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# COMBINING MICROSCOPY AND PHYSICAL TECHNIQUES IN THE STUDY OF COCOA BUTTER POLYMORPHS AND VEGETABLE FAT BLENDS

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## Abstract

Transmission electron microscopy, differential scanning calorimetry and X-ray diffraction have been used to study the cocoa butter polymorphs and blends of cocoa butter with a hydrogenated vegetable fat. The results indicate the presence of six polymorphs and confirm observations made by other workers. Vegetable fat addition affects both the molecular structure and the morphology of the crystals observed. After temperature cycling, a blend containing 50% vegetable fat developed two crystal types and differences in the X-ray pattern were apparent. Correlations could be made between the known molecular structure and the morphology observed in most of the polymorphs. In selected cases, and particularly the blends containing vegetable fat, knowledge of the polymorphic form did not always enable an accurate prediction of morphology.

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Key Words: cocoa butter polymorphs, hydrogenated vegetable fat, fat blends, Transmission Electron Microscopy, X-ray diffraction, Differential Scanning Calorimetry, freeze-fracture, chocolate.

## Introduction

The study of cocoa butter continues to be of interest to the food industry due to its commercial importance in the manufacture of chocolate.

Although cocoa butter is a mixture of triglycerides it can exist in a number of distinct polymorphs (Wille & Lutton, 1966). A number of methods have been used to investigate them. Chapman et al. (1971) undertook a study comprising X-ray diffraction and Differential Scanning Calorimetry (DSC) on the polymorphism of cocoa butter. The carbon replica technique for Transmission Electron Microscopy (TEM) has been shown to be the most suitable E.M. technique (Berger et al. 1979) to study the morphological characteristics of cocoa butter. It was apparent from all these studies that no single method can successfully provide the required data to allow characterisation. The work described, therefore, used X-ray diffraction, Differential Scanning Calorimetry and Transmission Electron Microscopy as complementary techniques on the same set of samples, in order to study cocoa butter polymorphs and the effect of vegetable fat addition.

The legislation in certain countries permits incorporation of vegetable fats up to 5% of the chocolate (15% of the fat phase). They are known commercially as Cocoa Butter Equivalents (CBE), and Cocoa Butter Substitutes (CBS). They have been described recently by Haumann (1984). CBE have triglycerides closely resembling those found in cocoa butter. CBS have some triglycerides not found in the cocoa butter system, but still have a limited compatibility with cocoa butter. The study involved the effect of a hydrogenated vegetable fat (CBS) on the properties of the cocoa butter.

## Nomenclature

Two systems of nomenclature have been derived from earlier studies on cocoa butter polymorphism. One is attributable to Wille & Lutton (1966) and based on melting point, the other on crystallographic structure and developed by Larsson (1966) Table 1.

For simplicity, this paper used the I-VI classification but reference will also be made to

the Larsson nomenclature in discussing crystal structure.

TABLE 1

| POLYMORPH | | MELTING POINT* |
|---|---|---|
| WILLE & LUTTON | LARSSON | |
| I | $\beta'_2$ ($\gamma$) | 17.3°C |
| II | $\alpha$ | 23.3°C |
| III | $\alpha + \beta'$ | 25.5°C |
| IV | $\beta'$ | 27.5°C |
| V | $\beta_2$ | 33.8°C |
| VI | $\beta$ | 36.3°C |

* (from Wille & Lutton, 1966)

## Materials and Methods

### Preparations

Polymorphs: The cocoa butter polymorphs were prepared by a similar approach to that given by Wille & Lutton (1966). In all the preparations, the cocoa butter had been heated to 50°C with agitation until molten, and then kept at this temperature for at least 2 hours.

Form I: Approximately 5 cm³ of the cocoa butter melt was flash frozen in liquid nitrogen (-196°C).

Form II: Approximately 5 cm³ of the cocoa butter melt was flash frozen in liquid nitrogen (-196°C) and stored in aluminium foil at 0°C for 2 hours prior to analysis.

Form III: Approximately 10 cm³ of the cocoa butter was flash frozen in liquid nitrogen and stored at 6°C overnight.

Form IV: Approximately 10 cm³ of the cocoa butter was pre-cooled to 6°C for 15-30 minutes and then crystallised at 16°C for 4 hours.

