

## Polymorphic Phases of Natural Fat from Cupuassu (*Theobroma grandiflorum*) Beans: A WAXS/SAXS/DSC Study

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**ABSTRACT:** The polymorphic phases of natural fat extracted from cupuassu beans were studied by performing in situ temperature-dependent X-ray scattering experiments and differential scanning calorimetry (DSC) using synchrotron radiation. Cupuassu (*Theobroma grandiflorum*) is a native plant of the *Theobroma* species originally found in Brazil's Amazon region, with a great potential for utilization in the chocolate industry. The experiments and data analysis were performed using as a reference the known polymorphic phase behavior of cocoa butter. The results led to the identification of cupuassu fat polymorphic crystalline phases. Using the nomenclature generally found in the literature for triacylglycerols (TAGs) systems, these phases were labeled  $\gamma$ ,  $\alpha$ ,  $\beta'$ , and  $\beta$  in increasing order of melting temperature and stability. Differential scanning calorimetry measurements of fusion temperatures indicated the existence of two  $\beta$  states in cupuassu fat. These phases were labeled  $\beta_2$  and  $\beta_1$ , following the aforementioned nomenclature. In spite of the different melting points, the existence of a  $\beta_2 \rightarrow \beta_1$  transition could not be proven, due to the great deal of structural similarity of the corresponding X-ray patterns obtained in our experiments. A comparison of our results with those reported for several pure compounds and ternary mixtures of TAGs indicated that these components are mainly responsible for the cupuassu fat phase behavior. This study provided the first experimental results of an in-situ follow-up of the polymorphic phase transitions and crystallization of fat from Brazilian cupuassu beans, an industrially important natural product.

### Introduction

Natural fats consist of complex mixtures of triacylglycerols (TAGs). These mixtures crystallize in different forms, depending on the chemical composition of their long chains. This phenomenon is well-known in the industrial production of cosmetics, pharmaceuticals, and foods containing natural oils and fats, mainly because it affects significantly the physical properties of the end products.

The polymorphism of mixed saturated–unsaturated TAGs has been extensively studied in view of their industrial applications, in particular those related to the confectionery and food industry.<sup>1</sup> Polymorphism results from a variety of molecular conformations and molecular packings (lamellar stacking and lateral chain packing). These two levels of organization are identified by their long and short spacings observed by X-ray diffraction (XRD) at low and wide-angle, respectively.<sup>1–4</sup> According to the literature,<sup>1–3</sup> three basic polymorphs of the TAGs are observed related to the lateral packing of the fatty acid chains called  $\alpha$ ,  $\beta'$ , and  $\beta$ , in increasing order of melting temperature. The crystalline structure of each phase has characteristic wide angle Bragg reflections as follows: (1)  $\alpha$ -form: hexagonal subcell (H) with one strong short spacing line at  $\sim 4.2$  Å; (2)  $\beta'$ -form: orthorhombic perpendicular ( $O_{\perp}$ ) subcell with two strong short spacing lines near 4.2–4.3 Å and 3.7–4.1 Å; and (3)  $\beta$ -form: triclinic parallel ( $T_{\parallel}$ ) subcell with a strong short spacing line at  $\sim 4.6$  Å. An additional form, labeled the

$\gamma$ -form (orthorhombic subcell), quite similar to the  $\beta'$ -form, has also been reported and it is characterized by a melting temperature lower than the  $\alpha$ -form. As a rule, when two or more thermal states are identified, all having similar crystallographic structures, they receive the same name, and are distinguished by subscripts, for example,  $\beta_1$  and  $\beta_2$ , numbered in decreasing order of melting temperature.<sup>4</sup>

Fat from cupuassu beans is extensively used in the production of candies and confectionery in the northern and north-eastern regions of Brazil, where the *Theobroma* species was originally found and is considered as an excellent raw material for utilization in the food industry.<sup>5</sup> Besides, cupuassu fat is considered a good candidate to partially substitute cocoa butter in candies and confectionery.<sup>5,6</sup> The main purpose of this work was to study the polymorphic behavior of cupuassu fat performing simultaneous low and wide-angle X-ray diffraction (XRD) experiments combined with DSC measurements. This study was performed using as reference the known behavior of cocoa butter as well as its main TAG components as described in the literature.<sup>1,4,7–16</sup> A cocoa butter sample from the same region was also studied following the same protocols for all experiments. After the cupuassu fat polymorphic phases obtained by isothermal annealing were identified and described, additional in situ experiments were made in an attempt to crystallize those phases following different thermal pathways.

### Materials and Methods

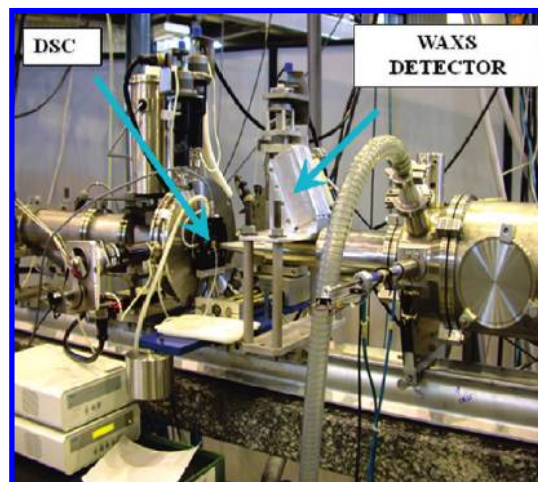
**Samples Description.** The fat utilized in this work was extracted from cupuassu beans. Cocoa butter and cupuassu fat for the experiments were kindly provided by Chocolates da Amazonia

