Real-Time X-Ray Powder Diffraction Investigations on Cocoa Butter. II. The Relationship Between Melting Behavior and Composition of β-Cocoa Butter

Kees van Malssen*, René Peschar, and Henk Schenk

Amsterdam Institute for Molecular Sciences (AIMS), Laboratory for Crystallography, University of Amsterdam, 1018 WV Amsterdam, The Netherlands

ABSTRACT: The melting behavior of twelve different cocoa butter samples, in the β-phase, has been investigated with real-time X-ray powder diffraction. The melting trajectory of each sample is characterized by three temperature values: a starting point, a point of maximum melting, and a point of complete melting. These points are determined by an analysis of the subsequent X-ray diffraction patterns. A least-squares analysis has been developed which allows the observed melting points to be related to the composition of the cocoa butter. This analysis shows that the melting behavior of cocoa butter can be described as a function of certain binary combinations of its major components.

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KEY WORDS: Cocoa butter, cocoa butter composition, melting behavior, polymorphism, X-ray powder diffraction.

In this series of papers, the results of real-time X-ray diffraction applied to cocoa butter are presented. Whereas the first paper (1) described the solidification of molten cocoa butter, this paper deals with the melting of cocoa butter in the β-phase, which is the polymorph commonly present in chocolate products.

Cocoa butter is a mixture of a large number of different triacylglycerols (TAG). TAG are denoted by means of a one- or two-character shorthand for each fatty acid. A simpler notation sometimes used for the composition of cocoa butter (2–4) classifies the fatty acids into two groups, saturated (s) and unsaturated (u), thus reducing the number of different components to six classes: sss, ssu, sus, suu, uuu, and usu. However, the advantage of this simplified description with respect to the study of polymorphism of complex mixtures, such as cocoa butter, still awaits experimental evidence.

At present, everyone agrees that the composition of cocoa butter influences its physical and polymorphic behavior. For example, Chaiseri and Dimick (5) explained differences in lipid and hardness characteristics of cocoa butters in terms of differences in chemical composition. A literature search concerning the melting of β-cocoa butter reveals a variety of melting points (1). Because differences in chemical composition are believed to influence melting behavior, we have carried out a series of real-time X-ray powder diffraction experiments on the melting of β-cocoa butter. The primary goal was to establish accurately the melting trajectories for samples of different origin and composition. Another aim was to search for a possible relation between melting behavior and the chemical composition of the butters and particularly, in what detail the composition of cocoa butter should be specified to be useful for the prediction of melting trajectories of the β-phase.

*To whom correspondence should be addressed at Amsterdam Institute for Molecular Sciences (AIMS), Laboratory for Crystallography, University of Amsterdam, Nieuwe Actergracht 166, 1018 WV Amsterdam, The Netherlands.

MATERIALS AND METHODS

Samples and sample preparation. Twelve cocoa butter samples were provided by Cacao De Zaan (Koog aan de Zaan, The Netherlands), originating from different countries on three continents. Each butter has its own characteristic chemical composition, depending on the country and the harvest period. In Table 1, the simplified TAG composition of the cocoa butters is given. A detailed characterization is presented in Tables 5–8. Stocks of the various cocoa butter samples have been stored for several years at temperatures just above 0°C and were completely in the β-phase. 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. By preparing samples in various ways, we ascertained that the diffraction characteristics are independent of the method of filling the sample holder.

Melting behavior. The experiments were conducted with a real-time X-ray powder diffractometer (1,6), which allows the recording of the d-spacings from 3.0 to 6.1 Å simultaneously. The diffractometer is equipped with a temperature-control unit so that the temperature of the sample can be varied in the range of −193 to 250°C, in steps of 0.1°C. The experimental settings are identical to those described in Part I (1).

