Effects of Minor Lipids on Crystallization of Milk Fat–Cocoa Butter Blends and Bloom Formation in Chocolate

R.A. Tietz[a] and R.W. Hartel[b,*]

[a]Kraft Foods, Glenview, Illinois 60025, and [b]University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT: Minor lipids, such as diacylglycerols, monoacylglycerols, cholesterol, and phospholipids, play a key role in crystallization of fats. In this study, the effects of minor lipid components on crystallization of blends of cocoa butter (CB) with 10% milk fat or milk-fat fractions, and on bloom formation of chocolate were investigated. Both removing the minor lipids from milk fat and doubling the level of minor lipids from milk fat resulted in longer nucleation onset time, slower crystallization rate, and rapid bloom development in chocolate. Removal of minor lipids resulted in the formation of irregular primary and secondary crystals with inclusions of liquid fat, whereas the crystals were spherical and uniform in shape in the presence of minor lipids. Minor lipids from milk fat, even at the low concentrations typically found in nature, affected the crystallization of milk fat–CB blends, impacted the chocolate microstructure, and affected bloom development in chocolate.

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KEY WORDS: Chocolate, cocoa butter, fat bloom, lipid crystallization, microstructure, milk fat, minor lipids.

Fat bloom in chocolate is still a major concern for many chocolate manufacturers as it results in an undesirable white, dusty surface appearance. However, the exact mechanisms involved in fat bloom formation have not been resolved. It has been hypothesized that fat bloom is the result of a combination of polymorphic transformation (from Form V to Form VI) and phase separation, and is related to the chocolate microstructure. When bloom is observed in chocolate, the most stable polymorph (Form VI) is always observed (1). However, the presence of Form VI does not necessarily lead to observable bloom (2,3). Adenier *et al*. (2) hypothesized that bloom formation was the result of liquid fat dissolving higher-melting triacylglycerols (TAG), which then migrated to the surface and recrystallized. Bricknell and Hartel (3) found that the morphology of sucrose particles in chocolate impacted the rate of bloom development, since amorphous sucrose particles (spherical) in chocolate inhibited bloom formation compared to chocolates made with irregular-shaped sucrose crystals.

Addition of milk fat to chocolate has been proven to inhibit bloom formation (3,4). However, the exact mechanism of how milk fat inhibits bloom formation has not been determined. Addition of high-melting milk-fat fractions inhibits bloom formation to the greatest extent (5). In addition to the TAG content, the presence of minor lipids (ML) in milk fat may affect crystallization of cocoa butter (CB) in chocolate and thereby affect bloom formation.

Approximately 2–4% ML are found in both milk fat (6) and cocoa butter (7). Also, different levels and types of ML have been found in the various milk-fat fractions, as the ML also fractionate somewhat according to melting point. Lower-melting fractions contain higher levels of diacylglycerols (DAG), monoacylglycerols (MAG), and cholesterol (8) than higher-melting fractions. Metin (9) also showed that lower-melting fractions contained higher levels of DAG, particularly 1,2-DAG. These different levels may be responsible for crystallization differences between milk-fat fractions and also may help to explain why different milk-fat fractions have different effects on bloom formation in chocolate (5). For example, DAG may either promote or delay crystallization, depending on composition and concentration (10,11). Savage and Dimick (12) postulated that minor components serve as seed crystals and promote crystallization of CB. CB containing higher levels of DAG crystallized at a slower rate, prolonging the solidification of fat (13). Hernqvist *et al*. (14) and Hernqvist and Anjou (15) also showed that the crystal growth was retarded in the presence of DAG.

The physical behavior of chocolate, or any food system, is determined by its microstructure, including the amount, size, shape, and polymorphic form of any crystals present. The nature of this crystalline microstructure is dependent on the mechanisms of crystallization (16). Formation of the appropriate fat crystal network (17) is important to obtain the desired physical properties of lipid-based foods, like chocolate. The relationships between the chemical composition, crystallization behavior, crystalline microstructure, physical behavior, and bloom formation in chocolate were investigated in this study.

EXPERIMENTAL PROCEDURES

Materials. Winter anhydrous milk fat (WAMF) and five different milk-fat fractions from dry fractionation of WAMF were obtained from Grassland Dairy (Greenwood, WI).

*To whom correspondence should be addressed at University of Wisconsin, 1605 Linden Dr., Madison, WI 53706. E-mail: hartel@calshp.cals.wisc.edu

The different milk-fat fractions were categorized as very high-melting (VHM), high-melting (HM), middle-melting 2 (MM2), middle-melting 1 (MM1), and low-melting (LM), with clear points of 47.4, 42.2, 28.4, 24.4, and 18.5°C, respectively.