Form V: Approximately 30 cm³ of the melt was hand tempered by stirring at 15°C until crystal seed formed and then at 24°C until a thick slurry was produced, poured into a mould and placed in a fridge at 6°C. It was demoulded and stored for 24 hours at 20°C.

Form VI: A tablet of Form V cocoa butter was stored in an incubator for one week, the temperature was cycled between 20°C and 30°C with the sample spending 4 hours at each temperature.

Vegetable fat: Blends containing 10%, 20%, 30%, 40% and 50% (w/w) commercially available hydrogenated vegetable fat with cocoa butter were prepared. The blends were melted at 50°C and kept at this temperature for at least 2 hours. The liquid fat blend was tempered using the same regime employed to prepare Form V. Once demoulded it was stored for at least 24 hours before examination. The 50% blend was also temperature cycled, as described in Form VI preparation.

### Techniques

X-ray diffraction: The sample was mounted in an aluminium holder for examination. The more unstable polymorphs were sandwiched between aluminium foil frozen in liquid nitrogen (-196°C) and secured into a cold block (-20°C). The experiments were performed on a Philips 1011/00 X-ray generator operating at 40 kV and 20 mA to give Cu radiation of X-ray wavelength $\lambda$= 1.54180 (Å). A D.P.T camera was used to record the patterns on Kodak X-ray film. A 15 minutes exposure was given. The long spacing data was determined to within ± 1 Å, the short spacings to ± 0.02 Å. No obvious transformations occurred during the experiment, although it is possible that some might occur in the more unstable systems.

Differential Scanning Calorimetry: A 15 mg sample was put in an aluminium pan and placed in the temperature controlled chamber. DSC measurements were taken on a Dupont 1090 Thermal Analyser operating at 2°C/min, between 15°C and 45°C, recording at 2 points/second. The protocol used Indium as the standard, the melting point being defined as the peak maximum.

Transmission Electron Microscopy: The TEM was performed using a pre-shadowed carbon replica technique. A freshly prepared sample (≅ 1 cm³) was flash frozen in liquid nitrogen (-196°C) and placed on a pre-cooled block. Fracture faces and surfaces were prepared. The block was then transferred into a Polaron coating unit, and a vacuum of better than $6 \times 10^{-6}$ Torr drawn. A second pre-cooled (-196°C) block acted as an anti-contaminator within the chamber. Platinum was evaporated at an angle of 40° to a thickness of 30 Å, carbon was evaporated from directly over the sample producing a 350 Å layer. After removal from the unit, the replica was cleaned using ether and 2:1 chloroform/methanol. The replicas were examined using the JEOL 1200EX TEM operating at 80 kV.

## Results

The X-ray data are listed in Table 2 and examples of the X-ray patterns are given in Fig. 1a-c. The DSC data are in Table 3. Although mixed triglycerides have a melting range (Timms, 1984), rather than a distinct melting point, the results presented are termed "melting points" in common with other workers, as they are observed as single distinct peaks. The TEM micrographs are in Figs. 2-10 and 12. They are negative prints, making the shadows appear dark.

### Form I

Few distinct features existed in the morphology of this polymorph. Some lamellae were observed, although with only limited ordering into layers (Fig. 2). The X-ray data corresponded with those observed in the literature (Wille & Lutton, 1966), with short spacings of 3.70 and 4.19 Å characteristic of the sub $\alpha$-structure of the polymorph. The DSC gave a melting point of 17.9°C.

### Form II

The sample had a morphology consisting of lamellae with layering of the sheets as shown in Fig. 3. The individual sheets or lamellae

