Research Article

Acceleration of polymorphic transition of cocoa butter and cocoa butter equivalent by addition of D-limonene

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The essential oils obtained from citric fruits have been used as aromatizing agents in various foods. However, the addition of orange essential oil, D-limonene, to cocoa butter (CB) has been shown to have an influence on its crystallization behavior. The objective of this study was to evaluate the influence of adding D-limonene at concentrations of 1.5 and 5% w/w on the polymorphic transitions in Brazilian CB and in a commercial cocoa butter equivalent (CBE). X-ray diffraction (XRD) was used to identify the polymorphic forms in the samples over 27 days of storage at 25°C. The results showed that D-limonene addition was able to accelerate the polymorphic transition in CB and CBE. The addition of 5% w/w D-limonene to Brazilian CB accelerated the complete transition from βV to the more stable βVI polymorphic form in only 11 days. Under the same conditions, pure CB remained in βV form and the transition was not completed in CBE samples. The polymorphic transition of CBE samples appeared to be governed by the characteristics of the triacylglycerol (TAG) profile and only intensified with D-limonene incorporation.

Practical applications : D-Limonene is an essential oil that has been used industrially to give orange flavor in chocolate products. Our results showed that D-limonene addition to CB and CBE accelerates the polymorphic transition from polymorphic form V to VI in a short period. As the polymorphic form VI is more stable than form V, the addition of D-limonene to chocolates and the like allows them to keep their melting properties and sensorial quality for long storage periods.

Keywords: Cocoa butter / Cocoa butter equivalent / D-Limonene / Polymorphism / X-ray diffraction

Received: October 21, 2014 / Revised: March 1, 2015 / Accepted: March 13, 2015

DOI: 10.1002/ejlt.201400557

1 Introduction

Brazilian cocoa butter (CB) is softer than African and Malaysian products because of the relatively low mono-unsaturated triacylglycerol (TAG) content, mainly SOS, and significant amounts of di-unsaturated TAG, principally SOO and POO [1–3]. This characteristic makes the Brazilian CB has the lowest heat resistance. In some countries, cocoa butter equivalents (CBEs) have been used by the chocolate industry to improve CB melting properties. These alternative fats have major TAG compositions and crystallization properties similar to those of CB. As they do not promote a eutectic effect, CB and CBE can be mixed in any proportion [4].

Soy lecithin and sorbitan esters are primarily used as emulsifiers and they are known to act as crystallization modifiers [5, 6]. Recent studies have demonstrated the use of D-limonene as a potential additive in CB crystallization processes [7]. D-Limonene is a monoterpene and is a constituent of citric fruit essential oils. Owing to its citric aroma, D-limonene has been widely used as an aromatizing

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Abbreviations: A, arachidic acid; **CB,** cocoa butter; **CBE,** cocoa butter equivalent; **FA,** fatty acids; **L,** linoleic acid; **M,** myristic acid; **O,** oleic acid; **P,** palmitic acid; **SSS,** tri-saturated; S_t, stearic acid; S_2U **(or SUS),** di-saturated or monounsaturated; **TAG,** triacyylglycerol; U_2S, di-unsaturated; U_3, tri-unsaturated; **XRD,** X-ray diffraction

agent in beverages, chewing gum, perfumes, and chocolate, conferring an orange aroma [8]. According to Ray et al. [7], the addition of 5% D-limonene to CB promotes the complete polymorphic transition from the βV to βVI form when storage during 21–28 days at 20°C. Do et al. [8] stated that D-limonene may be located between the fatty acids chains in the TAG and influences the properties of fat crystal network.

Many techniques and additives affect polymorphic transition of fats mainly by changing the degree of molecular mobility of the TAG in fat crystal network. Among them are emulsifier addition, essential oil addition, high temperature of fat crystallization, and ultrasound technique [5, 7, 9–12]. The study of polymorphic transitions has become important because it influences the melting properties of chocolates and fats. Despite the increased use of CBE in chocolate recipes, few studies have been carried out on the polymorphic transitions of CBE [13, 14]. In particular, there are no studies on the influence of D-limonene addition on the crystallization behavior of CBE and Brazilian CB. Thus, the aim of the present study is to evaluate the effects of different D-limonene concentrations on the polymorphic transitions of these fats.