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Company (CHOCAM), Amazonas State, Brazil. Its fatty acid (FA) composition was determined by the American Oil Chemists' Society (AOCS) official method<sup>17</sup> 1-62 with a Konik 4000 A HREC model gas chromatography (GC) unit equipped with a flame ionization detector (FID) and on-column injector. The column used was a Chrompack WCOT fused silica, stationary phase CP-sil 88, with a length of 50 m and an internal diameter of 0.25 mm. Helium was the carrier gas at a flow rate of 1.5 mL/min with hydrogen gas and air also being supplied to the FID. To separate the different FA the following temperature profile was used in the GC: initial hold at 180 °C for 2 min followed by heating at a rate of 10 °C/min until a temperature of 225 °C was reached. The detector was held constant at 300 °C. Injector temperature was 250 °C with a split ratio of 1/100. The 1- $\mu$ L injections were from a solution of methyl esters at approximately 200 mg/mL hexane. FAs were identified by using FA methyl ester standards from SIGMA. Composition (area percent) was based on the area integrated by Workstation Borwin 4 chromatography software. Its TAGs composition was determined in a Perkin-Elmer 250 high-performance liquid chromatography (HPLC) system using a Sicon Analytic refractive index detector following the AOCS official method<sup>17</sup> Ce 5b-89. The solvent system was acetone/acetonitrile (60:40 vol/vol) at a flow rate of 1 mL/min. The sample (20  $\mu$ L) was injected in acetone at 5 wt %/vol. A Lichrosorb RP-18 MERCK column of 25 cm length and 4.6 mm i.d. was used at a column oven temperature of 37 °C. TAGs were identified with commercial standards. Composition (area percent) was based on the area integrated by Hewlett-Packard HP 3395 chromatography software. Solid fat content (SFC) of cupuassu fat and cocoa butter and of blends of cocoa butter with 5 and 10 wt % cupuassu fat was evaluated using a Minispec mq20 unit equipped with temperature control (Bruker). NMR tubes of 9 mm diameter were filled with 4 mL of melted fat and capped. Pretreatment (tempering) of all samples was carried out using AOCS official method<sup>17</sup> Cd 16b-93 (Method II- Stabilizing confectionary fats). The tempering procedure was as follows: melt and store for 15 min at 100 °C followed by 5 min at 60 °C. Then, 90  $\pm$  5 min at 0 °C, 40  $\pm$  0.5 h at 26 °C, and finally 90  $\pm$  5 min at 0 °C. Finally, 60 min at each crystallization temperature. SFC was determined at 5, 10, 15, 20, 25, 30, and 35 °C. Duplicate runs were performed on consecutive days for each set of samples.

**X-ray Scattering and Calorimetric Experiments.** The samples were investigated using two linear position sensitive gas detectors to register the low and wide-angle X-ray patterns simultaneously. The experimental set up was installed at the D11A-SAXS Beamline of the Brazilian Synchrotron Light Laboratory (LNLS, Campinas, SP, Brazil), using a wavelength ( $\lambda$ ) of 1.7433 Å, and two sample-to-detector distances: 1344.50 mm (for low-angle scattering experiments) and 755.35 mm (for wide-angle scattering experiments). The samples for the XRD-DSC experiments were encapsulated in modified DSC aluminum pans with mica windows. The wide-angle scattering ( $2\theta$ ) detection range was 12–35° ( $d$ -spacing ranging from 2.90 Å up to 8.34 Å) and the detector was calibrated using  $n$ -tetracosane as a standard sample. The low-angle X-ray patterns were registered in a  $q$  range from 0.008 up to 0.5 Å<sup>-1</sup> (where  $q$  is the magnitude of the  $q$ -vector defined by  $q = (4\pi/\lambda) \sin \theta$  and  $2\theta$  is the scattering angle). This corresponds to a  $2\theta$  range of 0.1 to 8°. The calorimetric measurements were carried out simultaneously with XRD experiments using a DSC cell<sup>18</sup> (THM 600, Linkam). Temperature calibration was made with an indium standard sample. The experimental setup is shown in Figure 1.

The in situ polymorphic transitions of the fat were registered following specific thermal protocols to attain the desired polymorphic phases as explained in the following paragraphs. Common to all experiments was a high temperature (60 °C) warm-up period of 5 min to erase the sample's thermal history. Then the samples were quenched at a rate of 20 °C/min to reach the final temperature ( $T_f$ ) at which they were kept until the desired polymorphic phase was completely obtained. Isothermal structural transformations were then observed keeping the samples at fixed  $T_f$  during a period of time ( $t$ ). Measurements to determine the melting temperatures of the phases were performed by heating the samples up to 40 °C at a rate of 2 °C/min. Some phase transformations were also observed during



**Figure 1.** Instrumental setup for combined SAXS–WAXS–DSC measurements at the SAXSI Beamline of the LNLS.

**Table 1.** FA Composition of Cupuassu Fat and Cocoa Butter Samples

FA <sup>a</sup>	cupuassu fat (wt%)	cocoa butter (wt %)
C16:0	6.97	25.11
C16:1	0.12	0.29
C18:0	33.08	33.33
C18:1	42.32	36.47
C18:2	3.55	3.52
C18:3	0.13	0.08
C20:0	11.10	1.02
C20:1	0.41	0.09
C22:0	2.32	0.09
S	53.47	59.55
MU	42.85	36.85
PU	3.68	3.60

<sup>a</sup>Abbreviations: S = saturated, MU = monounsaturated, PU = polyunsaturated.

the heating process. For all experiments, the low and wide-angle X-ray patterns were collected at 1 min intervals. The melting temperature of each polymorph was taken at the minimum of the corresponding endothermic differential scanning calorimetry (DSC) peak.

## Results and Discussion

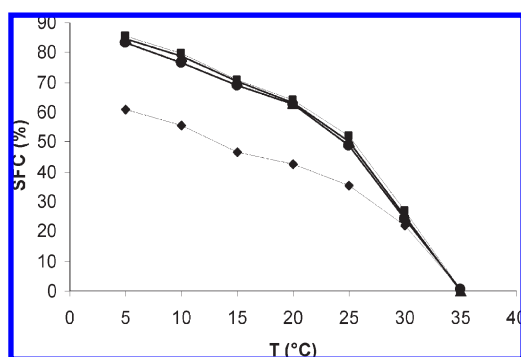
**Chemical Composition.** FA and TAG compositions are shown in Tables 1 and 2, respectively. The results shown in Table 1 indicated that the unsaturated fatty acid content was 46.53%. Table 2 reported that SOS-type TAGs (POP, POS, SOS, and SOA) were 55.39%. These values do not exactly agree with EU standards to qualify cupuassu fat as a cocoa butter equivalent (CBE) material.<sup>19</sup> Cupuassu fat does not reach two of all requirements: to have <45% unsaturated fatty acids (UFAs) content and >65% SOS-type TAGs. According to the geographical region,<sup>19</sup> cocoa butter palmitic acid content (C16:0) may vary from 24 to 26%, while stearic acid (C18:0) values may be between 33 and 38%. As shown in Table 1, C16:0 content in cocoa butter is significantly higher than that found in cupuassu fat. It is generally accepted that palmitic acid raises cholesterol levels in blood while stearic acid is considered neutral. The lower level of C16:0 of cupuassu fat may be considered an advantage from the nutritional point of view.