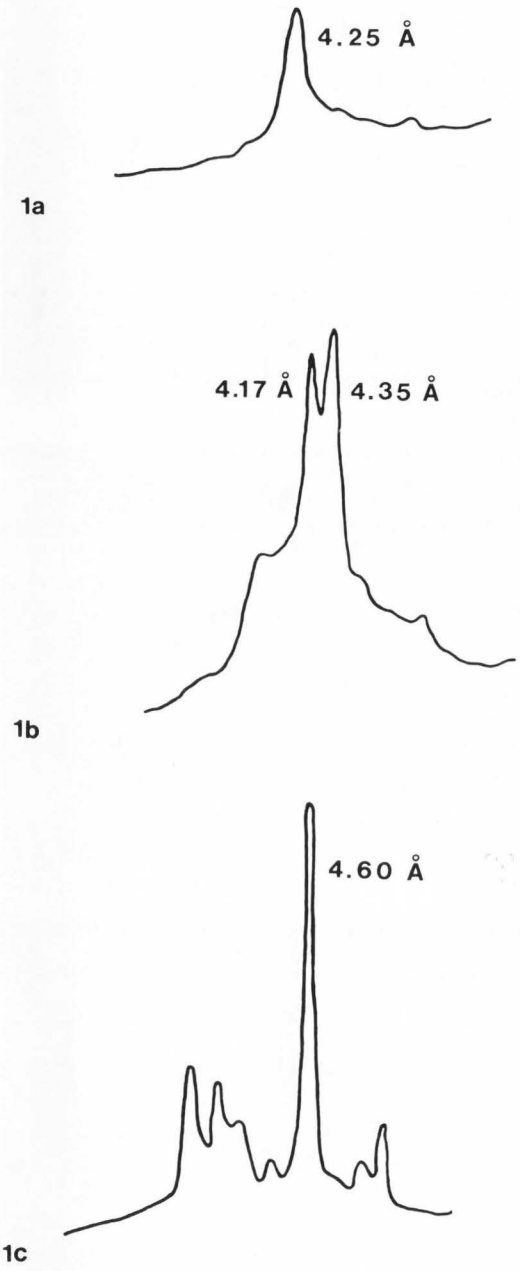
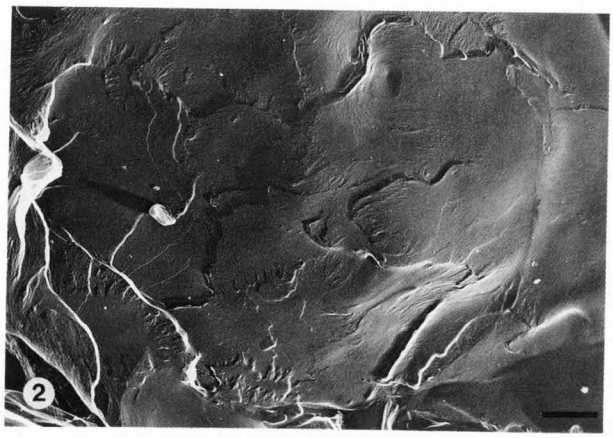



Figure 1. X-ray short spacings for Forms II, IV and VI. a. Short spacings of Form II. b. Short spacings of Form IV. c. Short spacings of Form VI.

appeared to be quite extensive spreading through the bulk of the sample. The individual lamella had a number of sheets stacked on one another. The X-ray pattern showed the disappearance of the 3.70 Å peak seen in Form I, and the development of a long spacing at 49 Å. The pattern typified the α-structure, Fig. 1a. The melting point was 24.4°C.



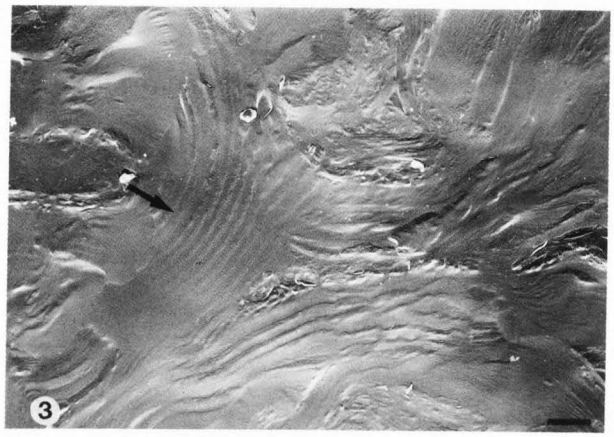





Figure 2. Form I Cocoa Butter, illustrating the lack of a distinct morphology. (Bar = 1 μm).

Figure 3. Form II Cocoa Butter, showing ordered lamellae (arrow). (Bar = 1 μm).

Figure 4. Form III Cocoa Butter, with protruding tubular crystals (arrow). (Bar = 1 μm).

