Cocoa-Butter Long Spacings and the Memory Effect

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ABSTRACT: Depending on the maximum temperature before cooling, cocoa butter recrystallizes in the β(V) and/or β(VI) phase. In order to obtain structural information on the seed material initiating this recrystallization process, experiments with cocoa butter were performed at a small-angle X-ray scattering station. Crystallization of cocoa butter at various crystallization temperatures provided long *d*-spacing values ($d > 45$ Å) that can be attributed to the polymorphic phases. From the recrystallization experiments it is concluded that the seeds initiating rapid-starting recrystallization have an 1,3-distearoyl-2-oleoylglycerol-dominated triple chain-length packing. Furthermore, the β(VI) phase of cocoa butter seems to adopt a similar packing. The seed crystals that initiate the slow-starting recrystallization and result in the β(V) phase are likely to be different from those giving the β(VI) phase.

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KEY WORDS: Cocoa butter, crystallization, long spacing, memory effect, seeding.

Cocoa butter, a vegetable fat, is widely applied in food products. It is valued not only for giving the product a good food structure but also for its melting characteristics. Since cocoa butter shows polymorphic behavior, a conditioned crystallization process is required to obtain the desired polymorphic phase and its associated melting range. The phase behavior of cocoa butter is very complex and is influenced by composition and thermal history. Therefore, the physical behavior of cocoa butter and related fats has been the subject of research for many years.

In spite of all efforts, there is still no consensus about the number and characteristics of cocoa butter phases. The nomenclature used in this paper [γ, α, β′, and two β phases β(V) and β(VI)] are taken from Vaeck (1) and Wille and Lutton (2). Cocoa-butter phases are identified by their typical X-ray powder diffraction (XRPD) pattern in the 3–6 Å region (Fig. 1), the so-called "fingerprint region," which originates from the different hydrocarbon chain-packings. Since a different chain packing corresponds with a different phase, the information provided by the fingerprint region is sufficient to distinguish between the various phases. Moreover, by monitoring this small region (from 15 to 30° 2θ in XRPD with Cu Kα radiation), it is possible to perform real-time experiments at laboratory scale (3). Recently, the full XRPD patterns, including peaks at long *d*-spacing values, were also used to characterize the various cocoa-butter phases (4,5).

From the phase-transition scheme of cocoa butter crystallizing under static isothermal conditions, it is concluded that the γ phase is very unstable, that α and β′ phases are metastable, and that the β phases have the highest stability (6). All the phases except the stable β phases can be obtained

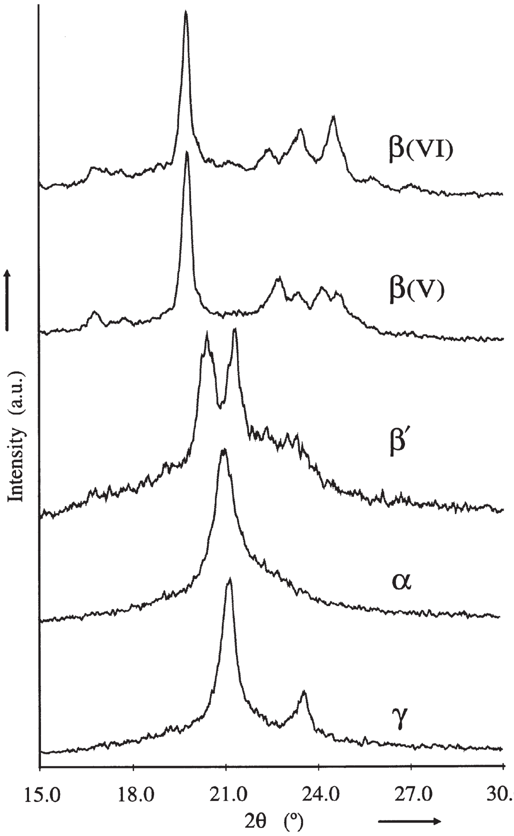

FIG. 1. Fingerprint region (λ = 1.5418 Å; *d*-spacing values 3.0 to 6.0 Å) of the X-ray powder diffraction (XRPD) patterns of the cocoa-butter phases. The characteristic γ, α, and β′ phases of Cameroon cocoa butter were measured after isothermal crystallization at –10.0, 0.0, and 20.0°C, respectively (6). The β(V) and β(VI) phases of the Bahia, Brazil, cocoa butter were measured after isothermal crystallization at 22.0°C and from bulk material, respectively. These are the clearest XRPD patterns of the β(V) and β(VI) phases observed so far with our XRPD equipment (8).