Ivory Coast cocoa butter and a low-fat, semisweet, dark chocolate base (28.1% fat) were supplied by Guittard Chocolate Co. (Burlingame, CA).

Separation of ML. ML (i.e., DAG, MAG, cholesterol, and phospholipids) were separated from TAG in the different milk-fat fractions and cocoa butter by using column chromatography according to a modified method of Carroll (18). A custom-made glass was filled with 60 g of 60–100 mesh Florisil® (Fisher Scientific, Pittsburgh, PA). After the milk fat was embedded in the packing material, the following lipid components were separated through the column using the following solvent profile: 1 L of hexane (hydrocarbons), 2.4 L of 2.5% ether in hexane (cholesterol esters), 3 L of 15% ether in hexane (TAG), 3 L of 25% ether in hexane (cholesterol), 3 L of 50% ether in hexane (DAG), 3 L of 2% methanol in ether (MAG), and finally 3 L of 4% acetic acid in ether (free fatty acids and remaining lipids). The TAG fractions were collected and kept separate. The ML components were pooled, leaving a fraction of TAG and a fraction of non-TAG components for each milk fat

High-performance liquid chromatography (HPLC) and gas chromatography (GC) were used to characterize the purity and composition of the component fats, the purified TAG, and the ML obtained from each fat. Quantification of the neutral lipid composition in CB, anhydrous milk fat (AMF), and milk-fat fractions was done by using HPLC based on a modified technique of Liu *et al.* (19). Fatty acid profiles of the CB, AMF, milk-fat fractions, and the pooled ML for each fat were analyzed by GC according to a modified method of Iverson and Sheppard (20). Acyl carbon number profiles of all component fats and their ML were analyzed by GC according to a modified method of Lund (21). Further details can be found in Tietz (22).

Fat crystallization. To study the impact of ML from the different milk-fat fractions and to look at the impact of different levels of ML on CB crystallization, blends of 10% milk fat–CB were made with varying levels of ML collected from their respective milk-fat fraction. The levels of ML used in these experiments were no ML, natural levels of ML (*ca.* 2.5%), and twice the natural level of ML (*ca.* 5%). For each fat, the ML removed in the column were added back to the native fat to give twice the level of ML for that fat.

Induction time and crystallization rate of these milk fat–CB blends were determined based on turbidity changes, using a Spectronic® Genesys™ 5 Spectrophotometer (Spectronic Instruments, Rochester, NY) with a thermoelectric cuvette holder and internal agitator (250 rpm). A wavelength of 490 nm was used to document changes in turbidity due to crystallization.

Samples were melted at 60°C with 1.3 g of fat weighed out in a Fisherbrand™ rectangular optical glass cuvette (Fisher Scientific, Pittsburgh, PA) containing a 7 × 2 mm magnetic stir bar. The samples were then cooled to 25°C to obtain representative rates of CB crystallization. Absorbance readings were taken automatically every 30 s until a maximum absorbance was reached. A crystallization rate was determined by the slope of the line fitted to the initial linear increasing region of the absorbance vs. time curve using least square linear regression. The point where the fitted line intercepted the x-axis was determined as the crystallization induction time. This induction time represents the point at which massive crystallization occurs, whereas the crystallization rate (initial slope of absorbance) provides a measure of how rapidly the crystal phase develops under these conditions. Neither parameter provides a fundamental measure of nucleation or growth rate, but only provides an easily measured parameter for comparison of crystallization kinetics.

Microstructure analysis. Images of the crystalline microstructure of CB and 10% milk fat–CB blends with varying levels of ML were taken using a Bio-Rad MRC-600 Confocal Laser Scanning Microscope (CLSM) (Integrated Microscopy Resources, University of Wisconsin, Madison, WI). CLSM allowed for a detailed image of the effect of ML on microstructure, and gave better images than a polarized light microscope. Images were enhanced using a combination of fluorescent dyes of Nile red and Nile blue at levels of 0.5 and 100 mg, respectively, per 500 g of fat. These dyes were chosen based on previous work (16). It was verified in preliminary crystallization experiments (data not shown) that this dye combination at these levels did not have a significant effect on crystallization kinetics of the milk fat–CB system.

Samples were prepared by dissolving the dye in the fat at 60°C for 16 h. A 1.3-g sample was then crystallized using a Spectronic® Genesys™ 5 Spectrophotometer with a thermoelectric cuvette holder and internal agitator. A temperature of 25°C and an agitation of 250 rpm were used when crystallizing samples. Blends of WAMF and VHM with cocoa butter were left to crystallize for 1 h and 30 min, before placing several drops of the fat slurry on a microscope slide. Samples were cooled to 10°C and held for 16 h before taking images, to ensure a fully crystalline sample.

