Real-Time X-Ray Powder Diffraction Investigations on Cocoa Butter. III. Direct β-Crystallization of Cocoa Butter: Occurrence of a Memory Effect

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ABSTRACT: Direct β-crystallization of different samples of cocoa butter has been investigated. The influence of the thermal history of cocoa butter on its phase behavior is defined as a memory effect. The chemical composition of cocoa butter has been related to the occurrence of the short-term β-memory effect *via* statistical analysis of the results. We explain how this effect can be attributed mainly to stearic acid and its related triacylglycerols. The total phase behavior of cocoa butter is discussed on the basis of the results obtained from the series of three papers of which this is the last.
JAOCS 73, 1225–1230 (1996).

KEY WORDS: Cocoa butter, cocoa butter composition, memory effect, phase transitions, polymorphism, X-ray powder diffraction.

In industrial confectionary processes, tempering is used to improve the quality of chocolate products. The tempering process involves a complicated scheme of heating, cooling, stirring, and scrapping the liquid mass to promote crystallization of a particular polymorphic state, the β-phase. It has been pointed out in the literature that nuclei of triacylglycerols (TAG), either present in molten cocoa butter or added as seeding kernels, may influence the solidification speed of cocoa butter. Hernqvist and Larsson (1) state that a fat should be heated at least 30°C above its melting point to avoid any influence of structural "memories" on crystallization. They attribute these memories to partly ordered laminar structures in the fluid that originate from the solid before melting. In accordance with this opinion, it has been common practice to heat cocoa butter to rather high temperatures to melt all crystallites (2,3). However, seeding experiments of Hachiya *et al.* (4) showed that the seeding effect of nuclei on dynamic crystallization depends strongly on the similarity in polymorphic behavior between seeding crystallites and liquid mass (cocoa butter). The seeding effectiveness of high-melting nuclei of trisaturated TAG turned out to be much less than that of SOS, BOB, or β-cocoa butter. Therefore, it can be expected that, to eliminate memory effects, the temperature has to be raised only slightly above the melting point.

Because the β-phase cannot be formed directly from memory-free liquid cocoa butter, and the β′-phase crystallizes from the melt at 25°C (5), direct β-crystallization from the melt at 25°C must be attributed to a β-memory effect. Therefore, the conditions under which the β-phase may (re)crystallize from the melt have been investigated by a series of experiments. Two different (re)crystallization stages may occur, referred to as short-term and long-term memory effects, respectively. By means of a statistical analysis, the former has been related to the chemical composition of the cocoa butters. Finally, knowledge of the polymorphic behavior of cocoa butter, obtained with these experiments and those described in the previous papers (5,6), will be used for a discussion concerning phase characteristics, nomenclature, and the phase transition scheme of cocoa butter.

MATERIALS AND METHODS

Samples, sample preparation, and experimental set-up. Samples were taken from the same stocks of twelve cocoa butters, provided by Cacao De Zaan (Koog aan de Zaan, The Netherlands) and used earlier for determination of melting characteristics (6). The stocks of cocoa butter were completely in the β-phase due to storage for more than 12 y at temperatures just above 0°C. Samples were prepared by pressing cocoa butter into the sample holder, resulting in a sample size of 10 × 15 × 1 mm, with a flat surface.

All diffraction experiments were conducted with a real-time X-ray powder diffractometer, which allows the recording of the d-spacings from 3.0 to 6.1 Å simultaneously. Temperature control keeps the temperature within 0.1°C of preset values. The experimental settings are identical to those described in Part I (5). The diffraction characteristics of the samples used in the experiments are independent of the way of filling the sample holder and egalizing the surface, as ascertained by using various preparation techniques.

Fast recrystallization experiments. To establish any fast β-recrystallization from melted cocoa butter, the following

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protocol was devised. A cocoa butter sample in the β-phase was heated in 10 min from 25.0°C to a preset temperature T_{max} and was subsequently kept isothermally at that temperature for 10 min. Then, the sample was cooled in 10 min to 25.0°C and kept isothermally for 45 min. [Additional experiments carried out with the protocol time parameter (10 min) reset to a different value in the range 1–20 min did not lead to significantly different results.] The total process has been divided into equally spaced intervals of 5 min, and a diffraction pattern was recorded during the first 30 s of each interval. For each of the 12 cocoa butters, this experimental protocol was carried out with T_{max} = 30.0–39.0°C in steps of 0.5°C. Recrystallization to the β-phase that takes place within 45 min is referred to as a short-term (β-)memory effect. The β-memory point temperature (β-MPT) of a cocoa butter is defined as the lowest T_{max} that is not followed by β-solidification within 45 min.