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*To whom correspondence should be addressed at Laboratory for Crystallography, Institute of Molecular Chemistry (IMC), Universiteit van Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands. E-mail: rene@science.uva.nl by crystallization from molten cocoa butter that has been heated to a sufficiently high temperature. Direct crystallization of the β phase from the melt was only observed by solidification of cocoa butter that was heated only a few degrees above its melting end point (MEP) before cooling (6,7), the so-called "memory effect" of cocoa butter. Van Malssen *et al.* (7) defined the β-memory point temperature (β-MPT) as the temperature to which cocoa butter in the β phase has to be heated to prevent recrystallization into the β phase within 45 min when cooled at 25°C. Furthermore, they found a correlation between β-MPT and the content of 1,3-distearoyl-2-oleoyl-glycerol (SOS) and stearic acid in cocoa butter. Loisel *et al.* (4) discussed this correlation and stated, based on long-spacing information that was obtained under different experimental conditions, that the memory effect is correlated with the trisaturated triacylglycerol (TAG) concentration. Recently, we studied the effect of the maximal temperature before recrystallization (T_{max}) and the crystallization temperature (T_{cryst}) on the recrystallization behavior of cocoa butter (8). Depending on the composition of cocoa butter and on T_{max}, both a rapid-starting and a slow-starting recrystallization were observed. It was concluded that the rapid-starting recrystallization is induced by high-melting SOS-rich seed crystals. In view of this discussion, we investigated crystallization and recrystallization of cocoa butter with small-angle X-ray scattering (SAXS). So far, all our results have been based on XRPD studies in the fingerprint region, but the long *d*-spacing values may also present valuable information about the different polymorphs and their phase transitions. Time-resolved diffraction of long spacings (40 Å < *d* < 70 Å) is difficult to perform with our laboratory time-resolved XRPD (tr-XRPD) equipment because of the relative weak diffraction. These studies may be feasible only with high-resolution XRPD stations or SAXS stations at synchrotron sources. This study describes the long-spacing results of time-resolved diffraction experiments of Bahia cocoa butter performed at the SAXS station of the DUBBLE beamline at the ESRF in Grenoble (BM26a; 9).

EXPERIMENTAL PROCEDURES

Samples, sample preparation, and data collection. Experiments were performed with cocoa butter originating from Bahia Brazil, which was obtained from ADM Cocoa B.V. (Koog aan de Zaan, The Netherlands). The iodine value of the cocoa butter was determined by the Wijs method (IUPAC method 2.205; Table 1; 10), the triglyceride composition was determined with gas–liquid chromatography (GLC) (IUPAC method 2.323; 10) and the fatty-acid composition with GLC *via* fatty acid methyl esters (IUPAC methods 2.301 and 2.302; 10).

Samples for temperature-controlled SAXS experiments were prepared by pressing cocoa butter in standard aluminum sample pans for differential scanning calorimetry (DSC; TA Instruments, Leatherhead, United Kingdom). To allow X-ray access to the sample, holes were punched in the pan and lid, which were covered with mica windows having a thickness of 0.025 mm (11). Samples for tr-XRPD were prepared by pressing cocoa butter into a temperature-controlled sample holder, resulting in a sample size of 10 × 15 × 1 mm (~150 mg) with a flat surface.

TABLE 1
Iodine Value and Triglyceride and Fatty Acid Content of the Bahia, Brazil, Cocoa Butter (8)

Iodine value	40.3	Fatty acid	(%)
		$C_{16:0}$	23.4
Triglyceride	(%)	$C_{16:1}$	0.5
C_{48}	0.2	$C_{18:0}$	31.3
C_{50}	16.7	$C_{18:1}$	37.8
C_{52}	45.6	$C_{18:2}$	4.5
C_{54}	35.9	$C_{18:3}$	0.2
C_{56}	1.7	$C_{20.0}$	1.2
		$C_{20:1}$	0.0
		$C_{22:0}$	0.1
		Rest	0.3

Synchrotron X-ray diffraction images were made at the SAXS station mounted on beamline BM26a of the DUBBLE CRG (ESRF, Grenoble, France; 9). The instrument, having an approximate beam spot of 0.7 × 1.0 mm, is equipped with a DSC cell to perform temperature-controlled measurements (12). The wavelength was fixed at 1.24 Å, and the camera length was chosen such that the minimum observable *d*-spacing value was 40 Å. The detector was calibrated using the *d*-spacings of rat-tail collagen. The 40 Å < *d* < 70 Å region of the SAXS images were analyzed using the programs READSAXS (13), FIT2D (14), BSL (15), and XOTOKO (16). The standard deviation of the *d*-spacing values in this region is ±0.2 Å. The data were normalized by dividing the images by the value of the second ionization chamber. The integrated intensity $I_c(q)$ was determined by integrating the image over a quart-circle sector and multiplying the result with q^2. This gives a minimum observed *d*-spacing value of 45.6 Å for the integrated intensity. The q^2 correction (for geometrical considerations) is commonly used for lamellar systems (17). Contour plots of $I_c(q)$ vs. frame number and time were made using the program *GNUPLOT* v3.7 (18).

Real-time XRPD patterns were taken using the tr-XRPD equipment as used in earlier studies, with the same instrumental setting (3) and a beam spot of 0.4 × 12 mm (Philips Xray tube, type PW2273/20). The use of Cu Kα radiation and a diffraction range of 14.6–29.6° 2θ resulted in an observed *d*-range of 3.0–6.1 Å.