**Solid Fat Content Profiles.** Figure 2 shows the solid fat content (SFC) profiles for cupuassu fat, cocoa butter, and the blends of cocoa butter with 5 or 10 wt % of cupuassu fat.

**Table 2. TAG Composition of Cupuassu Fat and Cocoa Butter Samples**

TAG <sup>a</sup>	cupuassu fat (wt %)	cocoa butter (wt %)
PLP	1.56	1.80
OOO	3.63	
POO	7.40	3.51
POP	2.29	20.37
SOO	18.09	3.98
POS	13.19	41.43
OOA	8.63	
SOS	27.48	26.96
PSS	1.33	
SOA	12.43	1.95
other	12.60	
StUSt	56.95	92.51
StStSt	1.33	
StUU	25.49	7.49
UUU	3.63	

<sup>a</sup> Abbreviations: A: arachidic acid (C 20:0); L: linoleic acid (C 18:2); O: oleic acid (C 18:1); P: palmitic acid (C 16:0); S: stearic acid (C 18:0); St = saturated and U = unsaturated.



**Figure 2.** Solid fat content with temperature for (◆) cupuassu fat, (■) cocoa butter, (▲) cocoa butter with 5% cupuassu fat, (●) cocoa butter with 10% cupuassu fat. Errors were within the symbols size.

Cocoa butter has a unique characteristic profile that makes it such a valuable fat. It shows a sharp decrease in SFC at temperatures slightly lower than the body temperature. A comparison of the SFC curves indicates that cupuassu fat also showed a sharp decrease in solids content between 30 and 35 °C. Incorporation of cupuassu fat in up to a 10% addition level yielded SFC curves that were nearly indistinguishable from the results reported for cocoa butter alone. Thus, addition of cupuassu fat did not produce softening of cocoa butter. This compatibility makes it suitable for use in cocoa butter-based confections. Although SFC values for temperatures below 30 °C were lower than the ones for cocoa butter, it is likely that functionality of cupuassu fat for chocolate applications may be improved by fractionation. Most likely the stearin fraction obtained would meet regulation for CBE.<sup>19</sup> Despite the differences in chemical composition between cocoa butter and cupuassu fat, this material may be considered a good candidate to partially substitute cocoa butter in candies and confectionery.

**Crystallization Experiments with Cupuassu Fat and Cocoa Butter.** The low and wide-angle X-ray patterns for the crystalline phases observed for cupuassu fat are shown in Figure 3a,b. The long and short spacings determined for each phase, together with the melting temperatures, are presented in Table 3. X-ray patterns and *d*-spacings obtained for cocoa butter are also displayed in Figure 3c,d and Table 3. Please note that the different forms of the latter are generally described in the literature using Roman numerals (I–VI) in order of increasing melting point.<sup>4,7</sup> In this work, we are

using only Greek letters to identify the cupuassu fat phases and a combination of the Greek and Roman numerals for cocoa butter polymorphs.

In these experiments the I( $\gamma$ ), II( $\alpha$ ), IV( $\beta'_1$ ), V( $\beta_2$ ), VI( $\beta_1$ ) phases for cocoa butter were obtained by isothermal crystallization for the following final temperatures,  $T_f$ : –20 °C (10 min), 18 °C (15 min), 18 °C (35 min), 25 °C (5 days), and 25 °C (4 months), respectively. The time for the phase formation is presented in parentheses in each case. The melting temperatures were also obtained following the same protocol.

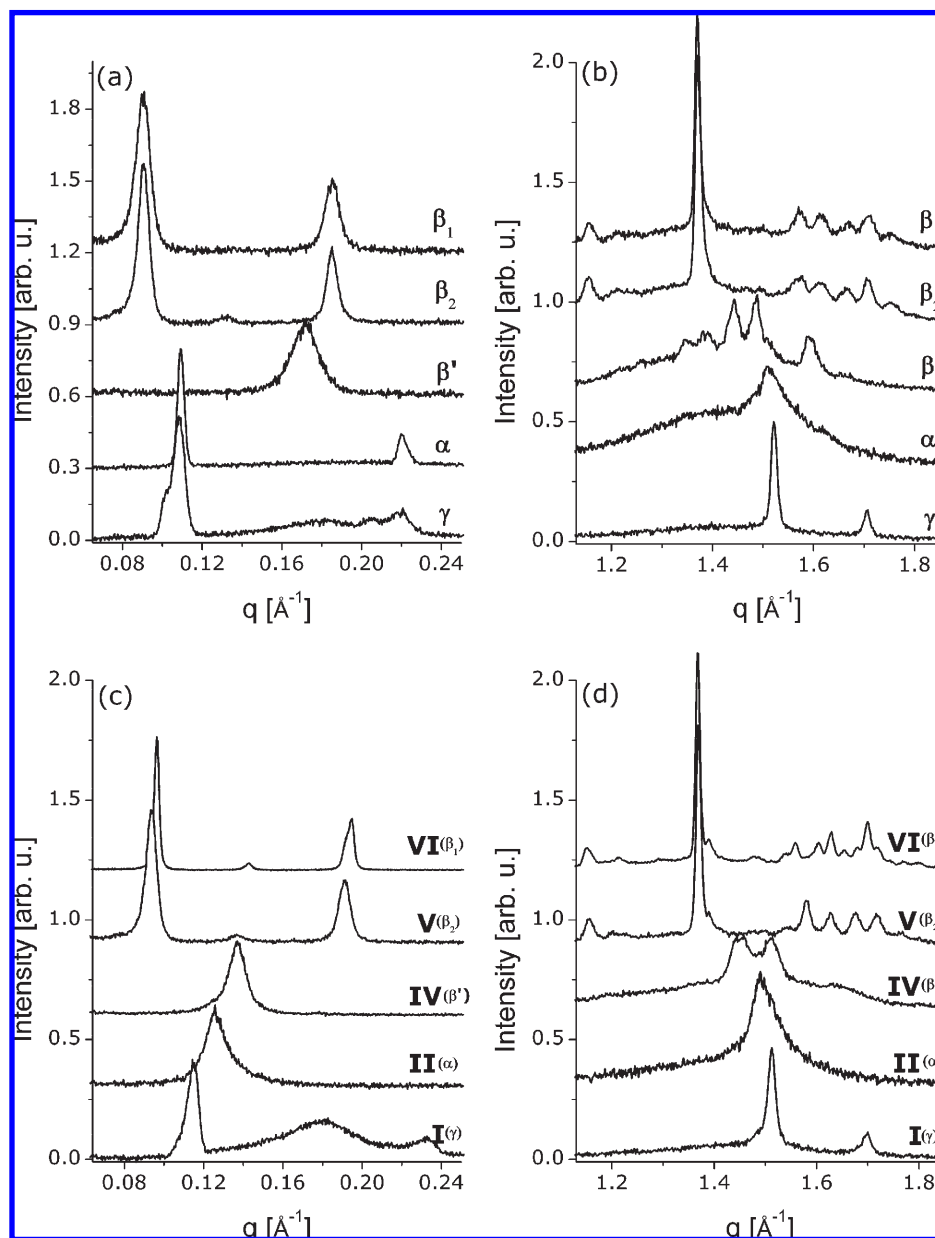
The  $\gamma$  phase for cupuassu fat was obtained quenching the samples from the melt (20 °C/min) to  $T_f = -20$  °C. At around 0 °C, the crystallization process started, and the  $\gamma$  phase was completely developed after a few minutes at –20 °C. The  $\gamma$  phase was also obtained after quenching the sample to  $T_f = -40$  °C. A long-spacing reflection at 46.6 Å, which was not observed when the sample was crystallized at  $T_f = -20$  °C, could be observed (see Figure 4a). This orthorhombic phase was observed in all experiments performed with an initial fast cooling to low temperatures (below 0 °C).