X-ray diffraction. To examine the effect of ML from milk fat on the polymorphic change of CB, a STOE Powder X-Ray Diffractometer (STOE and CIE GmbH, Darmstadt, Germany) was used. Fat (*ca.* 0.75 g) was weighed in Teflon discs (Siemens Industrial Automation, Inc., Madison, WI). Blends of 10% milk fat–CB with varying levels of ML were tempered according to IUPAC Method 2.150 (23). The diffraction intensity was measured between angles of 5 and 32° (2θ), with a count time of 2 s and a step width of 0.05° per count. Samples were stored in a temperature cycling chamber (19 and 29°C at intervals of 6 h) with x-ray readings taken every 2 d to determine the polymorphic change from Form V to Form VI. These spectra were compared to standard x-ray spectra of increasing levels of Form VI polymorph to estimate the ratio of Form VI to Form V by the method of Bricknell and Hartel (3).

Chocolate preparation. Chocolate samples (500 g) were made by adding the appropriate levels of milk fat or milk-fat fractions and CB to the low-fat chocolate base to make a chocolate with 33% fat and 10% milk fat (on a fat basis). Three levels of ML were studied (no ML, natural levels, and twice the natural level) as discussed previously. The chocolates were prepared and tempered in a 1-L jacketed tempering beaker (Cole Parmer, Chicago, IL) with a Master Servodyne motor head and controller (Cole Parmer) to maintain constant rpm and to monitor viscosity. A modified method of Kleinert (24) was used to temper the chocolate. The chocolate was melted at 60°C for 30 min at a mixing speed of 80 rpm. It was then cooled to 26°C until a constant viscosity was reached (*ca.* 1.5 h). After this, the chocolate was heated to 32.8°C until a constant viscosity was reached. It was cooled again to 29°C and held until viscosity reached a constant level. Finally, it was heated to 32.3°C, where it was held until a constant viscosity was reached. About 12.5 g of tempered chocolate was poured into molds, which were then stored at 13°C for 24 h to solidify the chocolate.

Bloom formation. An accelerated bloom study was conducted by exposing the chocolate samples to cycling temperatures (19 and 29°C at 6-h intervals). Samples were analyzed daily for surface bloom using a Hunterlab Color Quest Colormeter (model Q45/0; Hunter Association, Inc., Reston, VA) as described by Lohman and Hartel (5). L*, a*, and b* values obtained from the colormeter were used to calculate the whiteness index (WI) as described by Lohman and Hartel (5).

$$WI = 100 - \left[(100 - L^*)^2 + a^{*2} + b^{*2}\right]^{0.5} \quad [1]$$

A total of eight readings were taken for each chocolate sample with the sample being rotated 90° for each reading. Change in WI with cycling time followed an S-curve with an induction period, a linearly increasing region of WI, and a saturation value after a certain WI was reached. From the slope of the linear region of the bloom development curve using least square linear regression, bloom rate was determined. The point where this fitted line intercepted the x-axis was determined as the bloom induction time.

RESULTS AND DISCUSSION

Chemical analyses. The composition of TAG and ML in each component fat is seen in Table 1. ML content varied from 3.2% for the cocoa butter to 2.0% for WAMF. The different milk-fat fractions contained different levels and types of ML based on separation during fractionation. However, no trends in separation could be detected from the results in Table 1, since the fatty acid composition of each compound also influences its partition coefficient during fractionation.

The fatty acid profiles of the component fats, the purified TAG of each fat, and the ML separated from each fat are shown in Tables 2–4, respectively. The fatty acid distributions of CB, milk fat, and milk-fat fractions (Table 2) were consistent with previous studies. Long-chain saturated fatty acids were concentrated in the higher-melting fractions, whereas short-chain and unsaturated fatty acids were concentrated in the lower-melting fractions. The purified TAG (Table 3) had essentially the same distribution as the intact fats. The fatty acid profiles of the pooled ML for each fat are shown in Table 4. In CB, myristic acid (C14:0) is slightly concentrated in the ML and the longer-chain fatty acids have been reduced in concentration. In general, the fatty acid content of the ML of milk fats was reduced in the shorter-chain fatty acids. Other differences in fatty acid composition between the ML and the native fats were small.