Experiments on slow recrystallization. To establish whether β-memory remains present after heating cocoa butter to T_{max} or higher temperatures, some preliminary experiments were carried out for the cocoa butters from Equatorial Guinea and Malaysia. During the first 75 min, the protocol was identical to that of the fast recrystallization experiments described above, with T_{max} = 33.0–39.0°C in steps of 0.5°C. After 75 min, however, monitoring of the (re)crystallization process continued with a diffraction pattern being recorded during 30 s at the start of every 5 min. This process lasted until the sample solidified, either into the β-phase or into the β′-phase. The moment at which the first sign of β-crystallization can be observed was taken as the starting point of the crystallization while its end point was arrived at when the increase of integrated diffraction intensity between two subsequent patterns became less than 5%. Recrystallization to the β-phase that takes place after 45 min is referred to as a long-term (β)-memory effect.

Relationship between β-MPT and composition. A statistical analysis of the cocoa butter composition and β-MPT reveals the capability of cocoa butter components to be used for the prediction of β-MPT. Specific β-MPT values can be calculated as a function of the concentration of an individual component or a binary combination of two components. The quality of this calculation is expressed by the following parameters (6): *QP* (quality of prediction), which is an expression of the differences between calculated and observed values. A low *QP* corresponds with a low average error of prediction; *RPR* (relative predictive ratio), which represents the ratio of *QP* of the average β-MPT as predictor and *QP* of the corresponding component. A high *RPR* corresponds with a (relative) good prediction performance; *PrEx* (prediction extreme error), which expresses the largest absolute difference between observed and calculated β-MPT.

Melting characteristics of β-phase samples with different thermal histories. In our experiments four β-phase samples with different thermal histories have been observed: (i) The initial β-phase from the bulk, which has been stored at temperatures below 5°C for more than 12 y; (ii) the β-phase obtained *via* transformation of β′ in 2 d at 25°C after being heated to 60°C; (iii) the β-phase solidified from the melt as the result of a short-term memory effect; and (iv) the β-phase solidified from the melt after a long-term memory effect. The melting points of these are determined as described in Part II (6).

RESULTS

Short-term memory effect and β-MPT. For all twelve cocoa butter samples, the β-MPT values were established and are listed in Table 1. The β-memory effect is illustrated in Figure 1 with cocoa butter from Equatorial Guinea; it has a T_{max} of 35.0°C. It is clear that the sample is fluid at T_{max}, but the quick formation of β-cocoa butter is evidently the result of a short-term memory effect. On close observation, the diffraction intensities after resolidification appear to be increased when compared with the intensities before heating. However, an X-ray diffraction measurement, taken after renewed preparation of the cocoa butter sample, showed the increase in intensity to be a result of a change in preferred orientation of the crystallites.

Relationship between β-MPT and composition. The short-term β-memory effect clearly differs for cocoa butters from different origins: β-MPT varies from 32.5°C for the Brazilian cocoa butter to 38.0°C for the Malaysian cocoa butter. The capability of cocoa butter components to predict β-MPT values has been analyzed. The most interesting results are listed in Table 2. In Figure 2, β-MPT is shown as a function of the stearic acid content minus 2.5 times the arachidic acid content. The straight line is the least-squares result based on the complete data set. The dotted line is the least-squares result based on the data set from which data from Malaysia and Brazil, with the highest and the lowest β-MPT, respectively, are left out. It is clear that, even in this "worst" case, β-MPT of the Malaysian and Brazilian butters are predicted quite well.