According to the literature, SOS presents a low angle reflection corresponding to a 44 Å spacing in one of its low-temperature phases,<sup>20,21</sup> and POS also presents one reflection corresponding to a 47.6 Å spacing at low temperatures.<sup>22</sup> Therefore, the presence of this additional reflection could be attributed to the specific POS and SOS content in cupuassu fat. This reflection may thus be attributed to the existence of some domains formed by a combination of these two TAGs in binary mixtures crystallized at –40 °C, considering that these domains would not be present at –20 °C.

The formation of the  $\alpha$  phase for cupuassu fat was detected after quenching the melted fat to  $T_f = 10$  °C and keeping it at this temperature for about  $t = 5$  min. This phase was also obtained quenching the sample down to  $T_f = 18$  °C and keeping it at this temperature for the same period of time, but soon thereafter, it was noted that a transition to the  $\beta'$  phase had occurred. Notably, this transformation did not happen when the annealing temperature was 10 °C.

As mentioned above, the cupuassu  $\beta'$  phase was obtained by an isothermal transition from the  $\alpha$  phase. We expected that the  $\beta'$  phase formation would be easier to stabilize via this transition because several trials to obtain it directly from the melt seemed to be unsuccessful. The isothermal crystallization of the  $\beta'$  phase from the  $\alpha$  phase was carried out at different temperatures (8 °C for 1 h; 12.5 °C for 2 h; 15 °C for 2 h; 20 °C for 2 h; 25 °C for 1 h 40 min). In each case, some differences were observed in the XRD patterns (see Figure 5). At 8 °C, a small amount of the  $\beta'$  form was observed after 1 h. Transformation at 12.5, 15, and 20 °C was complete in 2 h. Surprisingly, when solidification took place at 25 °C, in addition to the  $\beta'$ -form, the  $\beta_2$  phase developed in 1 h 40 min. DSC melting diagrams showed two peaks indicating that both  $\beta'$  and  $\beta_2$  phase were present. This behavior differed from the one observed by van Malsen et al.<sup>10,11</sup> for cocoa butter using the same  $T_f$  and  $t$  conditions. According to these authors, cocoa butter solidified at 24 or 26 °C showing a very slow crystallization, and it took several hours before the process started. Besides, the  $\beta_2$  form only appeared after being kept for 5 days at 25 °C.

Concerning the  $\beta$  phase, two stable thermal states were detected for cupuassu fat, labeled  $\beta_2$  and  $\beta_1$  in order of increasing temperature. These cupuassu  $\beta$  phases were obtained keeping the sample at 25 °C for 5 days and 4 months,



**Figure 3.** (a) Low-angle and (b) wide-angle X-ray patterns corresponding to the  $\gamma$ ,  $\alpha$ ,  $\beta'$ , and  $\beta$  phases of cupuassu fat. (c) Low-angle and (d) wide-angle X-ray patterns showing the I( $\gamma$ ), II( $\alpha$ ), IV( $\beta'$ ), V( $\beta_2$ ), and VI( $\beta_1$ ) polymorphic phases of cocoa butter.

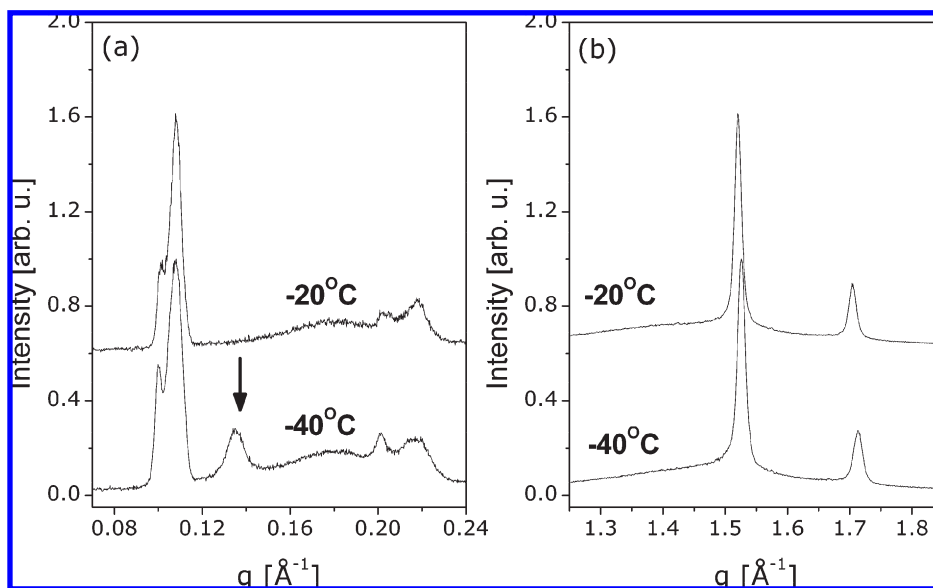
respectively. We note that their melting temperatures are similar to those of the V( $\beta_2$ ) and VI( $\beta_1$ ) phases of cocoa butter listed in Table 3. On the other hand, the structural parameters of the cupuassu fat for the states labeled  $\beta_2$  and  $\beta_1$  are very similar (see Table 3 and the X-ray patterns in Figure 3a,b). Consequently, there is no complete proof of a  $\beta_2 \rightarrow \beta_1$  transition in cupuassu fat. This transition takes place in cocoa butter and has been studied in great detail in the literature by Schenk et al. (2004)<sup>23</sup> using experiments with several recrystallization processes to prove that crystallization depends on the dynamics of the process. Their work on the characterization of the two  $\beta$  polymorphic phases in cocoa butter revealed changes in the supramolecular packing. More recently, van Mechelen et al. (2006)<sup>12,13</sup> used high resolution powder diffraction data and Rietveld refinement analysis to provide structural details of the crystalline  $\beta_2$  polymorph for several monounsaturated TAGs, as well as cocoa butter from Ivory Coast. This information is