2 Materials and methods

2.1 Materials

The Brazilian CB (Bahia, Brazil) and the CBE used in this study were kindly provided by the companies Barry Callebaut (Ilhéus, Brazil) and Fuji Vegetable Oil (Savannah, USA), respectively. CBE is made from domestic sunflower/safflower oil and Malaysian fractionated palm oil and is designed to mimic West African cocoa butter's physical characteristics. D-Limonene (4-isopropenyl-1-methylcyclohexene, 95–97%) was provided by the company BioCitrus (Mogi-Guaçu, Brazil). All the reagents and solvents used were of analytical grade.

2.2 Methods

2.2.1 Fatty acid composition

The fatty acid composition (FAME) was determined by gas chromatography after esterification, using the methodology described by Hartman et al. [15]. The analyses were carried out using an Agilent 6850 Series gas chromatograph system (Santa Clara, USA) connected with a flame ionization detector (FID) and an Agilent DB-23 capillary column (50% cyanopropyl- and 50% methylpolysiloxane; length of 60 m, internal diameter of 0.25 mm, and film thickness of 0.2 μm). The injector and detector temperatures were 250 and 280°C, respectively. The oven temperature was initially 110°C for 5 min, then increased at a rate of 5°C/min to 215°C, and was finally kept at this temperature for 24 min. The samples were injected automatically (sample volume of 1.0 μL) using helium as the stripping gas and a split ratio of 1:50. The qualitative composition of the samples was determined by comparison of the peak retention times with those of the respective fatty acid standards. The quantitative analysis was carried out by normalization of the peak areas, which were expressed as a percentage of the mass according to Method Ce 1f-96 [16]. The sample analyses were carried out in duplicate and the results are presented as mean values and standard deviation.

2.2.2 Triacylglycerol composition

The TAG were quantified using an Agilent 6850 Series capillary gas chromatograph system equipped with an FID and an Agilent DB-17HT capillary column (50% phenyl- and 50% methylpolysiloxane; length of 15 m, internal diameter of 0.25 mm, and film thickness of 0.15 μm). The sample preparation method and analytical conditions described by Ribeiro et al. [17] were used: split ratio of 1:100; initial column temperature of 250°C, increased at a rate of 5°C/min to 350°C; helium gas transport rate of 1.0 mL/min; injector temperature of 360°C; detector temperature of 375°C; injected sample volume of 1.0 μL; sample concentration of 100 mg/5 mL tetrahydrofuran. The TAG groups were identified by a comparison of their retention times according to Antoniosi Filho et al. [18] and were compared with data found in the literature [19]. The samples were analyzed in duplicate and the results are presented as mean values and standard deviation.

2.2.3 Sample preparation

The samples preparation and the crystallization process of fats without tempering were carried out according to procedures presented in Ray et al. [7] with crystallization temperature modified from 20°C to 25°C as a reference of room temperature. To prepare the samples containing D-limonene (1.5 and 5%, w/w), approximately 200 g of CB or CBE was melted at 60°C for 30 min and then homogenized with D-limonene for 10 min using a magnetic shaker (IKA Instruments RH basic1). The samples were placed in covered Petri dishes and stored in a controlled temperature chamber (TE-381, Tecnal, Piracicaba, Brazil) at 25°C to crystallize statically until the X-Ray diffraction analysis.

2.2.4 X-ray diffraction evaluation of the crystallization behavior of CB and CBE

The polymorphic forms, βV and βVI, of the CB and CBE lipid crystals (all samples) were determined by X-ray powder diffraction according to Method Cj 2-95 [20]. The diffractograms, *d*-spacings, and peak intensities of the obtained polymorphic forms from CBE and CB were compared with the characteristic diffraction patterns of CB presented in the literature [21, 22]. The classification of the peak intensities as

very strong (vs), strong (s), medium (m), weak (w), and very weak (vw) was based on these studies.