All parameter combinations, best capable of predicting β-MPT, have either stearic acid or the SOS content as one of the two components. Obviously, these are not independent parameters, as can be seen from the marginal increase of *RPR* when they are combined (*RPR* increases only from 2.3 to 2.4) and in view of the similarity in the parameter combinations with the highest *RPR* values. Because POS and POP together with SOS are the three major TAG of cocoa butter (total ± 73%), and because they are structurally closely related, the

TABLE 1
β-Memory Point Temperatures (β-MPT)

Country of origin	β-MPT °C ± 0.25°	Country of origin	β-MPT °C ± 0.25°
1 Peru	34.5	7 Ghana	35.5
2 Bahia, Brazil	32.5	8 Lome, Togo	35.5
3 Equatorial Guinea	36.0	9 Lagos, Nigeria	35.5
4 Sierra Leone	35.5	10 Cameroon	34.5
5 Liberia	35.0	11 Congo	35.5
6 Ivory Coast	36.0	12 Malaysia	38.0

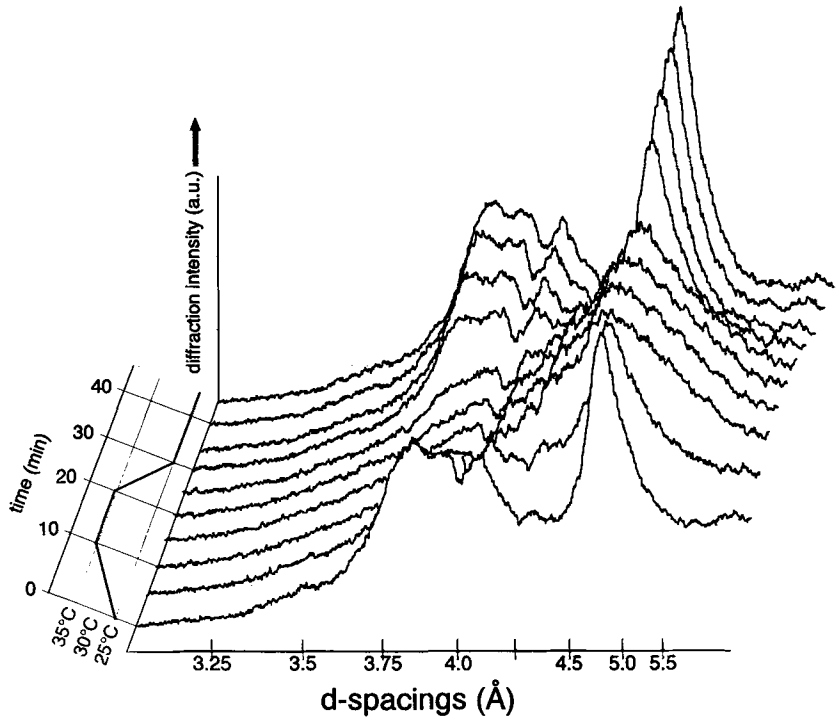

FIG. 1. β-Cocoa butter from Equatorial Guinea is melted by heating from 25.0 to 35.5°C in 10 min, kept for 10 min at 35.5°C, and cooled to 25.0°C in 10 min, at which temperature it is maintained.

results of POS, POP and palmitic acid are also included in Table 2.

Long-term memory effect and comparison of β-phases. The results of the slow recrystallization experiments are summarized in Table 3. The crystallization speed of the β-phase decreased dramatically when cocoa butter from Equatorial Guinea was heated to its β-MPT (36.0°C) or higher. It took several hours before the first sign of β-crystallization could be observed, and solidification required several hours to complete. Nevertheless, the β-phase still developed directly from the melt, as the result of a long-term memory effect. Malaysian cocoa butter kept a short-term memory effect to much higher temperatures, and it disappeared only above 37.5°C.