Acyl carbon number profiles are shown in Tables 5–7. CB is composed primarily of three TAG (C50–C54) with a minor amount of TAG with C34–C38. WAMF shows a bimodal distribution with peaks at C38 and C50. The higher-melting milk-fat fractions tend to have more higher acyl carbon number TAG and the lower-melting fractions have more lower acyl carbon number TAG, as expected. The purified fats have only slightly different acyl carbon profiles (Table 6). Interestingly, all of the shorter-chain TAG (C34–C38) were removed

TABLE 1
Composition of Neutral Lipids in Cocoa Butter, Milk Fat, and Milk-Fat Fractions[a,b,c]

Fat[d]	TAG	FA	1,3-DAG	Sterols	1,2-DAG	MAG
ICCB	96.8 ± 0.1	1.8 ± 0.0	1.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	Trace
SAMF	98.0 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	Trace
WAMF	98.0 ± 0.3	0.7 ± 0.1	0.7 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
VHM	97.6 ± 0.1	1.8 ± 0.1	0.4 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	Trace
HM	97.6 ± 0.2	1.6 ± 0.1	0.5 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
MM2	97.4 ± 0.2	1.5 ± 0.3	0.7 ± 0.1	0.2 ± 0.0	0.2 ± 0.2	0.1 ± 0.0
MM1	98.0 ± 0.2	0.8 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.7 ± 0.1	0.1 ± 0.1
LM	97.8 ± 0.8	1.2 ± 0.6	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.3	Trace

[a]Values determined by area percentage (peak area/total area).
[b]Average values determined by three replicates with standard deviations taken at 99% confidence intervals.
[c]Triacylglycerols (TAG), fatty acid (FA), 1,3-diacylglycerols (1,3-DAG), 1,2-diacylglycerols (1,2-DAG), and monoacylglycerols (MAG).
[d]Ivory Coast cocoa butter (ICCB), summer anhydrous milk fat (SAMF), winter anhydrous milk fat (WAMF), milk-fat fractions; very high-melting (VHM), high-melting (HM), middle-melting 2 (MM2), middle-melting 1 (MM1), and low-melting (LM).

TABLE 2
FA Content of ICCB, Anhydrous Milk Fat, and Milk-Fat Fractions[a,b]

FA	ICCB	SAMF	WAMF	VHM	HM	MM2	MM1	LM
C4:0	0.0	4.9	3.8	2.0	2.9	4.3	4.3	4.7
C6:0	0.0	2.2	2.3	1.6	1.7	2.4	2.5	2.7
C8:0	0.0	1.4	1.5	1.1	1.4	2.3	2.6	1.6
C10:0	0.0	2.5	3.0	2.2	2.6	3.1	2.7	3.2
C12:0	0.0	3.2	3.7	3.3	3.5	3.6	3.1	3.7
C14:0	6.8	17.9	11.4	12.4	13.7	12.2	12.1	10.9
C14:1	0.0	1.6	1.2	2.1	1.9	2.0	2.4	1.2
C16:0	29.3	30.5	31.4	38.5	35.0	29.9	33.8	29.5
C16:1	3.4	3.2	1.8	1.9	2.4	2.3	2.0	1.9
C18:0	30.6	11.1	14.1	20.4	16.2	12.3	13.9	12.2
C18:1	29.9	21.6	25.7	14.5	18.6	25.5	20.6	28.5

[a]Fatty acid values based on the average of three trials.
[b]Values given based on area percentage (peak area/total area). For abbreviations see Table 1.

TABLE 4
FA Content of Combined Minor Lipids Separated from Cocoa Butter, Milk Fat, and Milk-Fat Fractions[a,b]

Fatty acid	ICCB	WAMF	VHM	HM	MM2	MM1	LM
C4:0	0.0	1.9	2.1	2.2	2.3	1.9	2.2
C6:0	0.0	4.0	0.6	0.9	1.0	0.6	1.1
C8:0	0.0	0.8	0.8	0.8	0.8	0.9	1.0
C10:0	0.0	2.1	1.6	2.0	1.7	1.5	1.9
C12:0	0.0	4.4	2.8	7.2	8.2	3.0	3.3
C14:0	10.9	13.2	23.0	23.8	21.7	27.8	16.8
C14:1	0.0	1.6	0.0	0.0	3.2	0.0	3.0
C16:0	29.5	34.4	37.5	33.7	33.3	34.8	33.1
C16:1	9.4	2.6	4.2	4.7	8.9	6.5	7.9
C18:0	25.7	13.3	13.8	11.8	10.7	11.4	10.7
C18:1	24.5	21.7	13.7	12.8	14.9	11.6	19.1

[a]Fatty acid values based on the average of three trials.
[b]Values given based on area percentage (peak area/total area). See Table 1 for abbreviations.

from CB, indicating that these components were primarily the ML contained in CB. The acyl carbon number profiles of the ML for each fat are shown in Table 7. For CB, the TAG with C34–C38 were significantly concentrated in the ML, indicating the high content of DAG. In the milk-fat components, a bimodal distribution of acyl carbon number TAG was still found, but now the peaks had shifted slightly. In particular, the lower peak was shifted to a slightly lower acyl carbon number than the native milk fat.