The X-ray diffraction (XRD) measurements were obtained using a Philips powder diffractometer (PW1710, Almelo, Netherlands) with Bragg-Brentano geometry, a graphite monochromator for the diffracted band, step sizes of 0.03° (2θ), and an acquisition time of 2 s for each point measured. CuK_α radiation ($\lambda = 1.5418$ Å) was used, and the 2θ diffraction range was 15–27°, representing, through Bragg's law, $2d\sin\theta = n\lambda$ (assuming $n = 1$), *d*-spacing values of approximately 3–6 Å. The measurements were collected at 25°C using an Anton Paar TTK 450 temperature chamber (Richmond, USA).

The polymorphism of samples was monitored over 27 days at the following storage times: $d_4 = 4$th day, $d_6 =$ 6th day, $d_{11} = 11$th day, $d_{13} = 13$th day, $d_{18} = 18$th day, $d_{21} = 21$st day, $d_{25} = 25$th day, and $d_{27} = 27$th day.

As none of the CB and CBE samples was tempered, different degree of crystallizations was verified visually in some samples up to the fourth day of storage. To complement this information, previous tests were performed and more defined peaks were observed in the X-ray diffractograms of the control CBE and control CB samples from the second and fourth day of storage at 25°C, respectively. For this reason, and with the aim of standardization, the fourth day of storage (d_4) was defined as the initial reference time for the evaluation of the polymorphic transitions.

3 Results and discussion

3.1 Fatty acid composition

Table 1 shows the fatty acid compositions obtained for CB and CBE samples. In this table, it is possible to observe that the CB and CBE samples have the same major fatty acids, represented by the palmitic (C16:0), stearic (C18:0), and oleic (18:1) acid. In comparison to CBE, CB has a similar content of oleic acid, higher content of stearic acid and lower contend of palmitic acid (C16:0). These major fatty acids represent 95.19 and 93.37% of the total fatty acid content in CBE and CB, respectively. The sum of the saturated and unsaturated fatty acid contents present in CB and CBE is also similar.

The literature data, also presented in Table 1, show that the CBE fatty acid composition values for palmitic, stearic, and oleic acid have a wide range of values, and differ from fatty acid composition of CB. According to the literature, the variance in the fatty acid composition profiles of CBE could be due to the different categories that exist for this fat in the market, such as soft and hard types, which are made from different oil and fat sources [29]. For CB, the fatty acid composition found was in agreement with those reported in the literature (Table 1).

Table 1. Fatty acid composition (FA) (%) of the CB and CBE

FA	CBE	CBE[a]	CB	CB[b]
C12:0	0.12 ± 0.00		0.06 ± 0.01	
C14:0	0.49 ± 0.00	0.3–0.9	0.09 ± 0.00	0–0.2
C15:0	0.02 ± 0.02		0.04 ± 0.01	
C16:0	30.75 ± 0.05	14–59	25.45 ± 0.06	23–28
C16:1	0.03 ± 0.04	0–0.1	0.27 ± 0.00	0.2–0.4
C17:0	0.11 ± 0.01		0.21 ± 0.00	
C17:1	0.02 ± 0.03		0.07 ± 0.01	
C18:0	30.08 ± 0.06	8–46	32.93 ± 0.01	32–38
C18:1	34.36 ± 0.04	29–37	34.99 ± 0.03	33–38
C18:2t	0.06 ± 0.00		0.40 ± 0.02	
C18:2	2.83 ± 0.01	1–4	3.87 ± 0.08	2–4
C18:3	0.02 ± 0.03		0.23 ± 0.01	0.1–0.2
C20:0	0.48 ± 0.00	0.8–1.5	1.02 ± 0.01	0.9–1.2
C20:1	0.03 ± 0.04		0.08 ± 0.01	
C22:0	0.46 ± 0.01	0.1–0.5	0.20 ± 0.03	0.1–0.3
C24:0	0.17 ± 0.01		0.12 ± 0.01	
Σ Saturated	62.66 ± 0.09		60.12 ± 0.15	
Σ Unsaturated	37.32 ± 0.07		39.91 ± 0.29	

[a]Range of values for FA in CBE according to various authors [23–26].
[b]Range of values for FA in CB according to various authors [2, 3, 25–28].

3.2 Triacylglycerol composition

The TAG compositions of CBE and CB determined using gas chromatography are presented in Table 2. The major TAG were POSt, StOSt, POP, and PPS for CBE (11.51, 31.30, 31.33, and 10.35%) and POSt, StOSt, POP for CB (37.77, 21.68, and 19.01%). The literature data shown in this table demonstrate that the major TAG composition of CBE and CB has a large range of values, that is, high variability is found.