The melting behavior of the cocoa butter samples was monitored when heated from 27.0 to 42.0°C in 30 min. Starting from 27.0°C, the experiments were divided into equally spaced intervals of 30 s. Diffraction patterns were recorded during the first 15 s of each interval. For each sample, the series of diffraction patterns was analyzed by means of dynamic difference functions (DDF) (1). Three points are chosen to characterize the melting trajectory (Fig. 1): the melting start-

TABLE 1
Simplified Cocoa Butter Composition of 12 Samples[a]

Country of origin	Harvest date month-year	% A sss	% B ssu	% C sus	% D suu	% E uuu	Total % u	Total % s
1 Peru	11-81	2.0	0.2	85.6	11.1	0.3	36.2	62.9
2 Bahia, Brazil	11-80	1.0	0.4	71.4	24.6	2.1	42.4	57.1
3 Equatorial Guinea	2-81	2.0	0.3	80.8	15.5	0.8	38.2	61.2
4 Sierra Leone	2-81	2.7	0.4	83.4	12.4	0.4	36.6	62.7
5 Liberia	3-81	1.2	0.2	81.1	16.4	0.9	38.9	60.9
6 Ivory Coast	11-80	2.1	0.3	84.8	11.6	0.4	36.5	62.7
7 Ghana	12-80	1.7	0.2	85.2	11.3	0.3	36.3	62.4
8 Lome, Togo	12-79	1.5	0.1	85.1	12.3	0.5	37.1	62.4
9 Lagos, Nigeria	11-81	1.8	0.3	85.7	11.3	0.4	36.6	62.9
10 Cameroon	12-80	2.1	0.3	82.7	13.7	0.5	37.3	62.0
11 Congo	5-80	2.6	0.3	84.4	11.5	0.3	36.2	62.9
12 Malaysia	12-79	2.8	0.3	87.2	8.9	0.1	35.2	64.1
Average		2.0	0.3	83.1	13.4	0.6	37.3	62.0

[a]All fatty acids are considered to be either saturated (s) or unsaturated (u). This leads to six types of triacylglycerols (TAG): sss, ssu, sus, suu, uuu, usu. All TAG are assumed to be racemic.

ing point (MSP), the point of maximum melting (MP) and the melting end point (MEP). The MSP is defined as the temperature at which DDF$(j,1)$ passes a threshold level of 10%. The melting end point (MEP) is defined as the temperature at which DDF(j,m) goes below 1%. Finally, MP is defined as the temperature corresponding with the maximum in DDF$(j,j-1)$.

Data set analysis. Multivariate data analysis is one of the methods commonly used to establish (possible) relationships between observations (melting points) and parameters (chemical composition). Principal component analysis yields principal components that rank the most relevant parameters (assumed to be independent), while a subsequent least-squares minimization leads to parameter coefficients that fit the data set in the best way. To sort out individual relationships between compositional parameters and melting characteristics

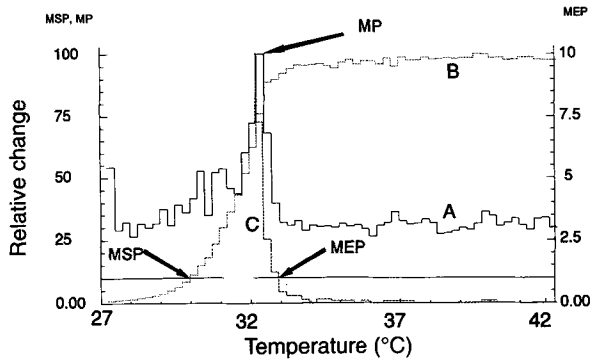

FIG. 1. Melting characteristics of β-cocoa butter from Lome, Togo. Curve A gives DDF$(j,j-1)$ as function of the temperature. The maximum corresponds with the melting point (MP). Curve B gives DDF$(j,1)$ as function of the temperature. The intersection with the threshold level at 10% corresponds with the melting starting point (MSP). Curve C gives the lower 10% DDF(j,m) as function of the temperature. The intersection with the threshold level at 1.0% defines the melting end point (MEP).