TABLE 2
β-MPT Predicting Parameter Combinations[a,b]

Main single components				
x_1		*QP*	*RPR*	*PrEx*
Average β-MPT (=35.3°C)		1.3	1	3.2
POP		2.0	0.7	6.1
POS		1.2	1.1	3.9
SOS		0.7	1.8	2.1
$C_{16:0}$		2.0	0.7	5.6
$C_{18:0}$		0.6	2.3	1.7
Best β-memory point prediction combinations				
x_1	Ratio	x_2	*QP*	*RPR*	*PrEx*
SOS	−1.05	SOL	0.52	2.5	1.6
SOS	0.95	PLS	0.51	2.6	1.5
SOS	2.5	PLP	0.49	2.7	1.4
$C_{18:0}$	−0.43	SOL	0.51	2.6	1.5
$C_{18:0}$	0.9	PLP	0.50	2.6	1.4
$C_{18:0}$	−1.25	SOA	0.49	2.7	1.3
$C_{18:0}$	−2.22	POA	0.47	2.8	1.1
$C_{18:0}$	−2.5	$C_{20:0}$	0.43	3.1	0.9

[a]Cocoa butter components x_1,x_2 used to predict β-MPT. β-MPT predicted by single components as: β-MPT = $b + a\ x_1$. β-MPT predicted by combined components as: β-MPT = $b + a\ (x_1 + \text{ratio} \cdot x_2)$. a,b are calculated by means of a least-squares fit.
[b]Abbreviations: β-MPT, β-memory point temperatures; *QP*, quality of prediction; *RPR*, relative predictive ratio; *PrEx*, predictive extreme error.

TABLE 3
Long-Term β-memory Effect[a]

	Resolidification time			
	Eq. Guinea		Malaysia	
T_{max}, °C	Start	End	Start	End
33.0	0 min	5 min	0 min	5 min
34.0	0 min	5 min	0 min	5 min
35.0	10 min	20 min	0 min	10 min
35.5	20 min	35 min		
36.0	4 h	8 h	5 min	20 min
36.5	5 h	8 h		
37.0	6 h	9 h	5 min	20 min
37.5			25 min	45 min
38.0	7 h	11 h		
38.5			1.5 h	4.5 h
39.0	x		x	

[a]Time required for resolidification into β-phase. Start and end of β-crystallization are measured from the moment the sample is brought to 25.0°C after being heated to T_{max}; x stands for formation of β′.

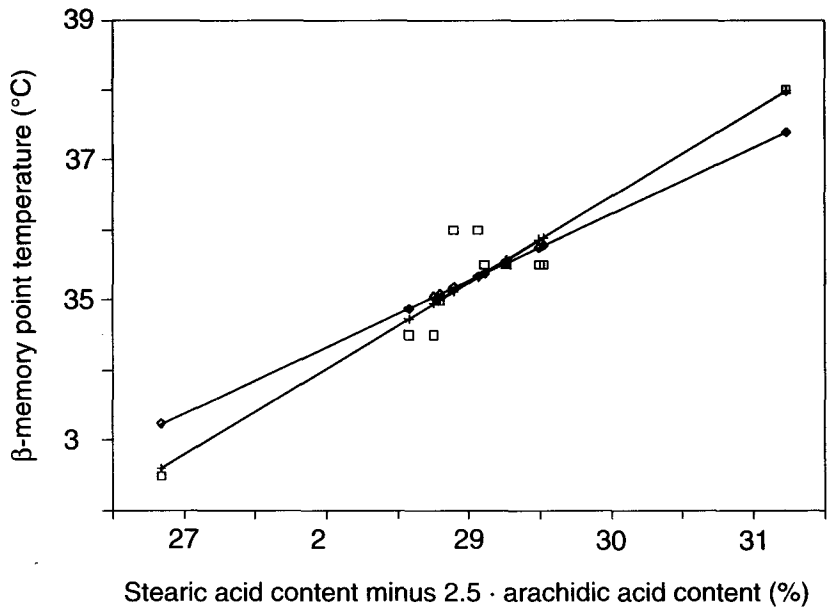

FIG. 2. β-Memory point temperature (β-MPT) as function of the stearic acid content (–2.5 times arachidic acid content). The straight line is the least-squares result based upon the complete data set; the dotted line is the least-squares result based upon the data set from which the highest and lowest β-MPT have been eliminated. □ = observed β-MPT; + = β-MPT calculated from the complete data set; ◇ = β-MPT calculated from the reduced data set.

Even though the short-term memory effect is different for both cocoa butters, the long-term memory effect disappears in both cases when the cocoa butter is heated above 39.0°C. The melting characteristics of the β-phase, obtained *via* a long-term memory effect, are, within experimental error, identical to those of the β-phases obtained *via* a short-term memory effect, *via* transformation of the β′-phase at 25.0°C, or after long storage at temperatures below 5.0°C.