Although the data shown in Tables 1–7 indicate the overall composition of the ML in these fats, the specific molecular composition (positional arrangement) is still unknown. Further analytical work will be necessary to completely characterize these components.

Crystallization kinetics. Differences in crystallization induction times were found with different levels of ML from milk fat in 10% milk fat–CB blends. Induction times were lowest for all milk fat and milk-fat fraction blends containing their natural level of ML, as seen in Figure 1. Both removing ML from milk fat and doubling the level of ML from milk fat resulted in a longer time for onset of nucleation to occur. The ML apparently serve as nucleating sites and aid in crystallization when present at lower levels; however, at higher levels they begin to interfere with crystallization. These results generally agree with previous studies on CB (12) and milk fat (25), which suggests that ML such as DAG, MAG, and phospholipids act as nucleating sites. It is interesting to see that different levels of ML in the different milk-fat fractions had varying impacts on induction time. This may be explained by the different composition and type of ML in each milk-fat fraction. For example, 1,2-DAG in palm oil and CB were found to delay crystal growth (10,26) and, in some cases, 1,3-DAG promoted crystallization. The 1,3-DAG may incorporate into the crystalline lattice more easily by mimicking more closely a CB TAG. These studies suggest that the levels of 1,2-DAG and 1,3-DAG may be more important than the over-

TABLE 3
FA Content of Purified TAG Components of Cocoa Butter, Milk Fat, and Milk-Fat Fractions[a,b]

FA	ICCB	WAMF	VHM	HM	MM2	MM1	LM
C4:0	0.0	4.6	3.5	4.7	3.7	5.1	4.7
C6:0	0.0	2.7	1.9	2.6	2.7	2.8	2.5
C8:0	0.0	2.3	1.4	1.9	1.8	2.1	2.3
C10:0	0.0	3.1	2.6	3.5	3.4	2.8	2.8
C12:0	0.0	3.6	3.4	4.2	3.7	3.0	3.0
C14:0	4.2	12.3	12.5	7.7	11.3	10.9	13.9
C14:1	0.0	2.8	2.5	2.4	1.9	2.9	3.2
C16:0	28.5	31.6	38.3	37.7	30.4	34.5	29.7
C16:1	4.9	2.8	1.3	1.7	1.9	1.6	2.3
C18:0	31.5	12.6	18.7	17.4	12.8	13.2	10.2
C18:1	30.7	21.5	13.6	16.2	26.5	19.4	25.2

[a]Fatty acid values based on the average of three trials.
[b]Values given based on area percentage (peak area/total area). See Table 1 for abbreviations.

TABLE 5
Acyl Carbon Content of Cocoa Butter, Milk Fat, and Milk-Fat Fractions[a,b]

Acyl carbon	ICCB	SAMF	WAMF	VHM	HM	MM2	MM1	LM
C26	0.0	2.9	4.4	2.4	3.1	3.3	1.5	3.0
C28	0.0	0.6	0.6	0.3	0.4	0.7	0.3	0.7
C30	0.0	1.0	1.1	0.6	1.7	1.2	0.6	1.3
C32	0.0	2.2	2.2	1.1	1.6	2.4	1.4	2.4
C34	0.1	5.0	4.6	2.7	3.7	5.3	3.8	5.9
C36	0.8	9.9	9.2	5.3	7.1	10.2	9.3	11.4
C38	1.3	12.7	12.3	5.8	9.7	14.2	11.0	15.0
C40	0.0	9.8	7.9	5.7	7.4	11.4	7.7	11.5
C42	0.0	6.4	6.2	5.3	6.4	6.7	4.7	6.6
C44	0.0	5.7	5.7	6.9	7.4	5.8	3.7	5.2
C46	0.0	6.4	6.5	10.3	8.7	5.4	3.5	5.2
C48	0.0	8.4	8.7	14.1	11.0	7.4	4.7	6.0
C50	21.5	11.8	12.4	19.1	14.3	9.8	7.4	9.1
C52	40.0	11.8	12.5	15.0	12.6	11.3	7.3	10.8
C54	36.3	5.5	5.8	5.6	4.9	5.1	3.3	5.8

[a]Acyl carbon values based on the average of three trials.
[b]Values given based on area percentage (peak area/total area). See Table 1 for abbreviations.