while taking into account the limited size of the total data set, we used the following procedure: Starting from a data set of N observations $O(i)$ in total (for MSP, MP, or MEP), p $(1 \leq p \leq N-1)$ independent observations can be singled out in M different ways:

$$M = \binom{N}{N-P} \quad [1]$$

Each set of N-p observations constitutes a data test-set that is subjected to a least-squares analysis. Subsequently, estimates $E(i,j)$ are calculated for the p independent observations on the basis of the least-squares results. Finally, the overall quality QP of the estimates is assessed by comparing the squared difference between estimates and independent observations, averaged over all data test-sets:

$$QP^2 = \frac{1}{pM} \sum_{i=1}^{M} \sum_{j=1}^{p} [E(i,j) - O(j)]^2 \quad [2]$$

In view of the limited number of observations, only linear relations of a single parameter

$$f(x) = a \cdot x + b \quad [3]$$

and of two parameters

$$f(x_1, x_2, r) = a(x_1 + r \cdot x_2) + b \quad [4]$$

were used. In the latter case, it turned out to be convenient to adopt the functional form with a ratio factor r.

In the least-squares analysis of a data test-set, the ratio $r = x_2/x_1$ is kept fixed, and the coefficients a and b are determined. Consequently, a series of least-squares analyses has to be carried out for various r values. In this way, the estimates $E(i,j)$ depend not only on the parameters x_1 and x_2 but also on their ratio r.

The reference estimation function is the arithmetic average over the N-p observations in each data test-set:

$$E(j)_{avg} = \frac{1}{N-p} \sum_{i=1}^{N-p} O(i) \quad [5]$$

The *QP*, based upon these estimates, is referred to as QP_{avg}. The predictive quality of the parameters or combinations of parameters was assessed by means of the following two criteria: (i) The so-called relative predictive ratio *RPR*, defined as:

$$RPR = \frac{QP_{avg}}{QP_{par}} \quad [6]$$

Large *RPR* indicate parameters or parameter combinations that are relatively good predictors. (ii) The largest absolute difference between *E(i,j)* and *O(j)* in all *M* data test-sets, referred to as the predictive extreme error *PrEx*. It is necessary for good predictors to have a small *PrEx*.

RESULTS AND DISCUSSION

A detailed chemical analysis of the cocoa butters shows that over 30 different TAG are present at concentrations from 0.1%. Eleven TAG are present in a concentration greater than 1.0%. Only these TAG have been used for the statistical analyses of the experiments.

Melting characteristics. In Figure 1, the melting behavior from one of the butters is shown in terms of the resulting DDF functions. The precise MSP is difficult to determine and the DDF(*j*,1) curve, from which it is determined, shows that the melting process has already started below the 10% threshold. This 10% level was chosen so that an increase of the melting speed always takes place above the temperature corresponding with this level. The authors are aware of the fact that this 10% threshold is rather arbitrary.

The MP and, in particular, the MEP are much more sharply defined. The 1% level for the MEP corresponds with the noise level in the signal. In Table 2, the melting points of the cocoa butters have been summarized. The melting characteristics obtained have an accuracy of ± 0.05°C.

Relationship between melting characteristics and composition. Starting from the MP values of the 12 cocoa butter samples, summarized in Table 2, 66 data test-sets of 10 MP values each were constructed and analyzed by the method described above. The parameters used in the analyses are the iodine value, the fatty acid contents, TAG, free acids and

TABLE 2
Melting Characteristics of 12 β-Cocoa Butter Samples

Country of origin	MSP[a] (%) ± 0.05°	MP[a] (°C) ± 0.05°	MEP[a] (°C) ± 0.05°
1 Peru	29.8	31.9	32.75
2 Bahia, Brazil	29.4	31.75	32.20
3 Equatorial Guinea	29.9	32.25	33.25
4 Sierra Leone	30.0	33.0	33.4
5 Liberia	30.0	33.0	33.5
6 Ivory Coast	30.4	32.75	33.5
7 Ghana	29.9	32.25	33.5
8 Lome, Togo	29.9	32.25	32.9
9 Lagos, Nigeria	30.1	33.0	33.6
10 Cameroon	29.8	32.5	33.0
11 Congo	30.05	32.75	33.3
12 Malaysia	30.5	33.6	34.15
Average	30.0	32.6	33.2

[a]Abbreviations: MSP, melting starting point; MP, melting point; MEP, melting end point.