important to understand the mechanism of the phase transition that leads to the higher temperature phase  $\beta_1$ . In the case of the cupuassu  $\beta_2 \rightarrow \beta_1$  transition, phase segregation and formation of structural domains with different packing characteristics or specific rheological properties of the system may create the possibility of reaching the temperature for the  $\beta_1$  phase before the stable  $\beta_1$  superstructure sets in. Different thermal properties are not simple to explain in these complex solid fat systems and the X-ray data do not provide structural differences that would clearly identify the  $\beta_2$  and  $\beta_1$  phases in cupuassu fat as two different polymorphs. Additional studies should be performed to prove that these  $\beta$  states are distinct polymorphic phases. The knowledge of the thermal pathway to reach the  $\beta_2$  crystallization is very important for chocolate manufacturing. For this reason, the description of the behavior of the  $\beta$  phases is fundamental for the industrial use of cupuassu fat as a partial or total substitute for cocoa butter.

**Table 3. Long and Short  $d$ -Spacings of the Different Crystallographic Phases and Melting Temperatures of Cupuassu Fat and Cocoa Butter Obtained in This Work**

cupuassu fat			cocoa butter		
phases	$d$ -spacings ( $\text{\AA}$ ) <sup>a</sup>	melt temp ( $^{\circ}\text{C}$ )	phases	$d$ -spacings ( $\text{\AA}$ ) <sup>a</sup>	melt temp ( $^{\circ}\text{C}$ )
$\gamma$	61.36(w) <sup>b</sup> , 57.97(vs) <sup>b</sup> , 46.6(m) <sup>b,c</sup> , 34.95(diff) <sup>b</sup> , 28.83(w) <sup>b</sup> , 4.13(vs), 3.68(s)	4.0	I ( $\gamma$ )	55.14(m) <sup>b</sup> , 35.12(w) <sup>b</sup> , 27.41(m) <sup>b</sup> , 4.15(s), 3.70(m)	14.2
$\alpha$	57.24(s) <sup>b</sup> , 28.35(w) <sup>b</sup> , 4.16(s)	17.7	II ( $\alpha$ )	49.76(s) <sup>b</sup> , 4.21(s)	22.7
$\beta'$	36.27(s) <sup>b</sup> , 4.56(m), 4.44(m), 4.26(vs), 4.13(vs), 3.83(s)	26.8	IV ( $\beta'_1$ )	45.90(s) <sup>b</sup> , 4.33(s), 4.16(s)	28.2
$\beta_2$	69.38(vs) <sup>b</sup> , 47.76(vw) <sup>b</sup> , 33.95(vs) <sup>b</sup> , 5.44(m), 4.58(vs), 4.00(m), 3.90(m), 3.77(m), 3.68(m), 3.59(w)	32.5	V ( $\beta_2$ )	64.50(vs) <sup>b</sup> , 45.94(vs) <sup>b</sup> , 32.88(vs) <sup>b</sup> , 5.43(m), 4.59(vs), 3.98(s), 3.86(m), 3.75(m), 3.66(m)	33.3
$\beta_1^d$	69.64(vs) <sup>b</sup> , 33.93(vs) <sup>b</sup> , 5.43(m), 4.59(vs), 3.97(m), 3.89(m), 3.76(m), 3.68(m), 3.59(vw)	36.0	VI ( $\beta_1$ )	65.22(vs) <sup>b</sup> , 44.04(s) <sup>b</sup> , 32.42(s) <sup>b</sup> , 5.45(m), 4.59(vs), 4.53(m), 4.03(m), 3.92(m), 3.86(s), 3.80(w), 3.74(m), 3.70(s), 3.66(m)	36.4

<sup>a</sup> Relative intensity: vw = very weak, w = weak, mw = medium to weak, m = medium, ms = medium strong, s = strong, vs = very strong, diff = diffuse. The nomenclature frequently used for cocoa butter forms is indicated between parentheses in the second column. <sup>b</sup> Long-spacings. <sup>c</sup> Only observed at  $-40^{\circ}\text{C}$ . <sup>d</sup> The melting temperature corresponds to a  $\beta_1$  phase, but the diffraction data do not show significant structural differences from the  $\beta_2$  phase.

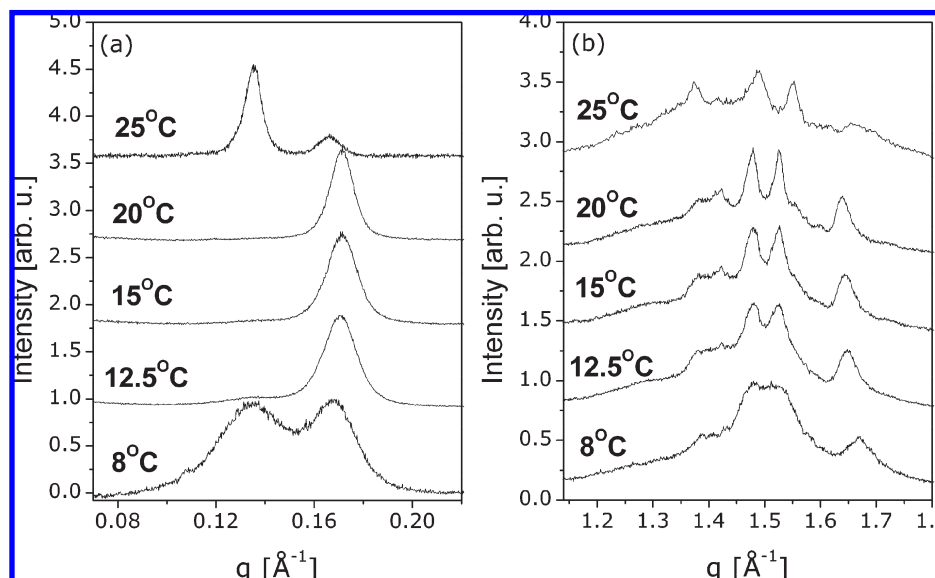


**Figure 4.** (a) Low-angle and (b) wide-angle X-ray patterns showing the  $\gamma$  phase of cupuassu fat crystallized in two different temperatures ( $-20^{\circ}\text{C}$  and  $-40^{\circ}\text{C}$ ). The arrow shows one extra peak in the low-angle pattern of the  $\gamma$  phase obtained from  $-40^{\circ}\text{C}$ .