Form III

The tubular crystals that protruded from the surface of the sample were very distinctive (Fig. 4). These crystals were distributed over all the surface, appearing as individual crystals (typically 2-3 μm long) though predominantly in clusters. The clusters appeared to involve 6 or more crystals within an area of about 4 μm. The background showed limited localised ordering. The X-ray pattern corresponded with that described by Wille & Lutton (1966). The development of two peaks at 15.24 and 16.6 Å, and a short spacing at 3.87 Å was characteristic. The melting point was 27.7°C.

Form IV

Form IV was characterised by needle-like crystals varying in length from 0.5 μm to 2 μm. They were distributed throughout the structure both on the surface and within the bulk (Fig. 5). The X-ray data had a doublet at 4.17 and 4.35 Å, and a long spacing of 46 Å, characteristic of the β' structure (Fig. 1b). The melting point was 28.4°C.

Form V

The crystals associated with Form V were up to 1 μm in length, well defined and regular in shape. They were frequently stacked on top of one another into multilayers of crystals (Fig. 6). The characteristic X-ray pattern had a strong 4.6 Å peak in the short spacing, and 66 Å in the long spacing - typical of the β structure. The melting point was 33.0°C.

Form VI

Significant differences in the morphology were observed in Form V following transformation to Form VI. Firstly, there was a general increase in the fat crystal size from 1 μm in Form V to 2-3 μm in Form VI. Secondly, a feature which was apparent on the surface was the protrusion of crystals (Fig. 7) often 3-4 μm or longer. Despite these significant differences in morphology the X-ray pattern only showed a small difference in relative intensities of some short spacing peaks from Form V. A single peak at 3.71 Å was observed, with lesser intensity peaks at 3.88 Å and 4.04 Å (Fig. 1c). The melting point increased to 34.6°C.

Addition of Vegetable Fat

The hydrogenated vegetable fat had a different morphology from that normally seen in cocoa butter, with large well defined crystals with an average size of 2-3 μm as seen in Fig. 8. The X-ray pattern was characteristic of a β' structure. The melting point at 38.1°C was significantly higher than for any of the polymorphs of cocoa butter.

Addition of the vegetable fat to cocoa butter at a level of 10% and 20% had little effect on the characteristic Form V morphology (Fig. 9). The X-ray pattern did show some differences. The peaks normally present in Form V were produced, but in addition a low intensity peak at 4.25 Å was present, and a 49 Å peak had developed. The DSC showed a single melting point at 32.1°C, close to the melting point of Form V. It was apparent, therefore, that some structural changes had occurred which did not have a marked effect on the morphology.

As the vegetable fat content was increased to 30% and above, significant changes occurred in morphology. Crystal size decreased from 1 μm to 0.25 μm, and the crystal outlines became less distinct (Fig. 10). The X-ray pattern showed a suppression of the characteristic β structure of the Form V, with finally, at the 50% addition, domination of the β' structure of the vegetable fat (Table 2). The DSC trace broadened with the increase of the vegetable fat content as shown in