TABLE 3
Prediction of Melting Values by Compositional Parameters[a]

Component	MSP[b] = 30.0		MP[b] = 32.6		MEP[b] = 33.25	
	QP	*RPR*	*QP*	*RPR*	*QP*	*RPR*
Average[c]	0.30	1	0.57	1	0.52	1
POP	0.41	0.73	0.76	0.75	0.75	0.69
POS	0.24	1.28	0.50	1.13	0.47	1.11
PLS	0.35	0.86	0.63	0.89	0.56	0.92
POO	0.27	1.11	0.59	0.96	0.54	0.96
SOS	0.20	1.54	0.40	1.40	0.35	1.46
SLS	0.38	0.79	0.67	0.85	0.56	0.91
SOO	0.30	1.02	0.62	0.91	0.59	0.88
$C_{16:0}$	0.41	0.74	0.76	0.74	0.74	0.69
$C_{18:0}$	0.19	1.63	0.41	1.39	0.33	1.55
$C_{18:1}$	0.29	1.05	0.66	0.85	0.56	0.91
$C_{18:2}$	0.19	1.61	0.43	1.32	0.36	1.43
sss	0.28	1.08	0.53	1.06	0.50	1.03
sus	0.22	1.39	0.54	1.05	0.41	1.25
suu	0.22	1.38	0.54	1.05	0.41	1.27
u	0.22	1.34	0.55	1.03	0.42	1.24
s	0.21	1.42	0.53	1.08	0.38	1.36
Iodine value	0.22	1.35	0.51	1.11	0.45	1.14
% Free acids	0.33	0.91	0.63	0.90	0.57	0.90
% Diglycerides	0.31	0.99	0.60	0.96	0.54	0.96

[a]Abbreviations: as defined in Table 1; *QP*, quality of prediction; *RPR*, relative predictive ratio. Cocoa butter components used to predict MSP, MP, and MEP. MSP; MP; MEP = b + a · component content a,b are calculated by means of a least-squares fit.
[b]Average value of complete data set.
[c]The average MSP; MP or MEP as prediction for individual values.

TABLE 4
Combinations of Compositional Parameters Best Capable of Predicting the Melting Values[a]

x_1	*Ratio*	x_2	*QP*	*RPR*	*PrEx*
Average MPS			0.30	1	0.7
$C_{18:1}$	−0.45	suu	0.15	2.0	0.3
$C_{18:1}$	1.2	s	0.15	2.1	0.3
SOO	1.7	s	0.15	2.1	0.3
Average MP			0.57	1	1.2
$C_{18:1}$	−0.4	suu	0.35	1.6	0.7
SOA	−0.7	$C_{18:0}$	0.35	1.6	0.7
SOA	−0.4	SOS	0.34	1.7	0.6
POA	−0.25	$C_{18:0}$	0.32	1.8	0.6
POA	−0.15	SOS	0.29	1.9	0.6
$C_{18:1}$	0.9	s	0.26	2.2	0.5
Average MEP			0.52	1	1.2
$C_{18:1}$	−0.45	suu	0.29	1.8	0.5
$C_{18:1}$	1.1	s	0.28	1.8	0.5
SOA	−0.3	SOS	0.28	1.9	0.5
POA	−0.17	SOS	0.27	1.9	0.5
SOO	1.7	s	0.26	2.0	0.5
POA	−0.3	$C_{18:0}$	0.25	2.0	0.4
SOA	−0.5	$C_{18:0}$	0.25	2.0	0.5

[a]Abbreviations: as defined in Tables 1 and 2; *PrEx*, predictive extreme error. Cocoa butter components x_1,x_2 used to predict MSP, MP and MEP. MSP; MP; MEP = $b + a(x_1 + \text{ratio} \cdot x_2)$. *a*, *b* are calculated by means of a least-squares fit.