**Phase Transitions  $\gamma$ – $\alpha$  and  $\alpha$ – $\beta'$  for Cupuassu Fat.** The X-ray patterns showing the phase transformation from  $\gamma$  to  $\alpha$  and thereupon from  $\alpha$  to  $\beta'$  are shown in Figure 6a–d. The corresponding DSC data are presented in Figure 6e. The  $\gamma$  phase was achieved quenching the sample down to  $T_f = -40^{\circ}\text{C}$  and keeping this temperature for  $t = 10$  min. No changes were observed. Then the sample was heated at  $2^{\circ}\text{C}/\text{min}$  up to  $40^{\circ}\text{C}$ . During this heating, the phase transformations were observed. The first endothermic broad peak in the DSC recording and the X-ray patterns showed the  $\gamma$  to  $\alpha$  phase transformation at around  $4^{\circ}\text{C}$ . The  $\alpha$  phase melting was not detected, because it had been overlapped by the exothermic peak of  $\beta'$  crystallization at around  $13^{\circ}\text{C}$ . The  $\alpha$  to  $\beta'$ -phase transition was confirmed by the X-ray patterns (Figure 6a–d). However, an estimation of the  $\alpha$  phase melting temperature was possible establishing the correlation between the X-ray patterns and DSC data and it is presented in Table 3. The final endothermic peak in the DSC recording, with a maximum at  $28^{\circ}\text{C}$ , indicates the  $\beta'$  melting temperature as presented in Table 3. The presence of an additional reflection corresponding to a long spacing of  $46.6\text{\AA}$  cited previously was observed in the low-angle X-ray pattern of the  $\gamma$ -phase.

**Crystallization of the  $\alpha$  Phase from the Melt for Cupuassu Fat.** Since the melting temperature of the  $\alpha$  phase was not clearly observed in the experiments cited above, another experiment was performed crystallizing the  $\alpha$  phase directly from the melt. The sample was quenched to  $T_f = 10^{\circ}\text{C}$  and kept at this temperature for  $t = 10$  min. As soon as the temperature reached  $10^{\circ}\text{C}$ , the  $\alpha$  phase quickly appeared and no changes were observed in the X-ray diffraction patterns during those 10 min. After subsequent heating at the rate of  $4^{\circ}\text{C}/\text{min}$  was performed, the phase melted at around  $17.7^{\circ}\text{C}$ , as can be seen in the calorimetric data of Figure 7c. In the X-ray patterns of Figure 7a,b, it is possible to observe the crystallization and melting process discussed above. Two low-angle and one wide-angle reflections were detected indicating the crystallization of the  $\alpha$  phase and its subsequent melting signaled by the disappearance of the X-ray reflections.

**Comparison of the Polymorphic Phases of Cupuassu Fat with Those of Cocoa Butter and the Ternary Mixture of TAGs (25% SOS, 50% POS, and 25% SOO).** The experiments with cocoa butter sample were performed under the same conditions used for the cupuassu fat study. They were very



**Figure 5.** (a) Low-angle and (b) wide-angle X-ray patterns showing  $\beta'$  and  $\beta'$ -type phases of cupuassu fat isothermally crystallized at different temperatures (8 °C for 1 h; 12.5 °C for 2 h; 15 °C for 2 h; 20 °C for 2 h; 25 °C for 1h 40 min).

useful for establishing the differences in the polymorphic behavior of cocoa butter and cupuassu fat. Structural and thermal information from other authors was also used as reference and is given in the Supporting Information (Table S1).

The main similarities appear in the short spacings of both fats, indicating equivalent dimensional parameters for their subcells. Important exceptions are noted in the case of the  $\beta'$  phase of cupuassu fat. The wide-angle X-ray patterns presented some reflections that were absent in the cocoa butter samples, indicating differences in the structure of the respective subcells. The differences in the low-angle X-ray patterns for this phase seem to be evidence of a different lamellar stacking for the TAGs in cupuassu fat.