TABLE 6
Acyl Carbon Content of the Purified TAG Component of Cocoa Butter, Milk Fat, and Milk-Fat Fractions[a,b]

Acyl carbon	ICCB	WAMF	VHM	HM	MM2	MM1	LM
C26	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C28	0.0	0.6	0.3	0.3	0.6	0.4	0.7
C30	0.0	1.2	0.5	0.6	1.0	0.9	1.3
C32	0.0	2.4	1.1	1.3	2.1	1.8	2.5
C34	0.0	5.5	2.4	3.1	5.0	5.3	5.2
C36	0.0	11.2	5.2	6.6	11.7	13.5	11.6
C38	0.0	15.2	7.2	9.2	14.3	14.6	15.6
C40	0.0	12.0	6.3	8.1	11.6	11.8	12.8
C42	0.0	7.5	4.9	6.7	7.0	7.4	7.1
C44	0.0	6.7	7.3	7.6	6.0	5.6	5.7
C46	0.0	4.1	10.7	9.3	6.1	5.3	5.5
C48	0.0	8.7	14.6	11.8	7.6	7.0	6.7
C50	19.6	11.6	18.9	15.4	10.1	10.9	9.3
C52	47.2	8.3	15.1	13.9	11.3	10.8	10.8
C54	33.2	5.1	5.6	6.0	5.7	4.8	5.4

[a]Acyl carbon values based on the average of three trials.
[b]Values given based on area percentage (peak area/total area). See Table 1 for abbreviations.

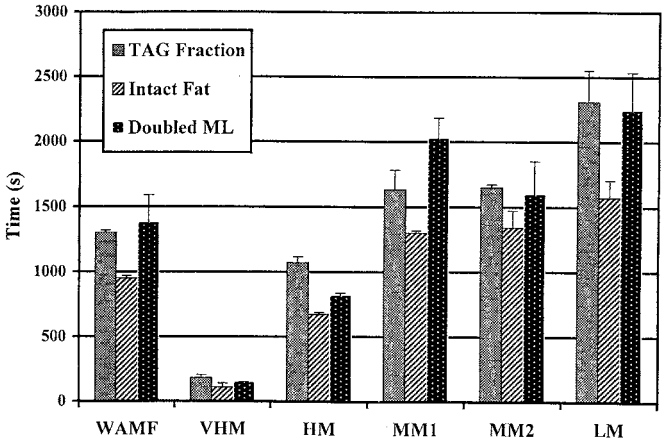

FIG. 1. Crystallization induction times for 10% milk fat–cocoa butter blends with varying levels of minor lipids (ML): no milk-fat ML [triacylglycerols (TAG) fraction], normal levels of milk-fat ML (intact fat) and twice the level of milk-fat ML (doubled ML). Winter anhydrous milk fat (WAMF) and milk-fat fractions; very high-melting (VHM), high-melting (HM), middle-melting 1 (MM1), middle-melting 2 (MM2), and low-melting (LM) milk-fat fractions. Samples were crystallized with agitation at 25°C. Induction time determined as the time when absorbance first began to increase.

all DAG concentration. ML play the greatest role in the initial nucleation step and play a smaller role in crystal growth. This was also seen in our results (data not shown) on crystallization rates, which were not very different between samples. Only the VHM, HM, and LM fractions showed statistically significant differences in crystallization rates with varying levels of ML. With these fractions, it was found that removing ML or doubling the ML level resulted in a slight decrease in crystallization rate.

Polymorphism. ML from milk fat not only affected nucleation and crystal growth but also had an impact on the polymorphic change of CB from Form V to Form VI. For all blends made with 10% milk fat in CB, the initial x-ray spectra showed that there was about 20% Form VI with the majority in Form V. After 4 d of temperature cycling, the blends made without ML contained about 80% Form VI, compared to only about 40% Form VI for blends with ML. After 10 d of temperature cycling, the blend without ML had reached *ca.* 100% Form VI, whereas the blends with ML had only reached about 60% Form VI. Higher levels of ML inhibited the polymorphic change from Form V to Form VI, which has been associated with bloom formation (1,27). Furthermore, the transition to Form VI in CB was inhibited by the presence of DAG (28).