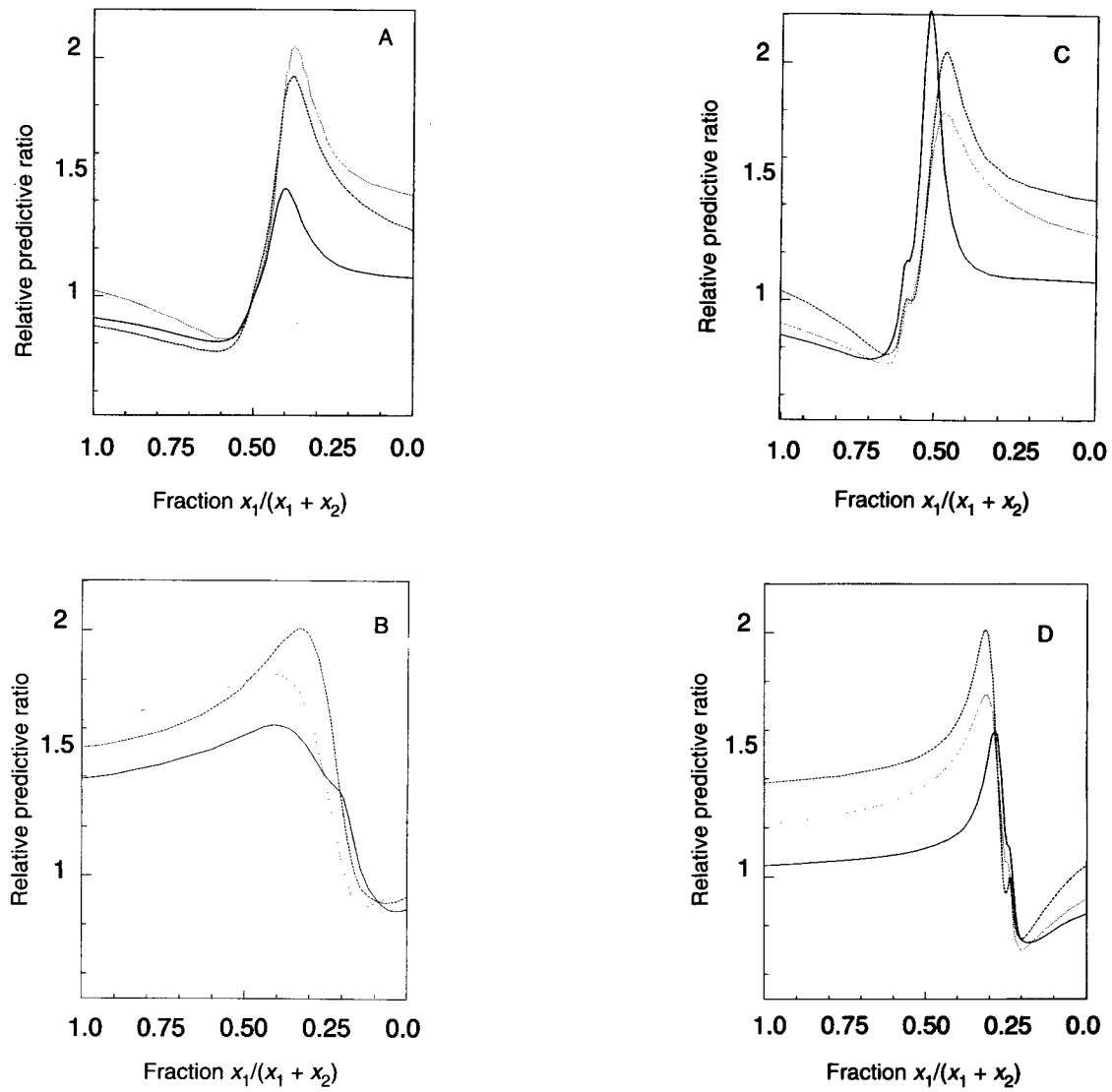

FIG. 2. Relative predictive ratios *(RPR)* of the four most promising two-parameter combinations (x_1,x_2), as function of the fraction of the first component (x_1). The three curves in each figure represent, respectively: (i) a dotted line for the *RPR* values for melting starting point (MSP); (ii) a straight line for the *RPR* values for melting point (MP); (iii) a striped line for the *RPR* values for melting end point (MEP). (A) x_1 = percentage saturated fatty acids, x_2 = percentage SOO; (B) x_1 = percentage stearic acid, x_2 = –percentage SOA; (C) x_1 = percentage saturated fatty acids, x_2 = percentage oleic acid; (D) x_1 = –percentage suu triacylglycerol, x_2 = percentage oleic acid. ····· MSP, —— MP, ------- MEP.