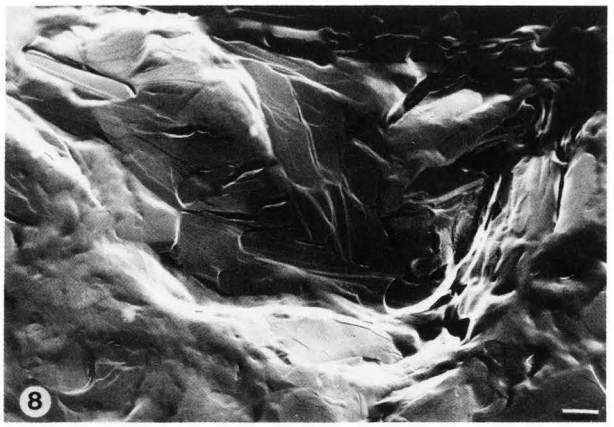
TABLE 2

X-RAY DIFFRACTION DATA

| | Short Spacing (Å) | Long Spacing (Å) |
|---|---|---|
| POLYMORPH I | 3.70 (S)<br>4.19 (VS) | 34 (W) |
| POLYMORPH II | 4.25 (S) | 16.6 (M)<br>49 (VS) |
| POLYMORPH III | 3.87 (M)<br>4.25 (S)<br>4.63 (M) | 15.24 (M)<br>16.6 (S)<br>49 (VS) |
| POLYMORPH IV | 4.17 (VS)<br>4.35 (VS) | 14.9 (S)<br>46 (VS) |
| POLYMORPH V | 3.68 (W) 4.6 (VS)<br>3.76 (M) 5.43 (M)<br>3.88 (W)<br>3.99 (M) | 8.08 (W)<br>13.15 (W)<br>16.20 (W)<br>34 (S)<br>66 (S) |
| POLYMORPH VI | 3.71 (S) 4.6 (VS)<br>3.88 (S) 5.16 (W)<br>4.04 (M) 5.47 (M)<br>4.28 (W) | 8.18 (W)<br>13.2 (W)<br>35 (S)<br>66 (S) |
| HYDROGENATED VEG. FAT | 3.87 (S)<br>4.08 (M)<br>4.24 (S)<br>4.39 (M) | 15.3 (S)<br>49 (VS) |
| 10% VEG FAT, 90% COCOA BUTTER | 3.68 (W) 4.6 (VS)<br>3.75 (M) 5.45 (M)<br>3.89 (W)<br>4.00 (M)<br>4.25 (W) | 8.18 (W)<br>13.26 (VW)<br>35 (S)<br>49 (M) |
| 50% VEG FAT, 50% COCOA BUTTER | | |
| UNCYCLED | 3.87 (S)<br>4.08 (M)<br>4.24 (S)<br>4.39 (M) | 15.3 (S)<br>49 (VS) |
| CYCLED | 3.89 (M)<br>4.25 (S)<br>4.6 (S) | 15.3 (W)<br>49 (VS) |

Key: S = Strong
M = Medium
W = Weak



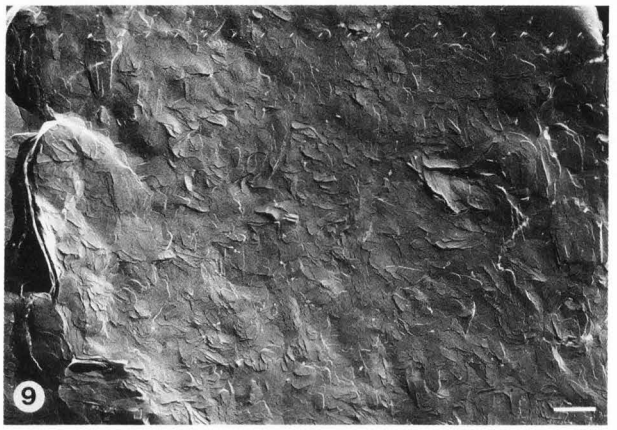





Figure 5. Form IV Cocoa Butter, made up of densely packed needle-like crystals. (Bar = 1 μm).

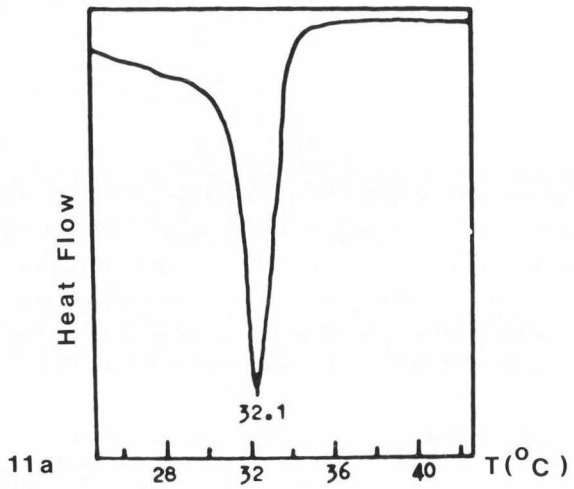
Figure 6. Form V Cocoa Butter, multilayered crystals (arrow), regular in shape. (Bar = 1 μm).

Figure 7. Form VI Cocoa Butter, crystals protruding (arrow) from a matrix of more regularly shaped crystals. (Bar = 1 μm).

Figure 8. Hydrogenated vegetable fat with long crystals arranged into layers. (Bar = 1 μm).