MEP determination and crystallization from the melt. In order to determine the MEP of Bahia cocoa butter, a sample was kept 20 min at temperatures in the range 20–40°C, as shown in Figure 2. The first 5 min of each of these 20-min periods was used for acclimatization, and in the successive 15 min a diffraction image was collected. Each succeeding temperature in the range between 27.5 and 34.0°C, in steps of 0.5°C, was reached from the preceding one by heating 20°C min^{-1}.

Crystallization from the melt and phase transitions of cocoa butter at various preset solidification temperatures T_p were investigated. Samples were heated to 60°C and kept at that temperature for several minutes and then cooled to 40°C at 5°C min^{-1}. Next, the measurements were started while

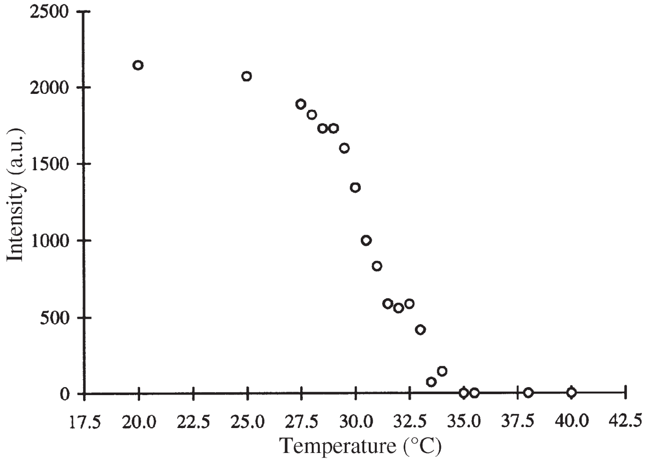

FIG. 2. Melting curve of β-cocoa butter from Bahia, Brazil, determined from the diffraction peak with a *d*-spacing value of 64.8 Å. The error in the intensity values is $\sqrt{I}$.

cooling the sample at 5°C min^{-1} to T_p and, subsequently, keeping it at that temperature for about 60 min. During the cooling and holding periods, images were made with a lifetime of 30 s and a dead time of 0.001 s.

To attribute the observed peaks correctly to the various cocoa-butter phases, the SAXS measurements are supported with laboratory tr-XRPD experiments that have been carried out using identical temperature profiles and an exposure time of 3 × 10 s per pattern.

Melting and recrystallization experiments near the MEP. The memory effect of cocoa butter was investigated by performing recrystallization experiments and comparing the results with the crystallization and phase transitions of the memory-free crystallization experiments. Each recrystallization experiment was started with a freshly prepared cocoa-butter sample. Samples were taken from the bulk of which the temperature had not exceeded its MEP during the last few years. After being at 20°C for 5 min, samples were heated to a maximum temperature (T_{max}) with a heating rate of 5°C min^{-1}. At T_{max} the samples were held for 5 min before cooling at 5°C min^{-1} to a crystallization temperature T_{cryst}, at which they were kept for 60 min. During the whole temperature profile, measurements were made with a lifetime of 30 s and a dead time of 0.001 s.

RESULTS

MEP. The first temperature T_f encountered without significant diffraction intensity is considered as the MEP. At the starting temperature T_f = 20°C, two diffraction peaks were observed (*d* = 64.8 and 47 Å). The intensity of the stronger peak (*d* = 64.8 Å) was determined at the various T_f values by subtracting the background from intensity *I* at the top of the diffraction peak. From the resulting temperature T_f vs. intensity *I* curve the MEP was determined as 35.0 ± 0.5°C (Fig. 2).

Crystallization from the melt. For the crystallization experiments, the time *t* (min) is relative to t_0, which is the time that the cocoa butter reaches T_p. Contour plots of $I_c(q)$ vs. frame number and time are shown for most crystallization experiments (Fig. 3).

(i) T_p = 25°C: Within the experiment time, no diffraction peaks have been observed after cooling liquified cocoa butter to 25°C, neither in SAXS nor in XRPD.

(ii) T_p = 20°C (Fig. 3A): At this T_p value a small diffraction peak with a *d*-spacing value of 49.6 Å was observed after 7 min. The intensity of this peak enhanced slowly until the end of the experiment time had been reached. No significant diffraction peaks were observed with XRPD.

(iii) T_p = 15°C (Fig. 3B): A sharp and rather intense diffraction peak at 54.9 Å was observed between *t* = 1 and 9 min. A broad diffraction peak with a *d*-spacing value of 49.6 Å started at *t* = 2 min and increased slowly. From *t* = 27 min, its intensity decreased slowly, until it was completely gone at *t* = 58 min. Between *t* = 28 min and the end of the experiment, a broad vague band of diffraction peaks was also observed around *d* = 44.8 Å. At the XRPD patterns, a peak with a *d*-spacing value of about 4.1 Å was observed from *t* = 1 min. From *t* = 28 min until the end of the experiment two diffraction peaks were observed at 3.8 and ~4.2 Å, respectively. Since the 4.1 and 4.2 Å peaks were very close to each other, it was not clear whether the peak at 4.1 Å slowly disappeared while at 4.2 Å a peak appeared or whether the 4.1 Å peak slowly shifted to 4.2 Å.