Explanation of memory effect. The results listed in Table 2 indicate SOS to be the most important TAG in relation to the memory effect. According to Sato *et al.*(7), SOS has melting points of 43°C for β_1 and 41.2°C for β_2. Probably, β-crystallites with a rather high SOS content stay present in the melt at temperatures below 39.0°C. However, in view of the absence of a β-memory effect when cocoa butter was heated up to or above 39°C, it is not likely that pure SOS crystallites are responsible for this effect. The results of the seeding experiments of Hachiya *et al.* (4) for dynamic crystallization support such a conclusion. Indeed, they found that seeding, even at 0.01 weight%, increased the crystallization rate remarkably. Such an amount of crystallites cannot be observed in X-ray powder diffraction measurements.

The results of the above experiments are in contradiction with the results of Chapman *et al.* (8). They reported that, when β-phase V is present, in partially liquid cocoa butter, β′ (IV) can be formed upon cooling at 0.5°/min. The results of our experiments lead to a different conclusion: even a very small percentage of β-components in cocoa butter is sufficient to result in a complete β-solidification of the cocoa butter sample.

DISCUSSION

In this series of papers, a number of results have been presented, making possible a renewed discussion on the polymorphic behavior of cocoa butter and the nature of the polymorphic states. In our experiments, only four significantly different solid states of cocoa butter have been observed, each characterized by an essentially different combination of melting trajectory and short-spacing X-ray pattern: (i) γ: Two strong diffraction peaks at 3.7 and 4.2 Å, obtained only by means of cooling liquid cocoa butter over 2°C/min and with a melting trajectory from –5 to 5°C. In all observed cases, γ was accompanied by some α. (ii) α: One strong diffraction peak at 4.2 Å, obtained by cooling liquid cocoa butter at more than 1°C/min, or after transformation of the solid γ-phase. The melting trajectory of α is from 17 to 22°C. (iii) β′: Strong diffraction peaks at 4.2 Å and near 4.3 Å and a medium peak at 3.7 Å. This phase is obtained by cooling liquid cocoa butter at 1°/min or less, or after transformation of the solid α-phase. The melting trajectory is from 20 to 27°C. (iv) β: One strong diffraction peak at 4.6 Å and a group of diffraction peaks between 3.5 and 4.0 Å. It is obtained only *via* a solid state transformation from β′ or seeding with β. It has a melting trajectory from 29 to 34 °C.

Although many different β′-patterns as well as a large variety of melting points have been reported in the literature, our experimental results (long melting trajectory but constant diffraction pattern) point towards only a single β′-phase in the cocoa butter phase scheme. Indeed, the β′-patterns reported can be easily classified together according to the criteria given

