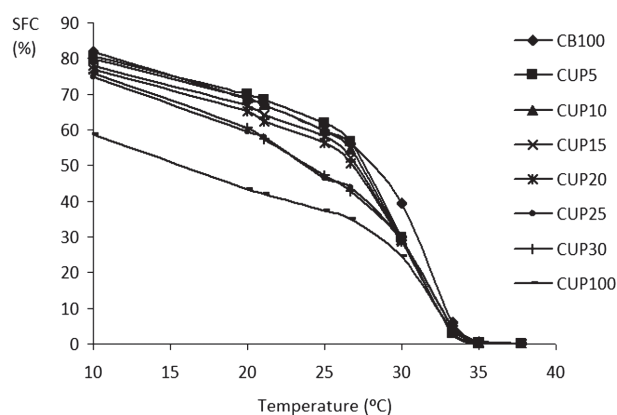


Figure 1
Solid fat contents (SFC) of cocoa butter, cupuassu fat and their mixtures at different temperatures.

of the curve of 100% cocoa butter. A considerable reduction in the SFC was observed in samples with 25% and 30% of cupuassu fat. Chocolates formulated with these proportions of cupuassu fat require an adjustment of the tempering process so as to adequately induce the formation of beta type crystal nuclei which have higher thermodynamic stability. Lannes *et al.*, (2003) studied the SFC curves in mixtures of cocoa butter and cupuassu fat and also observed that the cupuassu fat has a lower SFC curve compared to 100% cocoa butter. They also observed that the addition of 30% cupuassu fat to cocoa butter significantly lowered the SFC at 25°C. Figure 1 indicated that at 35°C all samples presented SFC values which are very close to zero. This corresponds to another important attribute of chocolate since the presence of solid fats at temperatures higher than 35°C, known as the “fatty residue”, is easily detected during the sensory evaluation.

3.5. X-Ray Diffraction

The X-ray diffraction curves for cocoa butter, cupuassu fat and some sample mixtures are given in Figure 2.

Figure 2 shows that the diffraction curves obtained for cocoa butter and cupuassu fat are very similar. The main peak for cocoa butter was observed at 19.48°. A similar result was obtained by Schenk and Peschar (2004) and Schenk *et al.*, (2006). They identified 5 polymorphic forms in cocoa butter obtained from different sources. They

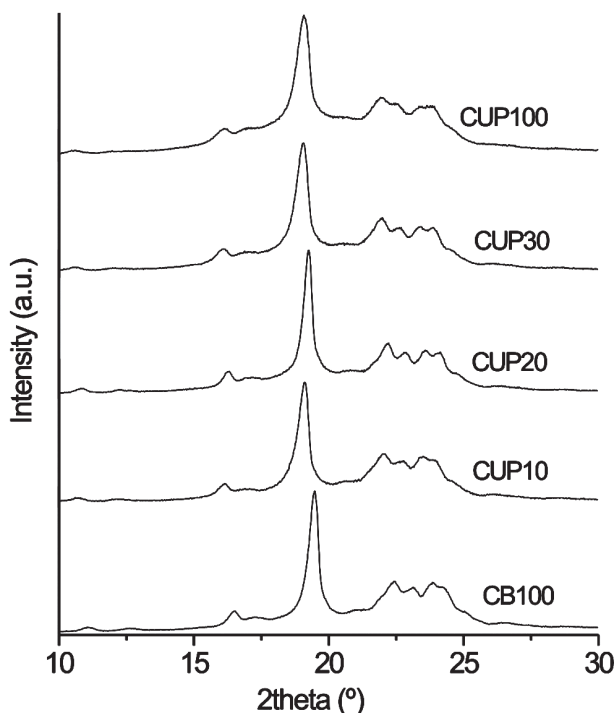


Figure 2

X-ray diffraction curves obtained for cocoa butter, cupuassu fat and CUP10, CUP20 and CUP30.

also found the presence of a main peak around 19.5° in a sample of cocoa butter from Bahia, Brazil and concluded that this behavior was caused by the beta crystalline form. The beta crystalline form was also identified by means of X-ray diffraction by Marangoni and Narine (2002), who studied cocoa butter which was crystallized at 22°C and held at this temperature for 20 days. The X-ray diffraction curves they obtained are very similar to those obtained in this study. For cupuassu fat, the main peak was observed at 19.08°, which is very close to that of cocoa butter. The diffraction curves of samples with 10, 20 and 30% cupuassu fat did not vary significantly from the diffraction curves of cocoa butter. Analyzing the shape of the curves and comparing the behavior of the peaks of cocoa butter and of cupuassu fat, it is possible to infer that the crystalline form present in the pure fat samples studied and in the mixtures is the beta form. This confirms that the conditions used for the tempering were adequate for the formation of this type of crystal. The similarity between the diffraction patterns of cocoa butter and cupuassu fat was expected since both showed similar triacylglycerol composition and, botanically, both belong to the same *theobroma* species.

4. CONCLUSIONS

Mixtures of cocoa butter (Bahia + Indonesian blend) with cupuassu fat showed chemical and crystallization compatibility. The solid fat content at 25°C and the near zero SFC value at 35°C together with the results of other determinations confirm that mixtures with up to 20% cupuassu fat can be used in the formulations of chocolates without significantly changing the physical properties of the final product.

X-ray diffractions together with the results obtained for the melting point indicates that the tempering procedures used for crystallization induces the formation of the beta crystalline form in cupuassu fat as well as in pure cocoa butter and their mixtures.

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