(iv) T_p = 10°C (Fig. 3C): A sharp diffraction peak at 54.7 Å was observed between *t* = –1 and 27 min, and in the same period a peak at ~4.1 Å was seen. From *t* = 3 min a broad peak with a *d*-spacing value of 48.7 Å was observed, which became sharper near the end of the experiment. In the end of the experiment, a clear shoulder at 46 Å was observed as well. In the XRPD patterns a peak at 4.2 Å was observed from *t* = 3 min and remained until the end of the experiment. It was not clear from the XRPD patterns whether the 4.1 Å peak shifted to 4.2 Å or slowly disappeared. From *t* = 31 min until the end of the experiment a diffraction peak at 3.8 Å also was observed.

(v) T_p = 5°C (Fig. 3D): At this T_p value a diffraction peak at 54.8 Å was observed from *t* = –1 min, which shifted within 3 min to a *d*-spacing value of 53.8 Å and returned to its original *d*-spacing value within 12 min, at which it remained until it disappeared at *t* = 38 min. A diffraction peak with a *d*-spacing value of 51.0 Å was observed from *t* = 3 min, which broadened and shifted to a broad band of diffraction peaks with a *d*-spacing range of 45–50 Å remaining until the end of the experiment. In XRPD a peak at ~4.1 Å was observed from *t* = –1 min. Later, this peak was gone and a peak at 4.2 Å was observed, remaining until the end of the experiment. From *t* = 32 min until the end of the experiment a peak at 3.8 Å was observed as well.

(vi) T_p = 0°C (Fig. 3E): A sharp diffraction peak with *d*-spacing value of 54.6 Å, observed from 5 min after start of the cooling, shifted to 53.8 Å within 3 min. At *t* = 11 min the peak split up into two co-existing maxima with *d*-spacing values of 52.6 and 55.0 Å. The intensity of the diffraction peak at 55.0 Å decreased slowly until this peak disappeared at *t* = 54 min. The other one broadened and remained until the end of the experiment. In XRPD a diffraction peak at ~4.1 Å was