When examined in terms of the saturation degree of the TAG group, the CB and CBE samples had quite different characteristics. The sum of the trisaturated triacylglycerol (S_3) content in CBE was approximately eightfold greater than that in CB, principally owing to the high PPSt content in CBE. According to O'Brien [30], trissaturated TAG show highest melting point and they are able to start the first nucleations of fat crystals. The percentages of symmetrical or monounsaturated TAG found in CBE were a little lower than those found in CB. The literature states that a greater presence of symmetrical TAG in a fat induces a crystallization process and polymorphic transitions faster than when asymmetrical TAG are present [31–33]. Studies showed that the type of symmetrical TAG has also an influence on the crystallization velocity. Fats with a higher proportion of StOSt than POP have a greater crystallization velocity. The StOSt melting point (43.8°C) is higher than that of POP (36.6°C), and this difference is the driving force that promotes the liquid–solid crystallization process [31, 34]. The content of StOSt and POP in the CBE sample was

Table 2. Triacylglycerol (TAG) contents (%) of the cocoa butter equivalent (CBE) and cocoa butter (CB)

Group	TAG	CBE	CBE[a]	TAG	CB	CB[b]
C48	PPP	1.70 ± 0.01		PPP	0.20 ± 0.07	0.4–0.7
	MOP	1.13 ± 0.07		MOP	0.00 ± 0.00	
C50	PPSt	10.35 ± 0.09		PPSt	0.68 ± 0.08	0.2–0.9
	POP	31.33 ± 0.05	35–45	POP	19.01 ± 0.08	13–19
	PLP	3.79 ± 0.09		PLP	2.21 ± 0.04	
C52	PStSt	0.50 ± 0.07		PStSt	0.72 ± 0.06	
	POSt	11.51 ± 0.06	14–19	POSt	37.77 ± 0.03	34–42
	POO	2.83 ± 0.08		POO	4.71 ± 0.03	
	PLSt	0.88 ± 0.06		PLSt	3.41 ± 0.03	
	PLO	0.01 ± 0.00		PLO	0.53 ± 0.04	
C54	$StStSt_t$	0.80 ± 0.01		$StStSt_t$	0.00 ± 0.00	
	StOSt	31.30 ± 0.07	21–40	StOSt	21.68 ± 0.06	23–29
	StOO	3.21 ± 0.06		StOO	5.31 ± 0.07	
	OOO+StLSt	0.67 ± 0.03		StLO	2.11 ± 0.06	
	StLO	0.00 ± 0.00		OOO	0.71 ± 0.01	
C56	StOA	0.00 ± 0.00		StOA	0.99 ± 0.04	
	S_3	13.35 ± 0.02		S_3	1.59 ± 0.06	
	S_2U (SUS)	80.29 ± 0.03		S_2U (SUS)	85.06 ± 0.07	
	U_2S	6.05 ± 0.02		U_2S	12.66 ± 0.06	
	U_3	0.34 ± 0.01		U_3	0.71 ± 0.01	

[a]Range of values found for TAG in CBE by various authors [23–25].
[b]Range of values found for TAG in CB by various authors [2, 3, 25, 27, 28].

almost the same, whereas in CB sample the content of StOSt was slightly greater than the content of POP. The sums of the diunsaturated (U_2S) and triunsaturated (U_3) triacylglycerol contents in CBE were approximately half those found in CB. According to De Cock [35] and Vereecken et al. [31], fats containing trisaturated and symmetrical TAG accelerate fat crystallization and the polymorphic transition. Thus, the obtained results and literature information suggest that CBE may have greater crystallization rate and polymorphic transition velocities than CB.