Figure 9. 10% vegetable fat, 90% cocoa butter blend, with a crystal shape similar to Form V morphology. (Bar = 1 μm).

Figure 10. 50% vegetable fat, 50% cocoa butter blend with small ill defined crystals. (Bar = 1 μm).



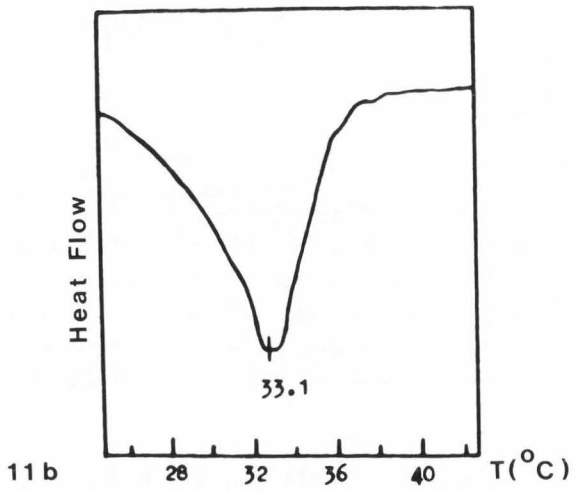




Figure 11. DSC traces for two of the vegetable fat blends. a. DSC trace - 10% vegetable fat addition. b. DSC trace - 50% vegetable fat addition.

Fig. 11a and b and a small increase to 33.1°C occurred in melting point. After temperature cycling two distinct crystals could be observed. One appeared to be rounded crystals 1-2 μm in length stacked on one another into multilayers (Fig. 12a). The other long elongated crystals up to 3-4 μm long, and stacked (Fig. 12b). The X-ray showed the development of a peak at 4.6 Å, and the loss of the 4.08 Å and 4.39 Å peaks from β' pattern (Table 2). The DSC gave a main peak at 35.8°C and a shoulder at about 38°C.

TABLE 3

MELTING POINT DATA

| | |
|---|---|
| POLYMORPH I | 17.9°C |
| POLYMORPH II | 24.4°C |
| POLYMORPH III | 27.7°C |
| POLYMORPH IV | 28.4°C |
| POLYMORPH V | 33.0°C |
| POLYMORPH VI | 34.6°C |
| HYDROGENATED VEG. FAT | 38.1°C |
| 10% VEG. FAT 90% COCOA BUTTER | 32.1°C |
| 50% VEG. FAT 50% COCOA BUTTER | |
| UNCYCLED | 33.1°C |
| CYCLED | 35.8°C |

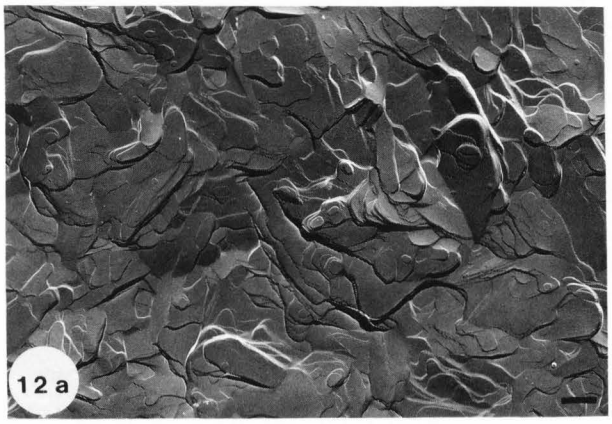




Figure 12. 50% vegetable fat, 50% cocoa butter blend after temperature cycling. Showing two types of crystalline form (a and b).
a. Rounded crystals stacked together. (Bar = 1 μm). b. Elongated crystals arranged into layers. (Bar = 1 μm).