diglycerides; the simplified TAG-composition contents and the total percentage of (un)saturated fatty acids. Only those components have been considered in the analyses that occur on average in the cocoa butters at least at 1.0%. In addition to the resulting 23 individual parameters, combinations of two parameters have been considered, with the ratio r of two parameters as an additional parameter. Similar analyses have been carried out independently for the MSP and the MEP.

An overview of the quality indicators *QP*, *RPR* and *PrEx* of the most relevant parameter (combinations) is given in Tables 3 and 4. An analysis of these results shows that some parameters, such as the iodine value or the percentage of (un)saturated fatty acids, are not necessarily adequate to predict melting points. However, some of the individual parameters that are insufficient to predict melting points may well lead to useful results in combination with other parameters. Indeed, some combinations of two parameters are capable of predicting the MSP, MP and MEP with an average error of less than 0.2°C and a maximum error of less than 0.5°C.

The four parameter combinations in Table 4 with the most promising *RPR* and *PrEx* results have been analyzed in more detail. Figure 2 illustrates how *RPR* values are a function of the ratio r of the two components. It is remarkable that these functions have a quite sharp maximum that quickly drops off-maximum to uniform values.

Figure 3 presents the melting characteristics MSP, MP, and MEP as functions of those four parameter combinations. Two different least-squares lines are presented for each MP, MEP,