3.3 X-ray diffraction analysis

Table 3 shows the polymorphic forms that were predominant in the samples at the initial time d_4, the intermediate time when the polymorphic transition to the βVI form was first observed, and at the final time of 27 storage days (d_{27}) at 25°C. Figure 1 presents the diffractograms obtained for the control samples of CBE and CB, and for the samples with 1.5 and 5% D-limonene. In this figure, the vertical bars indicate that the obtained values for the *d*-spacings of the βV and βVI polymorphic forms are consistent with the data presented by Wille et al. [22]. The βV form was identified using the peaks with medium intensity at 3.75 Å and with strong intensity at 3.98 Å, and the βVI form was identified using the peaks with weak intensity at 4.04 Å and with strong intensity at 3.70 Å. The changes in the diffractogram profiles (Fig. 1), whose differences were observed in the peak intensities and *d*-spacing values, indicated that the addition of 1.5 and 5% D-limonene accelerated the polymorphic transition of the βV form to βVI form in both the CBE and CB samples.

At d_4, the control CBE sample showed a mixture of the βV and βVI polymorphic forms (Table 3). The increase of peak intensity at d_{11} to a strong peak at 3.71 Å represents an increase in the βVI form present in the fat fraction. In addition, at d_{27}, a weak intensity peak was identified at 3.98 Å, which is also characteristic of the βVI form, thus confirming the increase in the βVI form in the control CBE sample (Fig. 1A).

A medium intensity peak at about 3.71 Å was observed for the CBE sample containing 1.5% D-limonene at d_4, whereas at times d_6 and d_{27}, the same peak was identified with a strong peak intensity, indicating an increase in the βVI form. In the CBE sample with 5% D-limonene, a signal with strong intensity was identified at 3.71 Å even at d_4, indicating that the higher concentration of D-limonene in the sample had a greater effect on accelerating the polymorphic transition from the βV to βVI form (Fig. 1B and C). The βVI form was predominant in the CBE sample with 5% D-limonene at the end of the storage period at 25°C.

When the diffractograms obtained for the control CB sample (Fig. 1D) were compared with the characteristic pattern of the βV form found in the literature [21, 36], the βV polymorphic form was found to be present throughout the

Table 3. Polymorphic forms and *d*-spacing values of the CB and CBE samples with addition of 1.5 and 5% D-limonene determined by X-ray diffraction at 25°C and different storage times

Samples/days	Polymorphic forms	*d*-Spacing (Å)							
	[a]β (V)	5.43 (m)	5.15 (w)	4.58 (vs)	4.23 (vw)	3.98 (s)	3.87 (m)	3.75 (m)	3.67 (w)
	[b]β (VI)	5.43 (m)	5.15 (w)	4.59 (vs)	4.27 (vw)	4.04 (w)	3.86 (m)		3.70 (s)
CBE (d_4)	β (V) + β (VI)	5.47 (vw)	5.17 (vw)	4.59 (vs)	4.27 (vw)	3.97 (m)	3.88 (m)	3.76 (m)	3.71 (m)
CBE (d_{11})	β (VI) > β (V)	5.48 (vw)		4.59 (vs)	4.27 (vw)	3.98 (m)	3.88 (m)	3.75 (m)	3.71 (s)
CBE (d_{27})	β (VI) >> β (V)	5.47 (w)	5.19 (vw)	4.59 (vs)	4.28(vw)	3.98 (w)	3.87 (m)	3.75 (m)	3.70 (s)
CBE + 1.5% w/w (d_4)	β (VI) + β (V)	5.48 (w)	–	4.60 (vs)	4.25 (vw)	–	3.89 (m)	3.76 (m)	3.71 (m)
CBE + 1.5% w/w (d_6)	β (VI) >> β (V)	5.46 (w)	–	4.60 (vs)	4.28 (vw)	–	3.88 (m)	3.76 (m)	3.70 (s)
CBE + 1.5% w/w (d_{27})	β (VI) >> β (V)	5.48 (w)	–	4.60 (vs)	4.28 (vw)	–	3.88 (m)	3.76 (m)	3.71 (s)
CBE + 5% w/w (d_4)	β (VI) >> β (V)	5.47 (w)		4.60 (vs)	4.28 (vw)	4.04 (w)	3.89 (m)	3.76 (m)	3.71 (s)
CBE + 5% w/w (d_{27})	β (VI) >>> β (V)	5.47 (w)	5.17 (vw)	4.59 (vs)	4.25 (vw)	4.05 (w)	3.88 (m)	3.75 (m)	3.71 (s)
CB (d_4)	β (V)	5.45 (w)	–	4.59 (vs)	4.24 (vw)	3.99 (s)	3.88 (m)	3.77 (m)	3.68 (m)
CB (d_{18})	β (V)	5.43 (w)	–	4.59 (vs)	4.25 (vw)	3.99 (m)	3.88 (m)	3.76 (m)	3.69 (m)
CB (d_{27})	β (V)	5.44 (w)	–	4.59 (vs)	4.25 (vw)	3.99 (m)	3.87 (m)	3.76 (m)	3.69 (m)
CB + 1.5% w/w (d_4)	β (V)	5.45 (w)	–	4.59 (vs)	4.25 (vw)	3.99 (s)	3.88 (m)	3.77 (m)	3.68 (m)
CB + 1.5% w/w (d_{25})	β (V) + β (VI)	5.41 (w)	–	4.59 (vs)	4.28 (vw)	3.99 (m)	3.87 (s)	3.74 (m)	3.70 (s)
CB + 1.5% w/w (d_{27})	β (V) + β (VI)	5.41 (w)	–	4.59 (vs)	4.28 (vw)	3.99 (m)	3.87 (s)	3.74 (m)	3.70 (s)
CB + 5% w/w (d_4)	β (V)	5.41 (w)	–	4.59 (vs)	4.28 (vw)	4.00 (m)	3.87 (m)	3.76 (m)	3.70 (m)
CB + 5% w/w (d_{11})	β (VI)	5.45 (vw)	–	4.59 (vs)	4.25 (vw)	4.04 (m)	3.87 (m)	–	3.70 (s)
CB + 5% w/w (d_{27})	β (VI)	5.40 (vw)	–	4.60 (vs)	4.28 (vw)	4.05 (w)	3.86 (s)	–	3.71 (s)