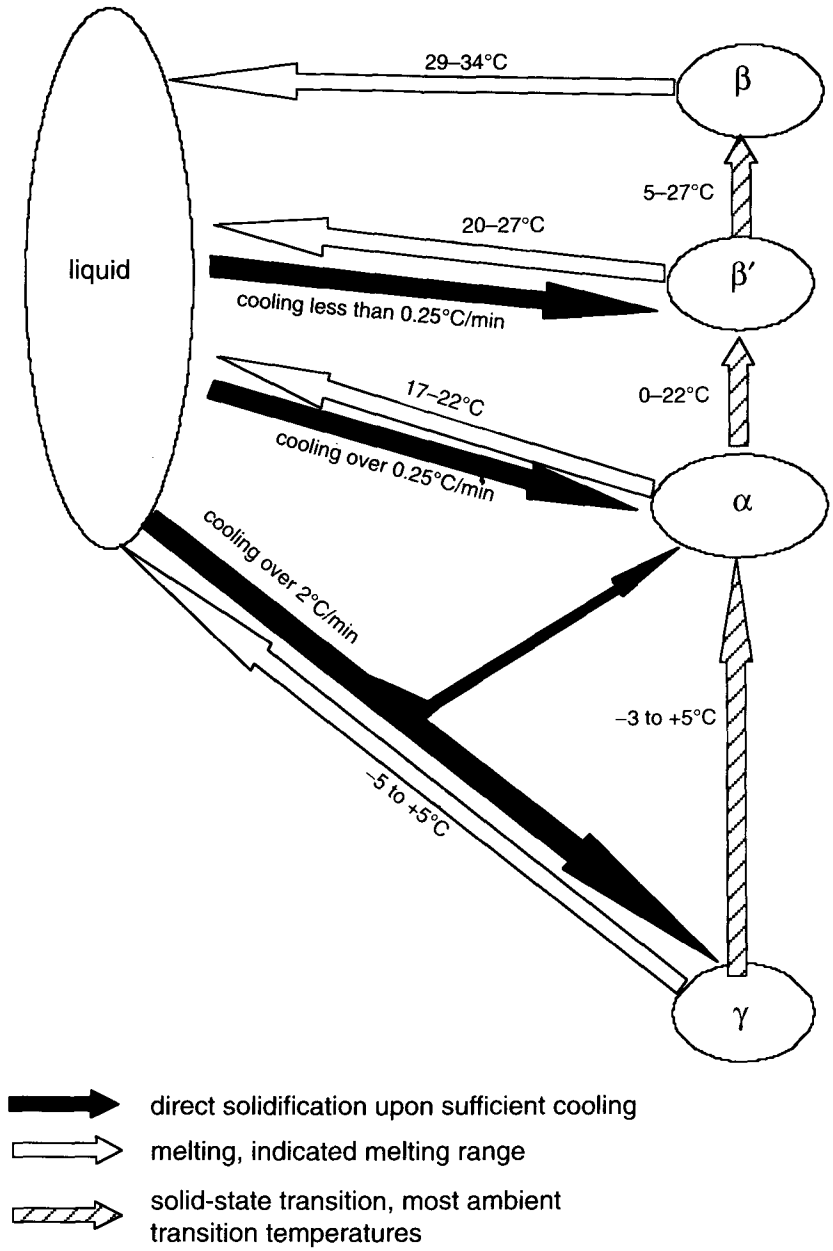

FIG. 3. Phase transition scheme for cocoa butter. Phase transitions are possible between the liquid phase and the four solid phases γ, α, β′, and β. Black arrows indicate direct solidification from liquid to a particular phase. White arrows indicate melting. Gray arrows indicate solid-to-solid phase transitions, with or without liquid intermediate state. Each arrow is accompanied by the relevant temperature range or cooling rate that applies to a cocoa butter of an average chemical composition.

above. This point of view is supported by the results of Schlichter Aronhime *et al.* (9). In the literature, two different β-cocoa butter states (V and VI) have been reported frequently, with clearly different melting points but almost identical diffraction patterns, at least in the short-spacing diffraction range (8,10). The four β-samples with very different thermal histories are of the same type, as can be concluded from the identical melting characteristics. Compared with the results of Wille and Lutton (10), our β-phase diffraction patterns look most like those of VI, while the melting points correspond better with those of V. The melting points reported for VI by Chapman *et al.* (8), however, do correspond with our results. The observation of the wide range of melting values for different cocoa butters and the small differences between the diffraction patterns lead us to the conclusion that phases V and VI should be regarded as two subphases of β. A

classification of V and VI as independent phases, instead of subphases only, cannot be made without additional evidence, e.g., the observation that in one of the two phases, oleic acid at the central position is stretched in the crystal packing, while it is bent in the other phase (11). The observed temperature-dependent phase behavior of cocoa butter has been summarized in Figure 3.

For each of the solid states, it has been observed that, during the melting process (a trajectory of 5 to 10°C or more), only the peak intensities change in the diffraction pattern. The peak widths, peak positions and intensity ratios, however, do not change significantly. This leads to the following two important conclusions: (i) the average crystallite size does not change during melting; and (ii) the average short-spacing chain packing does not change during melting. These conclusions suggest that each polymorphic state can be considered to consist of a conglomerate of individual crystallites, each having a sharp melting point that depends on the individual TAG composition. The melting trajectory of the solid state is then the integral representation of all of these individual melting points. Other observations support this picture, namely, (i) the initial α-diffraction intensity is higher at a solidification temperature of 18 instead of 22°C; and (ii) the occurrence of a β-memory effect whose strength is clearly a function of the composition of the cocoa butter.