## Discussion

The crystal structure of solid triglycerides in the α, β', β described by Larsson (1982) for triundecanoin, can be used to understand some of the morphologies observed in the cocoa butter polymorphs. The α structure is thought to consist of inefficiently packed bimolecular layers. These allow penetration of triglyceride chains between layers inhibiting interlayer packing. Growth will therefore occur laterally to form the lamellae structures as observed in Form II. The β' structure, though reducing interplane chain penetration by the tilt of the bimolecular units in the structure also places limitations on growth of the crystals. In one axis lateral growth is facilitated by the structure, but in the other it involves more complicated packing and so growth is slow. This causes more needle-like crystals as seen in Form IV. deMan (1982) described a similar effect in margarine fats examined by light microscopy. The β structure significantly reduces chain penetration, producing thicker and more equidimensional crystals, being less affected by the constraints on lateral growth found in the β' structure. This close correlation between structure and morphology is not always observed. Form III is not easily classified into an α or β' structure, morphologically. Its characteristic crystals protruding from a poorly structured background suggest two crystal types might be present. Form VI shows small differences in the X-ray pattern, but significant differences in morphology and melting point when compared with Form V.

This complex relationship between structure and morphology is shown in the study of the cocoa butter/vegetable fat blends. Differences in structure are observed at lower percent additions than differences observed in morphology. The structural differences observed may relate to the molecular packing of the triglycerides. Analysis of the hydrogenated vegetable fat shows it to contain a high proportion of triglycerides containing trans acids in comparison to cocoa butter in which the triglycerides have a predominant cis configuration in the oleo chain (Jewell, 1981). Precht (1977) postulated from work using X-ray diffraction on binary triglyceride systems that incorporation of triglycerides containing trans acids would preferentially stabilise the β' rather than β structure. The β' structure being more able to accommodate the lattice defects produced by these triglycerides.

The complexity of the cocoa butter and vegetable fat blends makes detailed interpretation of the X-ray data difficult. It is apparent, however, that the blends containing higher levels of the vegetable fat had a strong 49 Å peak and short spacings indicative of β' structure. This suggested that a change from the β structure in Form V cocoa butter to a β' structure had occurred. It is possible that some incorporation of triglycerides containing trans acids may have taken place causing preferential stabilisation of the β' structure in accordance with the model suggested by Precht. This difference in X-ray data might have been expected to cause the morphology to revert to that of the vegetable fat. It was observed, however, that crystal size diminished markedly. The DSC peak broadened at the higher levels of vegetable fat addition suggesting that the solid was not a purely crystalline phase. Overall this indicates that a β' structure was present in much of the solid phase, but a high level of disorder exists, inhibiting crystal growth and the development of a distinct crystal morphology. The effect of temperature cycling on such blends allows re-ordering of the triglycerides to form two distinct crystalline phases, with corresponding changes in X-ray and DSC data.

The TEM replica technique is a very effective method for examination of fat systems but some consideration of artefacts in the preparation is necessary. Freezing rate is not as critical in fats as in aqueous systems, liquid nitrogen being an adequate cryogen, and unlikely to cause distortions of the crystals during cooling. Melting during preparation can be a problem, the sample requiring to be kept at a low temperature (≅ -100°C). Despite these potential problems, the method has been shown to be relatively straightforward, and to provide useful data to complement DSC and X-ray results.

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## References

BERGER KG, JEWELL GG and POLLITT RJ. (1979). Oils and Fats, in: Food Microscopy, J.G. Vaughan (ed), Academic Press, London, 445-495.

CHAPMAN GM, AKEHURST EE and WRIGHT WB. (1971). Cocoa butter and confectionery fats. Studies using programmed temperature X-ray diffraction and Differential Scanning Calorimetry, Journal of American Oil Chemists Society 48, 824-830.

deMAN JM. (1982). Microscopy in the study of fats and emulsions, Food Microstruc. 1 (2), 209-222.

HAUMANN BF. (1984). Confectionery fats - for special uses, Journal of American Oil Chemists Society 61, 468-472.

JEWELL GG. (1981). Factors influencing the crystallisation of chocolate, in: Proceedings of the Thirty-Fifth Annual Production Conference, Pennsylvania Manufacturing Confectioners' Association, 1981, 63-66.

LARSSON K. (1966). Classification of crystal forms, Acta. Chem. Scand. 20, 2255-2260.

LARSSON K. (1982). Some effects of lipids on the structure of foods, Food Microstruc. 1 (1), 55-62.

PRECHT D and FREDE E. (1977). The crystal structure of fats. I. Molecular arrangements of saturated triglycerides and triglyceride mixtures, Kieler Milchw. Forschungsber. 29, 265-285.

TIMMS RE. (1984). Phase behaviour of fats and their mixtures, Prog. Lipid Res. 23, 1-38.