[a]The intensity is noted as very strong (vs), strong (s), medium (m), weak (w), or very weak (vw).
[b]Values for *d*-spacing for the identification of the polymorphic forms V and VI in the CB, according to Wille et al. [22].

entire storage period, and there was no indication of a polymorphic transition. However, the addition of 1.5% D-limonene to CB was shown to influence the polymorphic transition behavior because the βVI form was observed at d_{25}, identified by the strong peak intensity at 3.70 Å (Fig. 1E).

In the CB samples containing 5% D-limonene (Fig. 1F), the complete transition from the βV to βVI form took up to 11 days (d_{11}), as indicated by the characteristic diffractogram profile and according to the *d*-spacing values of the βVI polymorphic form (Table 3) [21]. These results are consistent with the data presented by Ray et al. [7]. These authors studied the influence of the addition of 5% D-limonene w/w on CB during storage for 28 days at 20°C and found that this additive promoted a complete polymorphic transition between 21 and 28 days (during the third storage week). Differences in the storage temperatures and TAG compositions of the two CB samples could explain the delayed time for the complete polymorphic transition; however, the authors did not present the TAG composition data. Also, they chose as reference time "weeks" instead of days or hours, which makes any kind of comparison hard to perform.

Comparing the results obtained for both the CBE and CB samples, it was concluded that the addition of 5% D-limonene to the samples resulted in a quicker polymorphic transition than that observed in samples with 1.5% D-limonene (Table 3). A mechanism that might explain how D-limonene accelerates the conversion from the βV to βVI polymorphic form could be related to the effect of D-limonene on the molecular mobility of TAG in the fat crystal network. As the melting point of D-limonene is extremely low (−73.5°C) [37], it is in the liquid state at 25°C, whereas the major TAG in the CBE and CB samples are still crystallizing at this temperature. As a consequence, the liquid fraction around the crystalline network may allow greater TAG mobility during the structural rearrangement into the more stable polymorph. Campos et al. [10] proposed this mechanism to explain the effect of LLL (trilinoleic), a TAG that also has a low melting point (−13.3°C) [38], on the polymorphic transition of the TAG in CB from the β′ polymorphic form into the β form. The authors also stated that the presence of liquid oil either occluded in the crystalline network or on the crystal surface accelerates polymorphic transformations. Do et al. [8] studied the impact of limonene on the physical properties of reduced fat chocolate. They found that the addition of D-limonene to chocolate significantly decreased its hardness when compared to chocolate produced with only CB. According to the authors, D-limonene may be located between the fatty acids chains in the TAG and around the fat crystal network, which in turn may influence the mobility degree of TAG and make the fat crystal network less cohesive, and consequently, the hardness of fatty product is decreased. Although D-limonene has a very different chemical structure than TAG molecules, molecular