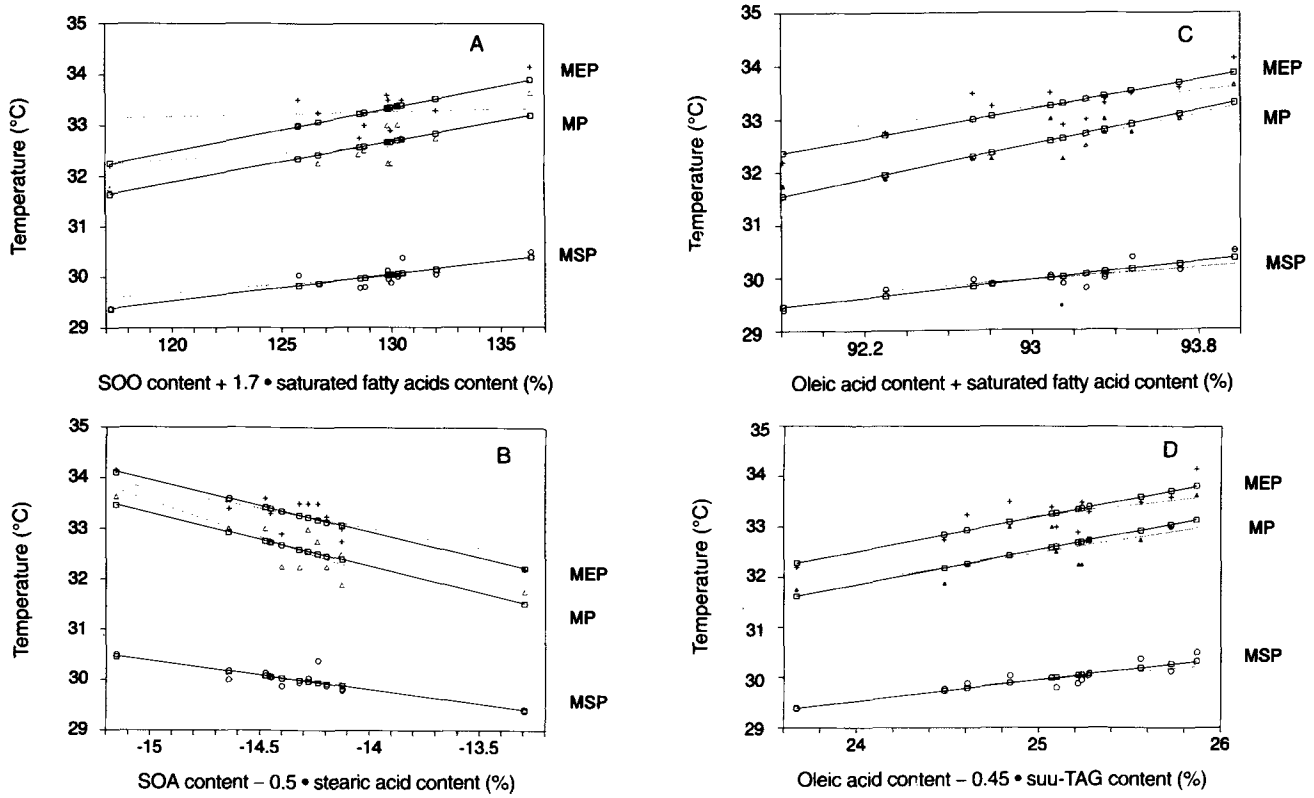

FIG. 3. Prediction of the melting behavior of β-cocoa butter by means of a linear relation between combinations of two components and melting temperature. Straight lines are least-squares functions based upon the complete data-set of 12 samples, dotted lines are least-squares functions based upon the data set from which the lowest and highest values are left out. ○ = observed melting starting point (MSP); △ = observed melting point (MP); + = observed melting end point (MEP); □ = calculated value. (A): melting characteristics as a function of (SOO content + 1.7 · saturated fatty acids content); (B): melting characteristics as a function of (SOA content –0.5 · stearic acid content); (C): melting characteristics as a function of (oleic acid content + saturated fatty acids content); (D): melting characteristics as a function of (oleic acid content –0.45 · suu triacylglycerol content).

and MSP: a straight line based on the complete data set of 12 melting points, and a dotted lined based on the data set without the highest and lowest melting points. In all four graphs of Figure 3, the MEP lines show the largest deviation, whereas the MP and MSP lines describe the data much more accurately. When comparing the dotted line (based on ten data) with the straight line (based on all data), for all graphs, the best results are from the combination of the oleic acid content and the saturated fatty acid content (Fig. 3C). It is clear that, in this case, even the dotted line coincides with the highest and lowest observed melting values quite well. This combination seems to be most interesting because it combines high *RPR* and low *PrEx* values with a maximum spread of data points over the whole melting range, as can be judged from the figure.

These experiments show that the melting process depends on the composition of the cocoa butter (Tables 5–8). It follows from the analyses of the experiments that the melting behavior of β-cocoa butter can be predicted quite accurately when the TAG composition of the cocoa butter is known. However, the 23 single components and their binary combinations constitute a largely collinear system.

TABLE 5
Analytical Data About the 12 Cocoa Butter Samples Used in the Real-Time X-Ray Diffraction Experiments[a]

Country of origin	Harvest date		Country of origin	Harvest date	
	Month	Year		Month	Year
1. Peru	11	81	7. Ghana	12	80
2. Bahia, Brazil	11	80	8. Lome, Togo	12	79
3. Equatorial Guinea	2	81	9. Lagos, Nigeria	11	81
4. Sierra Leone	2	81	10. Cameroon	12	80
5. Liberia	3	81	11. Congo	5	81
6. Ivory Coast	11	80	12. Malaysia	12	79

[a]The data have been collected by the laboratory of Cacao de Zaan, (Koog aan de Zaan, The Netherlands).