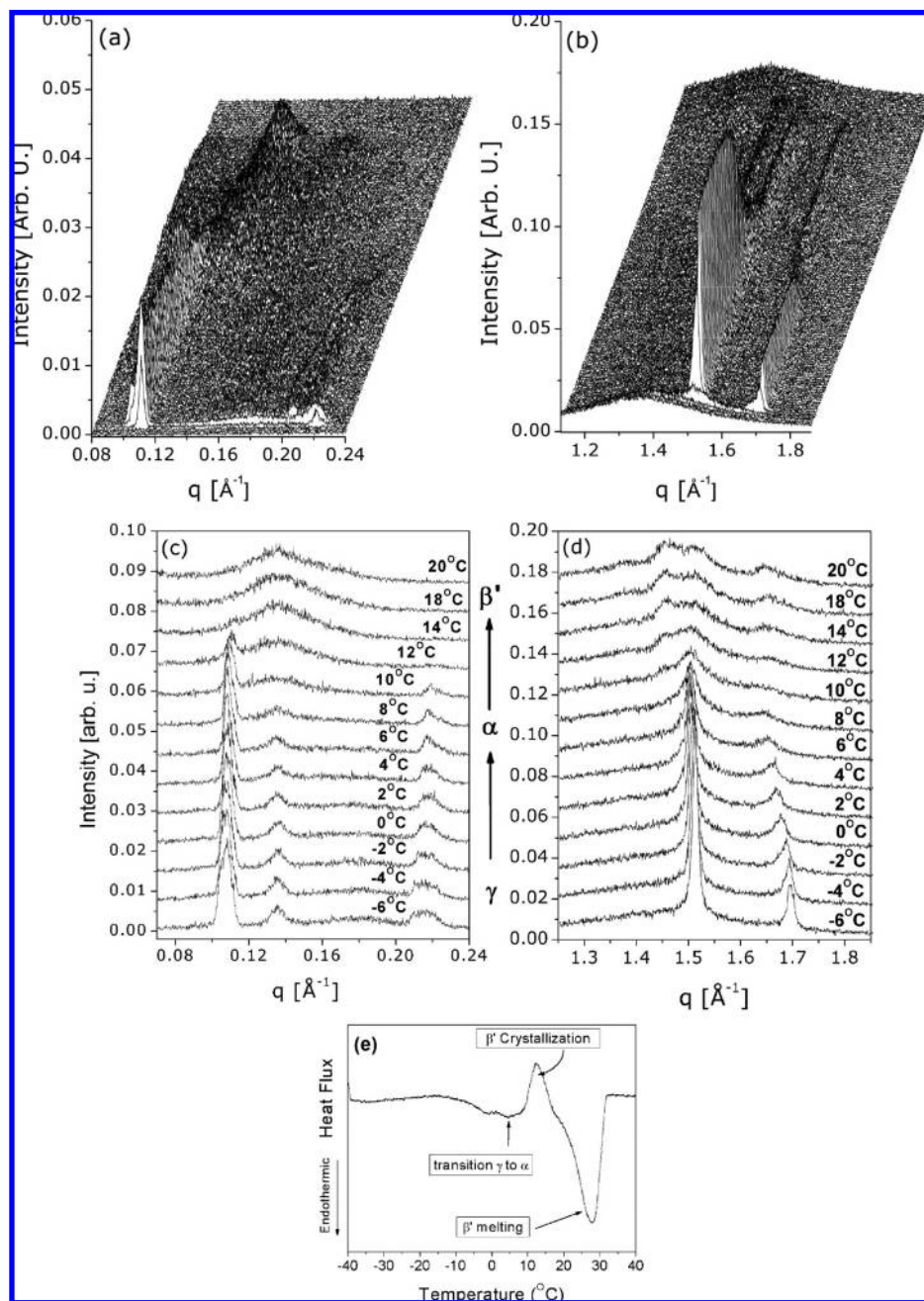
Following the order of thermal stability, the  $\gamma$  phase of cupuassu fat is here compared with phase I( $\gamma$ ) of cocoa butter. A 61.36 Å long spacing is observed in the cupuassu fat which is absent in the low-angle X-ray patterns for phase I( $\gamma$ ) of cocoa butter (see Figure 3 and Table 3). Moreover, depending on the temperature at which the  $\gamma$  phase is crystallized, a peak at 46.6 Å can be detected in the cupuassu samples (see Figure 4), also absent in the diffraction pattern for cocoa butter. It was observed that the melting temperature of the cupuassu  $\gamma$  phase was below that of cocoa butter (Table 3), which is in agreement with a higher content of unsaturated fatty acids and a lower content of symmetric TAGs, especially POP and POS. The measured POP and POS percentages were 2% and 13%, respectively, in cupuassu fat (Table 2), and 14–20% and 35–45% in cocoa butter depending on the origin of the beans.<sup>19</sup> The melting points reported for POP and POS are 40.8 °C and 34.7, respectively.<sup>4</sup>

When the  $\alpha$  phase of the cupuassu fat is compared with the II( $\alpha$ ) phase of cocoa butter, we note that two long spacings, corresponding to 57.24 and 28.35 Å, could be observed for cupuassu fat, whereas only one peak appears in the cocoa butter X-ray patterns (Figure 3, Table 3 and Supporting Information, Table S1). The melting temperature of the  $\alpha$  phase is also lower than the one in phase II( $\alpha$ ) of cocoa butter. This fact was expected given the differences in chemical composition.

Regarding the cupuassu  $\beta'$  phase, a long spacing at 36.27 Å was observed in the X-ray patterns. This value is smaller than

that reported for phases III( $\beta'_2$ ) and IV( $\beta'_1$ ) in Table S1, Supporting Information and the value measured for cocoa butter sample in Table 3. van Malssen et al.,<sup>9</sup> studying cocoa butter, concluded that the variation found in  $\beta'$  phases was remarkable. Seven different and apparently continuously varying  $\beta'$  X-ray patterns were observed. They had small, but significant differences in the number of peaks and peak shape, position, and intensity. Moreover, each  $\beta'$  phase showed a different melting range. They interpreted this phenomenon by considering that the  $\beta'$  phase of cocoa butter is not a single phase but rather a range of several subphases. According to these authors, the physical basis for the existence of a  $\beta'$  phase-range depends eventually on cocoa butter being a mixture of TAGs, and solid cocoa butter existing as a conglomerate of crystallites with individual TAG composition. The different cooling rates or crystallization temperatures would influence this distribution. Since the distribution of TAGs over the individual crystallites can vary continuously, the resulting characteristics will also vary. As may be observed in Figure 5, cupuassu fat did not show the same behavior as reported for cocoa butter by van Malssen et al.<sup>9</sup> Most likely, this was due to the differences in chemical composition between both fats, which although being quite similar, presented some critical differences.