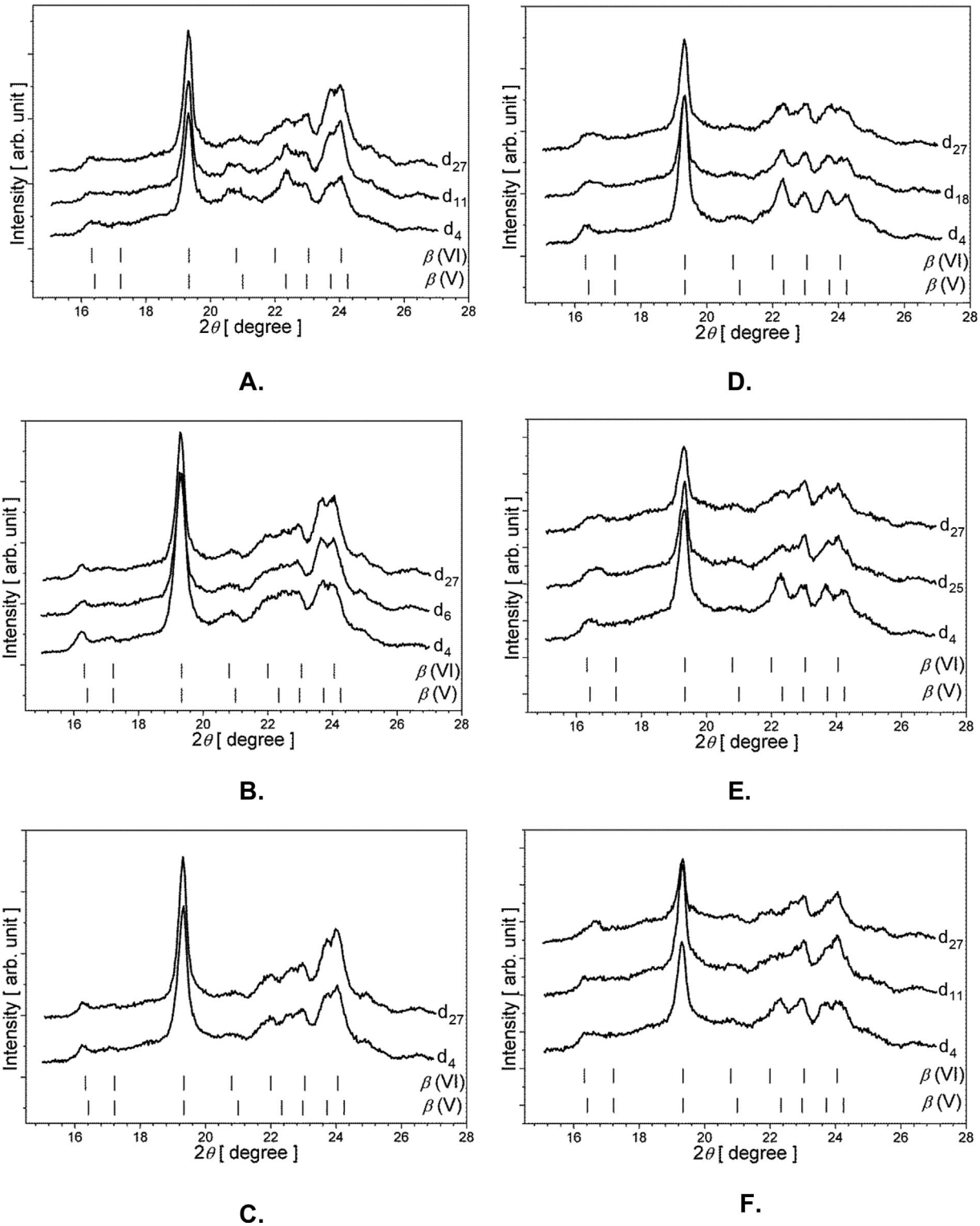

Figure 1. X-ray diffraction patterns of the CB and CBE samples during storage at 25°C. (A) CBE (B) CBE+1.5%, (C) CBE+5%, (D) CB, (E) CB+1.5% and (F) CB+5%.

mobility seems to be suitable mechanism to explain the effect of D-limonene on polymorphism change.