TABLE 6
Fatty Acid Composition (%) of 12 Cocoa Butter Samples[a]

	$C_{14:0}$	$C_{17:0}$	$C_{16:0}$	$C_{18:0}$	$C_{20:0}$	$C_{18:1}$	$C_{16:1}$	$C_{18:2}$	$C_{18:3}$
1	0.21	0.21	23.75	30.63	0.82	29.40	0.23	3.35	0.30
2	0.05	0.06	20.04	29.37	1.01	34.69	0.22	3.67	0.18
3	0.08	0.35	21.72	31.17	0.91	31.54	0.34	3.01	0.06
4	0.08	0.15	22.26	32.05	1.01	30.61	0.20	2.68	0.06
5	0.08	0.06	22.20	30.75	0.78	32.19	0.14	3.28	0.06
6	0.08	0.12	22.40	31.84	1.11	30.73	0.20	2.46	0.06
7	0.08	0.12	22.20	31.81	1.08	30.27	0.20	2.74	0.06
8	0.00	0.06	22.43	31.78	1.01	30.71	0.39	2.83	0.06
9	0.26	0.15	23.35	31.14	0.75	30.78	0.14	2.59	0.09
10	0.00	0.15	22.80	31.03	0.91	31.21	0.23	2.69	0.06
11	0.08	0.15	22.14	32.27	1.11	30.40	0.20	2.51	0.06
12	0.00	0.00	21.65	34.08	1.14	29.82	0.20	2.16	0.06
Average	0.08	0.13	22.25	31.49	0.97	31.03	0.22	2.83	0.09

[a]See Table 5 for sources of cocoa butter samples.

TABLE 7
Iodine Value, Free Acids (%), Mono-, and Diglycerides (%) of 12 Cocoa Butter Samples[a]

	Iodine value	Free acids	Monoglycerides	Diglycerides
1	36.2	1.40	0.0	0.9
2	40.8	0.84	0.1	1.4
3	37.0	1.20	0.1	1.5
4	35.3	1.34	0.1	1.7
5	34.5	0.90	0.1	0.7
6	34.7	1.45	0.1	1.3
7	35.2	1.27	0.1	0.8
8	33.0	1.57	0.1	1.0
9	35.2	0.80	0.1	0.8
10	36.6	0.78	0.1	1.2
11	35.0	2.00	0.2	2.4
12	33.9	0.75	0.2	1.4
Average	35.6	1.19	0.1	1.2

[a]See Table 5 for sources of cocoa butter samples.

TABLE 8
Triacylglycerol Composition (%) of 12 Cocoa Butter Samples[a]

	POS	SOS	POP	SOO	POO	PLS	SLS	SOA	PLP	POA	SOL	Others
1	36.5	21.7	15.4	3.6	3.1	3.6	2.1	1.2	1.5	1.0	1.3	8.1
2	29.9	20.2	11.0	10.9	8.1	3.2	2.2	1.4	1.2	1.0	1.3	7.9
3	34.3	22.6	13.0	6.7	5.1	3.5	2.3	1.4	1.3	1.0	0.6	7.6
4	35.8	23.7	13.5	5.5	4.2	3.3	2.2	1.4	1.2	1.1	0.5	7.0
5	35.0	22.3	13.8	7.1	5.6	3.4	2.2	1.1	1.3	0.9	1.0	6.1
6	36.6	23.9	14.0	5.3	4.0	3.0	2.0	1.7	1.2	1.3	0.5	5.7
7	36.3	23.8	13.8	5.1	3.9	3.5	2.3	1.6	1.3	1.2	0.4	5.5
8	36.7	23.9	14.1	5.2	4.0	3.4	2.2	1.5	1.3	1.2	0.6	5.4
9	37.1	22.9	15.0	5.1	4.1	3.2	2.0	1.1	1.3	0.9	0.5	6.3
10	37.5	23.4	15.0	4.5	3.6	1.6	1.0	1.4	0.6	1.1	2.4	7.2
11	37.5	25.1	13.9	3.9	2.9	1.9	1.3	1.7	0.7	1.3	1.9	7.0
12	37.8	27.4	13.0	3.9	2.7	2.5	1.8	1.9	0.9	1.3	0.7	5.4
Average	35.9	23.4	13.8	5.6	4.3	3.0	2.0	1.45	1.1	1.1	1.0	8.1

[a]The triacylglycerol composition has been calculated from the total fatty acid composition and the fatty acid at the 2 position, as described by Coleman (Ref. 7). See Table 5 for cocoa butter sample sources. P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; A, arachidic acid.

Therefore, definite conclusions concerning causal relations between componenets and melting behavior are premature.

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