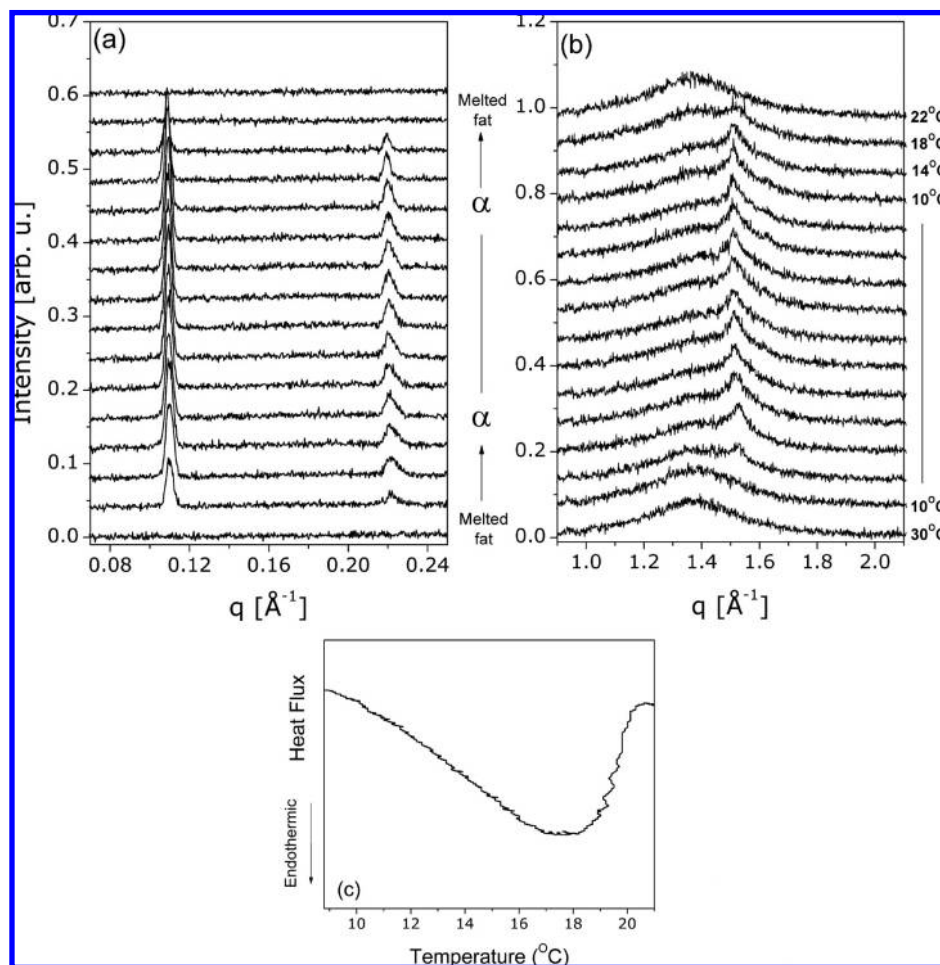
Finally, structural and thermal similarities were observed between the  $\beta_2$  phase of cupuassu fat and phase V( $\beta_2$ ) of cocoa butter. On the other hand, the X-ray pattern of the  $\beta_1$  phase of cupuassu fat presented very small differences when compared to phase VI( $\beta_1$ ) of cocoa butter. (see Figure 3, Table 3 and Table S1, Supporting Information). It is important to note that the differences between the  $\beta_2$  and  $\beta_1$  phases in cupuassu fat are mainly related to the thermal properties. This is not the case with the V( $\beta_2$ ) and VI( $\beta_1$ ) phases in cocoa butter, in which differences are related to thermal behavior and structure of the polymorphic phases. In cupuassu fat, the  $\beta_2$  state seems to be quite stable, suggesting that the phenomenon of “blooming” may not occur so easily in the products manufactured with total or partial substitution of cocoa butter by cupuassu fat, since this effect is related to the crystallization of the  $\beta_1$  phase of cocoa butter.



**Figure 6.** (a, b) 3D plots of low and wide-angle X-ray measurements. (c, d) The scans show the phase transitions from  $\gamma$  to  $\alpha$  to  $\beta'$  in cupuassu fat. (e) Simultaneous (DSC) recording. The endothermic peaks at around 4 and 28 °C indicate the  $\gamma$  to  $\alpha$  phase transition and the  $\beta'$  melting. The exothermic peak represents  $\beta'$  crystallization, around 13 °C. The melting of the  $\alpha$  phase was not detected because this transition is energetically lower than the  $\beta'$  crystallization.

The  $\beta$  phase crystallization process of cocoa butter is extremely important for the chocolate industry. Properly processed chocolate will appear glossy since the cocoa butter form V( $\beta_2$ ) crystals are small (length  $< 5 \mu\text{m}$ ). Uncontrolled recrystallization of cocoa butter TAGs into form VI( $\beta_1$ ) often leads to larger crystals that scatter reflected light, conferring a “dusty” appearance to chocolate.<sup>19</sup> Thus, the so-called bloom effect is attributed to the presence of the most stable polymorph (form VI( $\beta_1$ )). According to the literature, a fat system with a high content of symmetrical TAGs has a strong tendency to crystallize with high structural order.<sup>19</sup> Fat bloom is still the major concern for many chocolate manufacturers because of the resulting undesirable white, dusty surface appearance.

Since SOS, POS, and SOO are three of the major components of cupuassu fat, it is worth mentioning that the melting temperatures as well as the long and short spacings of the phases reported by Willie and Lutten<sup>4</sup> for a mixture of three different TAGs (25% SOS, 50% POS, and 25% SOO) presented great similarities when compared with the values obtained by us for the cupuassu fat as can be seen in Table 3 and Table S1 (Supporting Information). The main differences appeared in the phases with the  $\beta'$ -type subcells. Only one  $\beta'$  phase was observed in cupuassu fat, while the ternary mixture presented two  $\beta'$  forms (III and IV). This brings us back to the aforementioned possible existence of more than one  $\beta'$  phase in cocoa butter and some natural fats mixtures presented by van Malssen et al.<sup>9</sup>



**Figure 7.** (a) Low-angle and (b) wide-angle X-ray patterns showing the crystallization and melting of the  $\alpha$  phase of cupuassu fat. (c) Simultaneous DSC recording. The endothermic peak represents melting of the  $\alpha$  phase, around 17.7 °C.

The structural similarities between the (SOS-POS-SOO) ternary mixture of TAGs and cupuassu fat seem to confirm that this main TAGs composition dominates the polymorphic behavior in cupuassu fat.

### Conclusions

These are the first results reported on the polymorphic behavior of Brazilian cupuassu fat. This work represents a valuable contribution to Food Technology Science, since there is a great potential for the use of this fat as raw material in the chocolate industry. The polymorphic phases observed for cupuassu fat were identified as  $\gamma$ ,  $\alpha$ ,  $\beta'$ , and  $\beta$  in order of increasing melting temperature. This study proved the existence of great thermal and structural similarity between the phases of cupuassu fat and cocoa butter. It is important to note that although thermal properties of the two  $\beta$  states detected for cupuassu pointed to an analogy with the  $\beta_2$  (V) and  $\beta_1$  (VI) phases of cocoa butter, the structural transition between the  $\beta_2$  and  $\beta_1$  phases in cupuassu fat could not be proven. More experiments are needed to prove this fact. In cupuassu fat, the  $\beta_2$  phase seems to be more stable, suggesting that the phenomenon of “bloom-ing” may not occur so easily in products manufactured with total or partial substitution of cocoa butter by cupuassu fat, since this effect is related to crystallization of the  $\beta_1$  phase.

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**Supporting Information Available:** X-ray  $d$ -spacings and melting temperatures of the cocoa butter polymorphic forms and the SOS-POS-SOO ternary mixture according to references cited in this paper (Table S1). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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