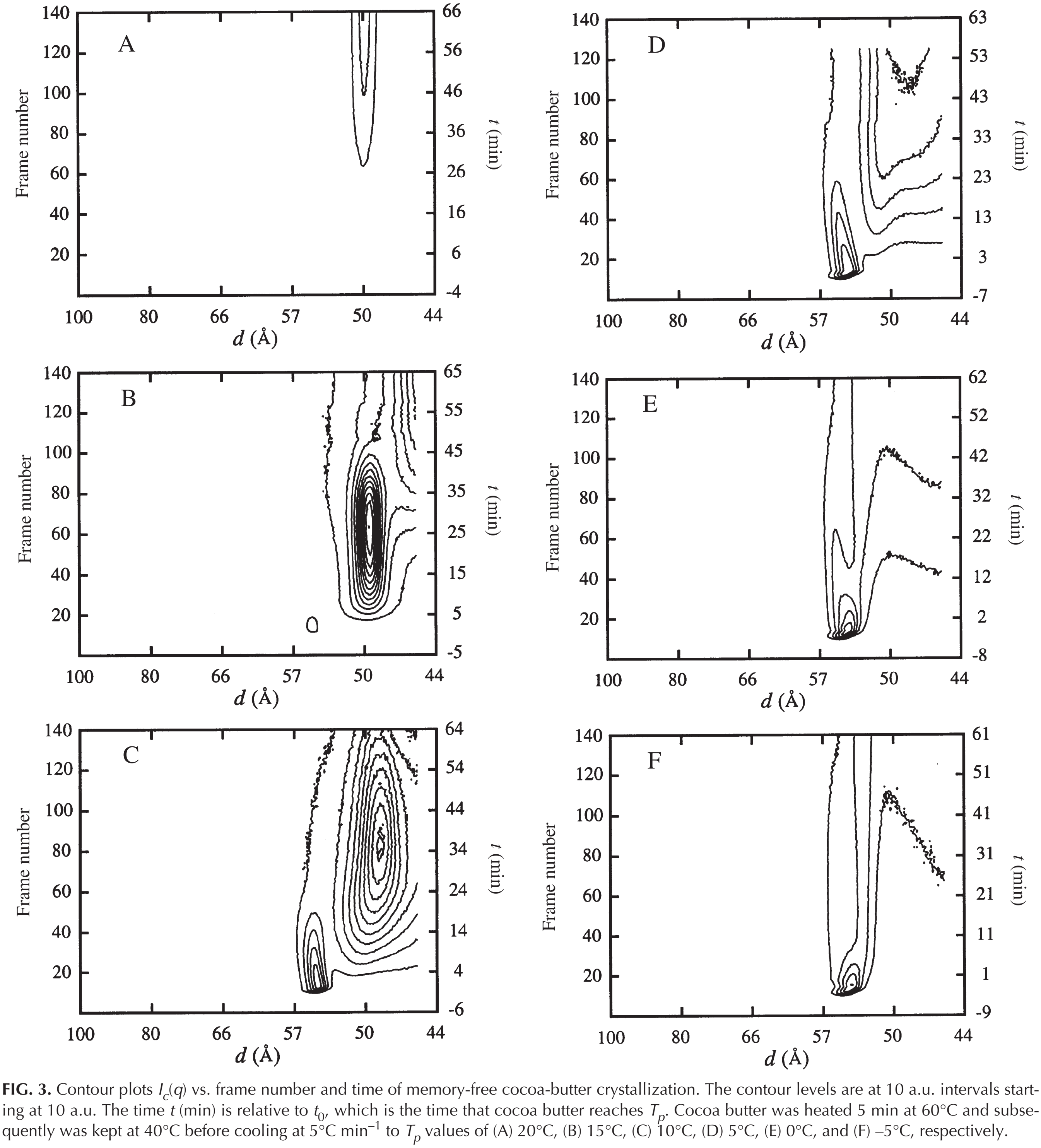

FIG. 3. Contour plots $I_c(q)$ vs. frame number and time of memory-free cocoa-butter crystallization. The contour levels are at 10 a.u. intervals starting at 10 a.u. The time t (min) is relative to t_0, which is the time that cocoa butter reaches T_p. Cocoa butter was heated 5 min at 60°C and subsequently was kept at 40°C before cooling at 5°C min^{-1} to T_p values of (A) 20°C, (B) 15°C, (C) 10°C, (D) 5°C, (E) 0°C, and (F) –5°C, respectively.

observed in the same period as the diffraction peak at 54.6 Å. From $t = 0$ min until the end of the experiment diffraction peaks were also observed at 4.2 and 3.8 Å. At $T_p = -5$°C (Fig. 3F) and $T_p = -10$°C the observed (changes in the) diffraction patterns are quite similar to those at $T_p = 0$°C, except for the peak at $d = 55.6$ Å that was still clearly present at $T_p = -5$°C, but that almost disappeared at $T_p = -10$°C.

Melting and recrystallization experiments near the MEP. For the recrystallization experiments, the time t (min) is relative to t_0, which is the time that the cocoa butter reaches T_{cryst}. Before the start of each experiment, a diffraction peak with a d-spacing value of 64.8 Å was observed. In Figure 4 relevant contour plots of $I_c(q)$ vs. frame number and time are shown for most recrystallization experiments.