WILLE RL and LUTTON ES. (1966). Polymorphism of Cocoa Butter, Journal of American Oil Chemists Society 43, 491-498.

## Discussion with Reviewers

Reviewer II: Is it correct to consider a mixed triglyceride system such as cocoa butter to have distinct polymorphic forms? To what extent does compound crystallisation occur?
Authors: Natural fats are often a complex mixture of triglycerides with intrinsic variations in composition and physical properties. The studies undertaken by Wille and Lutton (1966) and others, however, indicate that cocoa butter has a polymorphism which can be defined by a series of distinct X-ray patterns. The exact composition of the solid phase is liable to change between polymorphs, all of which have some level of liquid content, allowing interchange between the liquid and solid states. The development of possibly two or more solid solutions (Timms, 1984) and compound crystallisation are likely to occur particularly in the less stable polymorphs. Despite this potential complexity, the behaviour of the system appears adequately defined by six states with specific X-ray patterns and crystal morphologies and provides useful data for chocolate technology.

D. Manning: Is it possible to have crystalline melting points ranging between those stated for Form I through Form VI? For example, after solidification is it possible to have a crystalline melting point of 30.7°C which lies between polymorphs IV and V? How would such a crystal be classified?
Authors: Yes, this is possible, and has been observed in thermal data (Jewell, 1981). Their characterisation is difficult as X-ray diffraction does not often show a corresponding difference. In the example given, the crystalline material probably consists of metastable mixtures of β and β' structures.

D. Manning: In reference to Form I polymorphs, is it possible to have a crystalline material without continuously repeating lamellae? How would the crystalline data of Form I polymorphs compare to the same sample of cocoa butter in the liquid state?
Authors: It is unlikely that a crystalline state would exist without continuously repeating lamellae. It is, however, possible to have limited repeating lamellae present which, although not crystalline material, would contrast with the liquid state which is highly disordered.

J. deMan: The electron micrographs of the crystals convey the impression of an almost continuous sheet of crystal. Is this the way we have to envisage the structure of cocoa butter?
Authors: Although the impression is of continuous crystalline material, in Form V, SFI data indicates that at 20°C it is ≅ 80% solid. It is likely that the fracture of the fat occurs preferentially through the crystalline material.

D. Manning: In what manner does interpretation of the X-ray data differ when investigating pure single triglycerides as compared to a triglyceride mixture like cocoa butter?
Authors: In a single triglyceride the exact composition of the solid phase is known. This is not always the case in a mixed triglyceride system. Full interpretation of the X-ray data is more complex therefore and information on the packing of individual triglycerides cannot be ascertained directly.

D. Manning: Was there any evidence of annealing or crystal transition with Form I or II polymorphs at the 2°C/min heating rate? Was there any experimentation conducted with faster heating rates?
P. Dimick: Would the 15 mg sample weight induce upward shifts in melting point?
Authors: Heating rates of 2 and 5°C/min have been used routinely in our laboratory without any apparent problems of annealing. The sample weight has been standardised for the protocol used.

P. Dimick: What were the SFI for Forms V and VI samples? Similarly what was the SFI for the blends?
Authors: SFI data are shown in Table 4. Form VI data were not obtained.

TABLE 4

| | 20°C | 25°C | 27.5°C | 30°C | 35°C |
|---|---|---|---|---|---|
| | % solids | | | | |
| Form V | 80 | 75 | 66 | 43 | - |
| Vegetable Fat | 67.8 | 60.2 | 56.0 | 49.6 | 31.4 |
| 10% | 73.2 | 65.5 | 54.9 | 31.7 | 1.3 |
| 20% | 69.4 | 60.5 | 51.3 | 30.2 | 1.7 |
| 30% | 69.8 | 59.7 | 44.4 | 21.7 | 2.2 |
| 40% | 66.7 | 55.8 | 40.9 | 18.5 | 3.0 |
| 50% | 62.2 | 48.3 | 36.3 | 19.1 | 5.2 |

P. Dimick: Does β' crystal formation occur satisfactorily at 16°C, and is β' formation likely to occur during the protocol used for production of Form VI?
Authors: The methods as described produce the required polymorphs satisfactorily, without any evidence for mixed products or unwanted transformations.