TABLE 7
Acyl Carbon Content of Minor Lipids Separated from Cocoa Butter, Milk Fat, and Milk-Fat Fractions[a,b]

Acyl carbon	ICCB	WAMF	VHM	HM	MM2	MM1	LM
C12	0.17	3.4	1.7	1.4	1.4	1.5	3.4
C18	1.5	4.6	5.5	2.5	4.3	2.9	5.7
C24	2.9	18.5	12.1	11.1	16.6	6.2	22.8
C26	0	2.4	1.8	3.3	4.5	2.2	1.2
C28	0	4.0	6.8	4.4	6.7	1.8	2.6
C30	0	6.1	5.5	8.6	4.8	1.9	5.3
C32	0	8.7	6.8	1.4	0.8	4.7	4.3
C34	1.9	6.0	7.9	8.4	8.3	2.4	0.4
C36	10.0	1.2	3.7	3.0	1.1	5.7	2.4
C38	7.5	0.5	0.6	4.0	2.3	0.8	3.2
C40	0.5	2.5	2.2	0.8	3.4	1.3	1.2
C42	0	1.4	1.5	2.0	2.7	1.8	1.4
C44	0.7	3.9	4.7	4.3	4.9	3.5	1.7
C46	9.7	9.8	7.5	10.6	9.3	8.1	11.6
C48	26.7	13.4	11.0	14.9	12.6	15.8	17.6
C50	27.8	5.8	13.8	14.0	9.7	17.8	8.7
C52	9.4	7.1	5.1	4.0	4.8	17.2	6.0
C54	1.3	0.7	1.9	1.4	1.9	6.9	0.7

[a]Acyl carbon values based on the average of three trials.
[b]Values given based on area percentage (peak area/total area). See Table 1 for abbreviations.

Crystalline microstructure. In this system, large primary crystals with spherulitic shape were formed during crystallization at 25°C. These spherulites were surrounded by many very small secondary crystals, which were most likely formed during cooling of the slurry to 10°C. The primary crystals contained most of the crystalline fat in this system, since the solid fat content of CB at 25°C is 70–77%. Figure 2 shows the effect of removing the ML from CB on the primary and secondary crystal structure. When the ML were present, the primary crystals were essentially uniform spherulites (200–300 µm) with filaments radiating from a central nucleus (Fig. 2A). A narrow band, or halo, appeared on the outside edge of the crystal, a phenomenon also observed by Rousseau *et al.* (29). Removal of the ML from cocoa butter resulted in a significantly different crystalline microstructure (Fig. 2B) even though there was no apparent difference in polymorph (similar x-ray spectra). When the ML were removed, the spherulites were more irregularly shaped and contained mul-

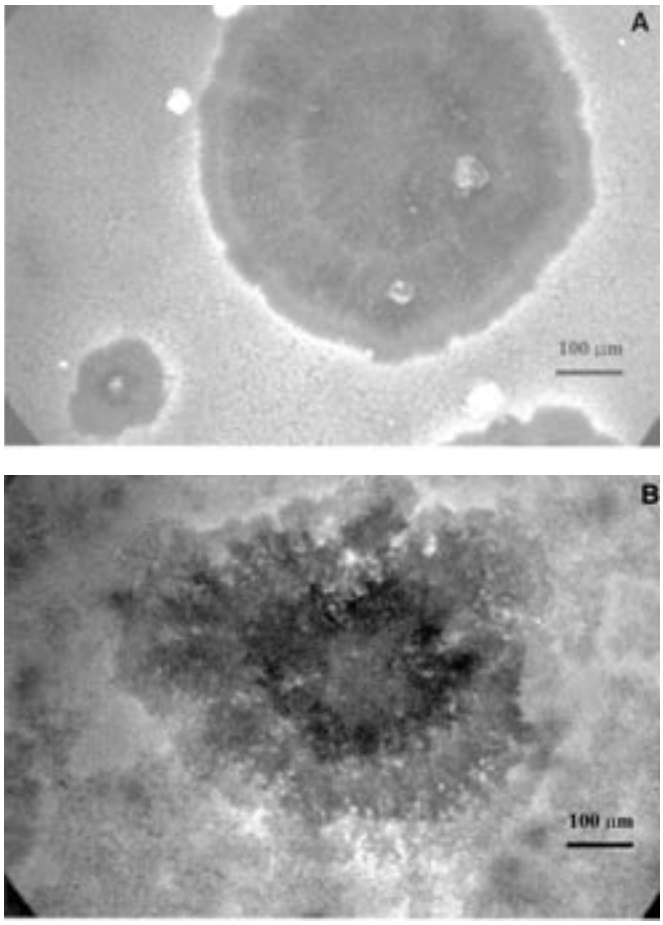

FIG. 2. Confocal laser scanning micrographs of (A) Ivory Coast cocoa butter, and (B) purified TAG component of Ivory Coast cocoa butter. Samples were stained with Nile red and Nile blue used in a ratio of 0.5:100. Magnification = 100×. Sample crystallized at 25°C for 1 h, placed on microscope slide and then further crystallized at 10°C for 16 h. For abbreviation see Figure 1.

tiple inclusions (potentially liquid fat). Apparently, the presence of ML helped organize the primary crystal structure within the spherulites in a more uniform way. The secondary crystal structure also was distorted upon removal of ML. In this case, secondary crystals were more diffuse and irregular. Similar results of the effects of ML on crystallization of milk fat were observed by Marangoni and Hartel (17). The effect of doubling the level of ML in CB was not studied.