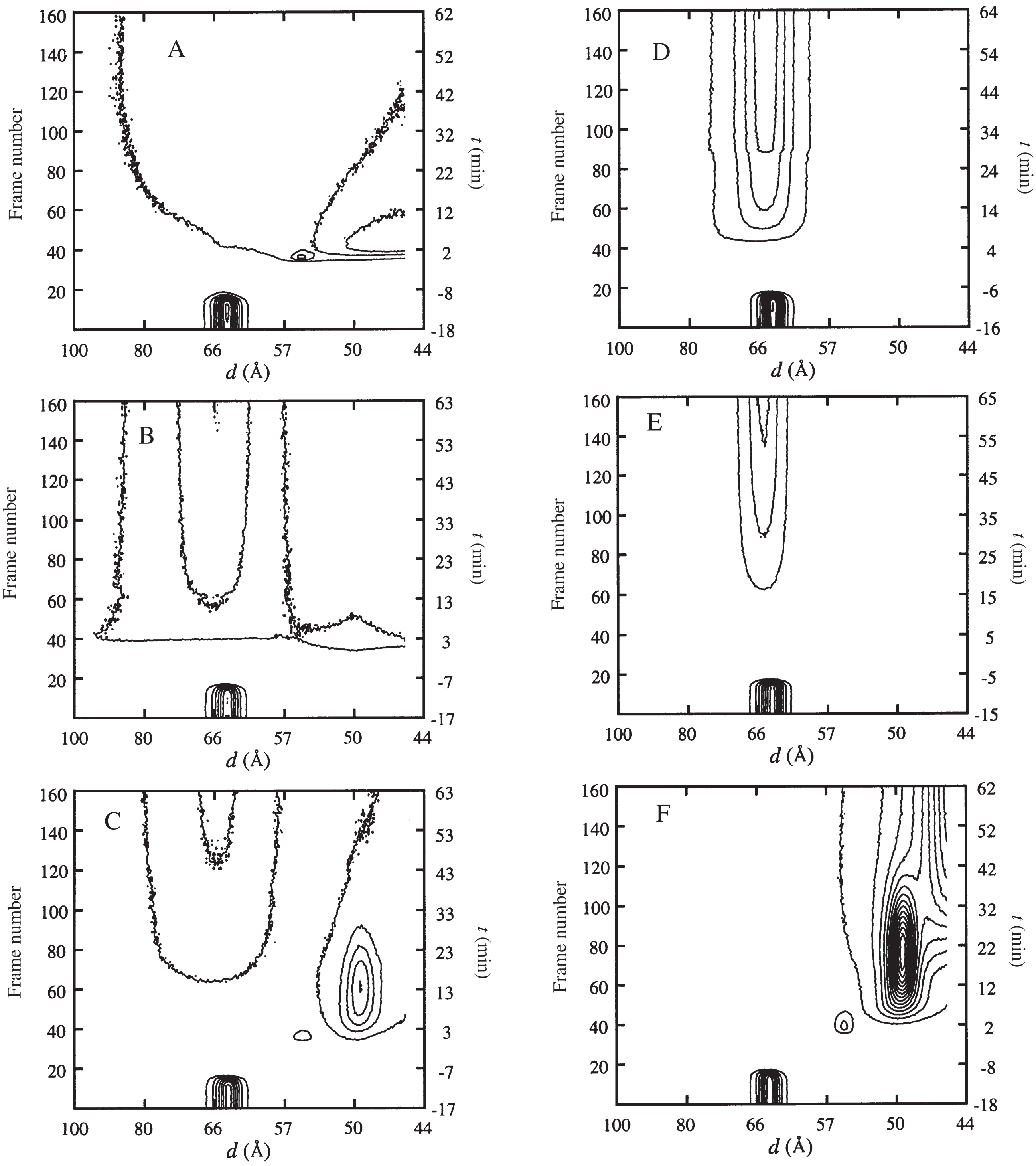

FIG. 4. Contour plots $I_c(q)$ vs. frame number and time of cocoa-butter recrystallization. The contour levels are at 10 a.u. intervals starting at 10 a.u. The time t (min) is relative to t_0, which is the time that cocoa butter reaches T_{cryst}. Cocoa butter was heated 5 min at T_{max} before cooling at 5°C min^{-1} to T_{cryst}. (A) T_{max} = 34.5°C and T_{cryst} = 10°C; (B) T_{max} = 35°C and T_{cryst} = 15°C; (C) T_{max} = 35.5°C and T_{cryst} = 15°C; (D) T_{max} = 36°C and T_{cryst} = 20°C; (E) T_{max} = 36°C and T_{cryst} = 25°C; (F) T_{max} = 37°C and T_{cryst} = 15°C.

(vii) T_{max} = 34°C and T_{cryst} = 15°C: The intensity of the peak at 64.8 Å decreased but remained observable during heating at T_{max}. Upon subsequent cooling to T_{cryst}, this peak became more intense again after t = 1 min.

(viii) T_{max} = 34.5°C and T_{cryst} = 15°C: Also during heating at this T_{max} value, the peak with a d-spacing value of 64.8 Å did not fully disappear. This peak became more intense again 3 min after cooling to 15°C.

(ix) T_{max} = 34.5°C and T_{cryst} = 10°C (Fig. 4A): The diffraction peak at 64.8 Å became slightly more intense between *t* = –2 and 1 min and remained until the end of the experiment, while from *t* = –2 min a diffraction peak at 49.2 Å was observed. This peak shifted and broadened within 3 min to a *d*-spacing value of 45.5 Å, while its intensity slowly decreased until the end of the experiment. Between *t* = 0 and 4 min a diffraction peak with *d*-spacing value of 54.7 Å also was observed.

(x) T_{max} = 35°C and T_{cryst} = 15°C (Fig. 4B): During heating at $T_{max} \geq$ 35.0°C no diffraction peaks were observed at all; even the peak at 64.8 Å fully disappeared at these temperatures. After heating at this T_{max} value and subsequent cooling to 15.0°C a diffraction peak at 49.4 Å was observed from *t* = –1 min. This peak disappeared at *t* = 28 min. A very broad and vague diffraction peak with a *d*-spacing value of 66.0 Å was observed from *t* = 4 min and remained until the end of the experiment.

(xi) T_{max} = 35.5°C and T_{cryst} = 15°C (Fig. 4C): Heating cocoa butter at this T_{max} value and afterward cooling to 15.0°C resulted in a diffraction peak observed at 49.2 Å between *t* = 0 and 51 min, a peak observed at 54.7 Å between *t* = 0 and 3 min, and a broad peak observed at 66.0 Å from *t* = 16 min until the end of the experiment.

(xii) T_{max} = 36°C and T_{cryst} = 20°C (Fig. 4D): A vague diffraction peak at 49.8 Å was observed from *t* = 1 min. Although the intensity of this peak was decreasing, it remained until the end of the experiment. A diffraction peak at 65.7 Å, which was observed from *t* = 5 min, remained until the end of the experiment as well, but the intensity of this peak was increasing.

(xiii) T_{max} = 36°C and T_{cryst} = 25°C (Fig. 4E): Only one peak was observed in the recrystallization experiment with T_{max} = 36.0°C and T_{cryst} = 25.0°C. This peak with a *d*-spacing value of 65.5 Å was observed from *t* = 6 min until the end of the experiment.