The above restrictions apply especially when the precise composition and thermal history are not known. To describe apparant variations in β′- or β-phases, a simple division in III and IV (for β') or V and VI (for β) is not sufficient because the number of possible variations is much greater. Ranking different phases by melting point is not appropriate because, as discussed above, the influence of the chemical composition is too large.

Phase identification. A possible solution to the problem of distinction between subphases might be found in the angle of tilt τ between the fatty acid hydrocarbon chain and the metyl-end group plane, if proof can be found that it varies with the phases. If it is possible to determine τ accurately, by combining the information obtained from the long spacings, the chainlength multiplicity and the chainlength, or, for mixtures, the average chainlength, then various subphases can be identified by a subscript indicating τ, for example: β_{xx}. Such a notation might improve the consistency between reported phases because it is independent of the number of phases obtained. It provides the possibility of comparing β- or β′-phases of cocoa butter of different composition with each other or with β- and β′-phases of a pure TAG, fatty acids or mixtures. We are aware of the fact that, in our experimental set-up, τ could not be established and that further research in this direction is necessary. Additional research is also necessary to complete the phase transition scheme in Figure 3.

ACKNOWLEDGMENT

The authors thank Dr. Frans van Kleef (Verkade) and Drs. Hans Kattenberg (Cacao de Zaan) for their continuing interest in the project and stimulating discussions. They also thank Cacao de Zaan for providing the cocoa butter samples.

REFERENCES

1. Hernqvist, L., and K. Larsson, On the Crystal Structure of the β′-Form of Triglycerides and Structural Changes at the Phase Transitions Liquid -> α -> β′ -> β, *Fette, Seife und Anstrichmit. 84*:349–354 (1982).
2. Feuge, R.O., W. Landmann, D. Mitcham, and N. Lovegren, Tempering Triglycerides by Mechanical Working, *J. Am. Oil Chem. Soc. 39*:310–313 (1962).
3. Vaeck, S.V., Die Polymorphie (Vielgestaltigkeit) Einiger Naturfette, *Int. Choc. Rev. 6*:100–113 (1951).
4. Hachiya, I., T. Koyano, and K. Sato, Seeding Effects on Solidification Behavior of Cocoa Butter and Dark Chocolate. I. Kinetics of Solidification. II. Physical Properties of Dark Chocolate, *J. Am. Oil Chem. Soc. 66*:1757–1770 (1989).
5. Van Malssen, K.F., R. Peschar, and H. Schenk, Real-Time X-Ray Powder Diffraction Investigations on Cocoa Butter. I. Temperature-Dependent Crystallization Behavior, *Ibid. 73*:1209–1215 (1996).
6. Van Malssen, K.F., R. Peschar, and H. Schenk, Real-Time X-Ray Powder Diffraction Investigations on Cocoa Butter. II. The Relationship Between Melting Behavior and Composition of β-Cocoa Butter, *Ibid. 73*:1217–1223 (1996).
7. Sato, K., T. Arishima, Z.H. Wang, K. Ojima, and H. Mori, Polymorphism of POP and SOS. I. Occurrence and Polymorphic Transformation, *Ibid. 66*:664–677 (1989).
8. Chapman, G.M., E.E. Akehurst, and W.B. Wright, Cocoa Butter and Confectionary Fats. Studies Using Programmed Temperature X-Ray Diffraction and Differential Scanning Calorimetry, *Ibid. 48*:824–830 (1971).
9. Schlichter Aronhime, J., S. Sarig, and N. Garti, Reconsideration of Polymorphic Transformations in Cocoa Butter Using the DSC, *Ibid. 65*:1140–1143 (1988).
10. Wille, R.L., and E.S. Lutton, Polymorphism of Cocoa Butter, *Ibid. 43*:491–496 (1966).
11. De Jong, S., T.C. van Soest, and M.A. van Schaick, Crystal Structures and Melting Points of Unsaturated Triacylglycerols in the β-Phase, *Ibid. 68*:371–378 (1991).

[Received October 2, 1995; accepted May 6, 1996]