Along with effect of D-limonene on fats, it is necessary to emphasize the influence of fat melting properties on polymorphic transition trend. CBE samples possibly have higher melting temperature and enthalpy than CB because they have more saturated (S_3) and the content of symmetrical (SUS) TAG is approximated. Also, the literature indicates that the high content of both symmetrical and saturated TAG induces faster crystallization rates and polymorphic transitions than when asymmetrical and unsaturated TAG are present [35, 31]. These last statements are in agreement with the results obtained in this present study, since the CBE samples with and without limonene started polymorphic transitions prior to all the CB samples in the same conditions (Table 3). Moreover, these results also showed that the addition of D-limonene to CBE samples only intensified the polymorphic transition that already occurs in the control CBE samples. Thus, the characteristics of the TAG profile may be the main factor controlling the polymorphic transition. Following this trend, in CBE samples, the crystal fraction with form βVI should increase faster than in CB samples during the storage time. Interestingly, however, this behavior was not observed in samples with 5% D-limonene. CB samples at that concentration did not show the polymorphic βVI form before d_{11}, however, during the 5-day interval between d_6 and d_{11}, the CB samples underwent a full polymorphic transition. Meanwhile, CBE samples at that same condition did not present full polymorphic transition even though the CBE samples had begun to crystallize and change polymorphism earlier than the CB samples. These results indicated that the CB sample containing 5% D-limonene intensified the acceleration of polymorphic transition in comparison to those CB samples with low concentrations of that additive. This behavior also represents that the addition of 5% D-limonene in CB may quickly promote a change of standard packing of TAG and polymorphism trend. The profile of TAG composition of CB may have influenced this result in sample containing 5% D-limonene. As previously presented, Brazilian CB has a particular TAG composition, with a higher unsaturated TAG content than the CBE. Owing to this characteristic, at the same crystallization temperature, Brazilian CB has lower melting temperature and enthalpy, and solid fat content than CBE, and would be still crystallizing [28, 39]; consequently, TAG molecules could be freer to rearrange in the fat crystal network than the TAG molecules present in CBE. Similarly, this behavior may be found when the CB melting point is decreased by a D-limonene incorporation or when an high crystallization temperature is used. Ray et al. [7] showed that the addition of 5% D-limonene to CB samples decreased their melting points and the solid fat content [8], and contribute to accelerate complete polymorphic transition. Fernandes et al. [9] studied the thermal and structural properties of dark chocolate with different recipes. Their results showed that in all cases, the most stable polymorphic forms were obtained more quickly when high temperatures were used in the crystallization process. Following these trends, it is possible to regard that any crystallization temperature considered high for a specific fat melting profile represents a condition that allow TAG molecules to rearrange in fat crystal network with most mobility degree. All these points may clarify why Brazilian CB samples containing 5% D-limonene had quicker full polymorphic transition than CBE samples in the same analysis condition. Thus, the results showed that the profile of TAG composition and the D-limonene concentration affect the different behavior of the polymorphic transition of CB and CBE samples.

4 Conclusions

The addition of D-limonene to fats accelerated the polymorphic transition in comparison to the pure CB and CBE. In CB samples, the addition of 5% w/w D-limonene accelerated the full polymorphic transition from form βV to βVI in less than 2 weeks of storage at 25°C ($d_{11} = 11$th day). CB samples containing 5% D-limonene w/w intensified the acceleration of polymorphic transition in comparison to those CB samples with low concentrations of that additive, which indicates that the addition of D-limonene promoted a change of standard packing of TAG and the polymorphism trend. Pure CB remained in βV form throughout the period evaluated. In CBE samples, the polymorphic transition seemed to be governed principally by the characteristics of the TAG profile and was only intensified with D-limonene addition. Although all the CBE samples started the polymorphic transition before the CB samples, none of the CBE samples showed a complete transition of form βV to βVI during the storage period.

The authors wish to acknowledge the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP – Proc. 2009/53006-0) for the financial support.

The authors have declared no conflict of interest.

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