(xiv) T_{max} = 37°C and T_{cryst} = 15°C (Fig. 4F). Heating at 37.0°C and subsequent cooling to 15.0°C revealed an intense diffraction peak observed at a *d*-spacing value of 54.6 Å between *t* = 0 and 8 min, a slowly increasing peak with a *d*-spacing value of 49.2 Å observable between *t* = 1 and 46 min, and a peak with a *d*-spacing value of 45.2 Å observed from *t* = 15 min until the end of the experiment.

DISCUSSION

Crystallization from the melt. The starting phase of the Bahia, Brazil, cocoa butter used in our experiments was β(VI) (8) with long spacings at 64.8 and 47 Å. The phase-transition scheme (6) and XRPD patterns (compare with Fig. 1) support the attribution of the other diffraction peaks, which were observed in the crystallization experiments, to various cocoa-butter phases.

The diffraction peak at 53.8 Å observed for T_p values of 5.0, 0.0, –5.0, and –10.0°C is attributed to the γ phase of cocoa butter. This phase is formed at low crystallization temperatures (T_p < 5.0°C) and shows diffraction peaks at 3.8 and 4.2 Å, as is known from mechanically static isothermal phase-transition studies (6,19). Remarkably, the diffraction peak at 53.8 Å split up into two co-existing peaks with *d*-spacing values of ~52.7 and ~55.6 Å for T_p values of 0.0, –5.0, and –10.0°C (Figs. 3D–F), whereas under the same isothermal conditions no changes were observed at the XRPD patterns. Interestingly, in a quenching experiment (from 80 to –10°C at about 100°C/s) carried out with cocoa butter from the Ivory Coast, Loisel *et al.* (4) observed diffraction peaks at *T* = –10°C at 52.6, 3.77, 4.19, and 4.22 Å but neither a peak at 53.8 Å nor a peak splitting as described above. This may be due to differences in the cocoa-butter composition or to the different temperature gradient used. These observations can be explained by assuming lipid segregation to occur in the γ phase (4). Triacylglycerol (TAG) molecules with similar chain length and conformational likeness aggregate to crystalline compounds, but without changing the lateral γ-chain packing of the TAG molecules. Lipid segregation has also been observed by others using different techniques (20,21).

The α phase was crystallized at T_p values of 15.0, 10.0, and 5.0°C, having diffraction peaks at *d*-spacing values of 49.6, 48.7, and 51.0 Å, respectively (Figs. 3B–D). Therefore, it seems that the exact long *d*-spacing values of the diffraction peaks of the α phase depend on the crystallization temperature. In contrast, the diffraction peak at short *d*-spacing of the α phase has a constant value of 4.2 Å. These observations may be explained by considering cocoa butter to be a conglomerate of crystallites (6). The TAG distribution of the crystallites formed at the various T_{cryst} values is expected to be slightly different and may result in slightly different long *d*-spacing values, but similar lateral chain packing.

The broad diffraction peak at 45 Å, its shoulder at 46 Å, and the broad range of peaks 45–50 Å that were observed at the T_p values of 15.0, 10.0, and 5.0°C, respectively (Figs. 3B–D), are attributed to the β′ phase, because under the same conditions diffraction peaks at 3.8 and ~4.2 Å were observed. Similar to earlier observations in the short *d*-spacing region (6), the diffraction peaks of β′ were observed at slightly different *d*-spacing values for cocoa butter crystallizing at different temperatures. Therefore, these peaks support the existence of a β′ phase range.

In line with the observed rapid-starting recrystallization of cocoa butter and the proposed existence of high-melting SOS-rich crystals in this Bahia, Brazil, cocoa butter (discussed below; 8), some of the diffraction peaks possibly may originate from an SOS-dominated crystalline lattice. In this respect it is of interest that, after cooling fluid SOS from 80°C to a room temperature of 12°C, Sato *et al.* (22) observed, using conventional XRPD equipment, diffraction maxima of the instable SOS α phase at 48.3 and 4.21 Å. On the other hand, after quenching highly pure liquid SOS from 50°C to 10 ± 1°C, Ueno *et al.* (23) observed in a synchrotron experiment long *d*-spacing diffraction maxima at 53 and 44 Å and short *d*-spacing maxima at 4.1 and 4.3 Å.

Cooling of totally molten cocoa butter at $T_p \leq$ 15°C with a cooling rate of 5°C min^{-1} resulted in all cases in the initial formation of crystalline material with diffraction peaks at ~54.7 and 4.1 Å (Figs. 3B–F). Although one may be tempted to interpret this to be the α phase, it is not likely to be so because the α phase has a diffraction peak at a short *d*-spacing value of 4.2 Å and a peak at a long *d*-spacing value of about 50 Å (the exact *d*-spacing value depends on T_p). Upon cooling of totally molten cocoa butter, nuclei apparently are formed with a chain-length packing of ~54.7 Å and a lateral chain-packing similar to the packing of the α phase. Since these nuclei transform rapidly to the well-known cocoa-butter phases, they are present only for a short time. Although real-time X-ray diffraction was applied in our earlier studies of cocoa-butter crystallization (6), formation of such nuclei was never observed before. The cooling rate and composition of the cocoa butter are the only two parameters that differ from the previous experiments. Since both will influence the formation of initial seed crystals, further research is necessary to determine what happens during primary nucleation of cocoa butter.

Melting and recrystallization experiments near the MEP. It is likely that, in the recrystallization experiments with low T_{max} values (34.0 and 34.5°C), cocoa-butter crystals of the original β(VI) phase of cocoa butter remained present during heating to T_{max} (Fig. 4A), because the diffraction peak at 64.8 Å did not fully disappear, and upon subsequent cooling to 10 or 15°C the cocoa butter recrystallized rapidly in its original β(VI) phase. On the other hand, when heating cocoa butter to $35.0 \leq T_{max} < 37.0$°C, the diffraction peaks of the β(VI) phase disappeared completely (Figs. 4B–E). Upon subsequent cooling to $15 \leq T_{cryst} \leq 25$°C, a diffraction peak at ~66 Å was observed. This peak, which is significantly different from the 64.8 Å peak of the β(VI) phase, is attributed to the β(V) phase. Moreover, recrystallization in the β(V) phase started a few minutes later compared to recrystallization of the β(VI) phase. This confirms earlier observations that the phase in which cocoa butter recrystallizes depends on the maximum temperature before recrystallization (8). Since the β phase was not observed in the memory-free cocoa-butter crystallization experiments (see *Crystallization from the melt)*, it is concluded that recrystallization *via* the memory effect largely accelerates the formation of β-cocoa butter.

In some T_{max}–T_{cryst} experiments the α phase also crystallized, but only for $T_{cryst} \leq 15$°C crystallization of the α phase started almost simultaneously with the formation of crystalline material *via* primary nucleation (diffraction peak at ~54.8 Å; see *Crystallization from the melt* section). Compared to memory-free cocoa-butter crystallization at $T_p \leq 15$°C, the formation of the α phase started at almost the same time. Apparently, the cocoa-butter crystalline material still present has to compete with the primary-nucleation process at lower T_{max} values. Only for $T_{cryst} = 10$°C was the diffraction peak at 45.5 Å of the β′ phase observed.

During heating at $T_{max} = 37$°C, all diffraction peaks disappeared (Fig. 4F). Subsequent cooling to 15°C resulted in the formation of an α and β′ phase *via* primary nucleation. In this experiment ($t < 62$ min) no β formation was observed. This T_{max}–T_{cryst} experiment is comparable to crystallization of memory-free cocoa butter at $T_p = 15$°C. Therefore, it is concluded that not enough crystalline material remained present for recrystallization of cocoa butter in the β phase.

Nature of cocoa butter's memory. The results obtained from diffraction at long *d*-spacing values confirm that the recrystallization behavior of cocoa butter depends on the maximum temperature before recrystallization (T_{max}). Since cocoa butter is considered to be a conglomerate of crystallites having individual triglyceride compositions, it is expected that the amount of remaining crystallites decreases and their composition changes with increasing T_{max} values. Our results provide information about the remaining crystallites at various T_{max} values, which are responsible for initiation of the recrystallization process.

For T_{max} values a few degrees Celsius above the MEP, the intensity of the peak at 64.8 Å decreased only during heating, and upon subsequent cooling the intensity of this peak increased rapidly (Fig. 4A). This peak at 64.8 Å, corresponding to the β(VI) phase of cocoa butter, originates from a crystalline lattice in which the TAG molecules are crystallized in a triple chain-length packing. SOS molecules crystallized in the β_2 phase adopt a similar packing with a long-spacing reflection of 65 Å. Therefore, these results are in agreement with our earlier interpretation that rapid-starting recrystallization of the β(VI) phase of cocoa butter is initiated by high-melting SOS-rich crystalline material (8). The crystalline lattice of the remaining seed crystals is also similar to the β(VI) phase of cocoa butter, and therefore it is assumed that the packing of the β(VI) phase is dominated by the triple chain-length packing of SOS-rich crystallites.

During heating of the cocoa butter at $35.0 \leq T_{max} < 37.0$°C, no significant diffraction peaks were observed in our experiments. It is concluded that almost all SOS-rich cocoa-butter crystallites with triple chain-length packing were molten. Because the cocoa butter still has good recrystallization properties, crystalline seed material must be present. Since no diffraction peak at $d = 64.8$ Å was observed in our experiments, it is not likely that the slow-starting recrystallization is initiated by seed crystals with the same compositional distribution as those present at $T_{max} < 35.0$°C. Upon cooling the cocoa butter recrystallizes in the β(V) phase, which does have triple chain-length packing. However, this triple chain-length packing seems to be different from the triple chain-length packing of the β(VI) phase or SOS-dominated crystalline lattice, because the diffraction peak at the long *d*-spacing value of the β(V) phase (66 Å) is significantly different from the 64.8 Å peak of the β(VI) phase. Furthermore, differences within the 3–6 Å region of the XRPD pattern also were observed (8).

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