The addition of 10% WAMF had only a minor effect on the crystalline structure of CB (Fig. 3A), although the primary crystals were slightly smaller than the pure CB due to inhibition of crystallization. In the milk fat–CB blends, removal of the ML from milk fat resulted in formation of primary crystals that were more random and open, with a branched structure, rather than filaments radiating from a central core (Fig. 3B). Once again, concentric rings were seen on the outside edge of the primary crystals. Secondary crystals were not affected significantly due to addition of 10% WAMF. Addition of twice the normal level of ML from milk fat had little effect

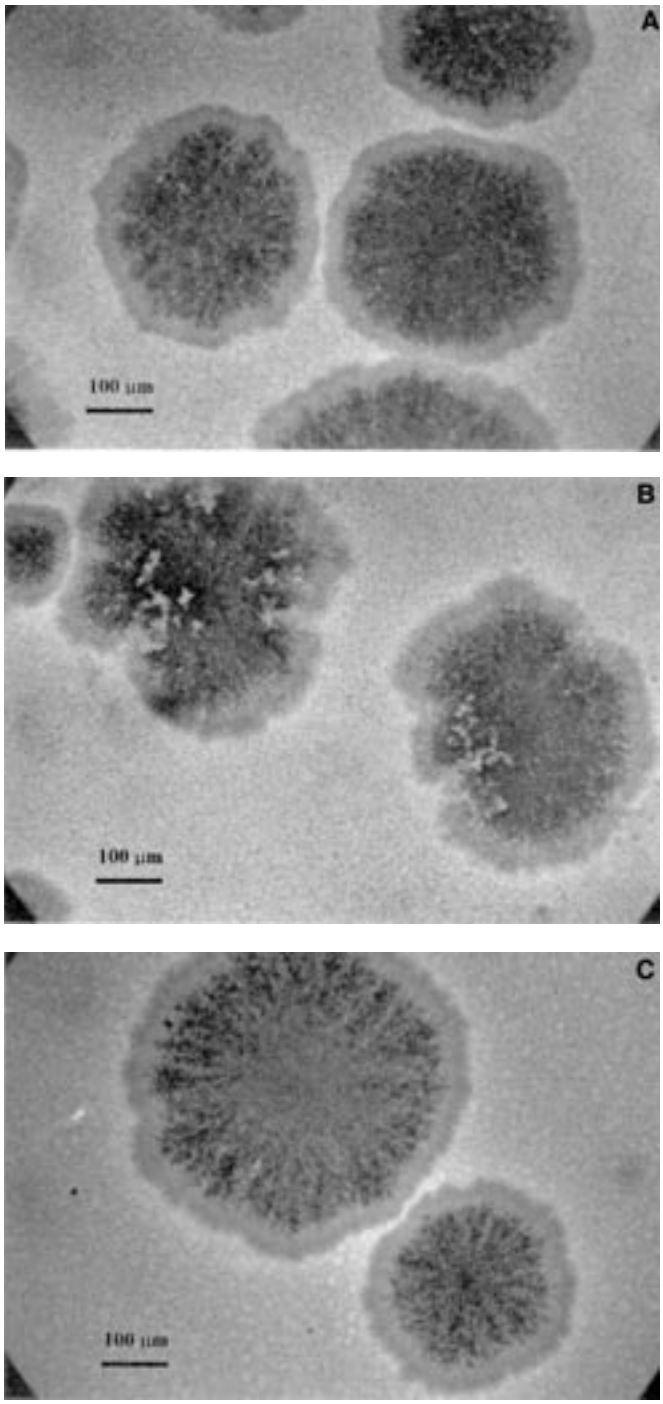

FIG. 3. Confocal laser scanning micrographs of Ivory Coast cocoa butter with (A) 10% winter milk fat, (B) 10% purified TAG of winter milk fat, and (C) 10% winter milk fat with twice the normal levels of minor lipids. Samples were stained with Nile red and Nile blue used in a ratio of 0.5:100. Magnification = 100×. Sample crystallized at 25°C for 1 h, placed on microscope slide, and then further crystallized at 10°C for 16 h. For abbreviation see Figure 1.

on the primary crystalline structure, but resulted in larger and more well-defined secondary crystals (Fig. 3C).

Addition of 10% VHM had a significant effect on CB crystal structure (Fig. 4A). Both primary and secondary crystals

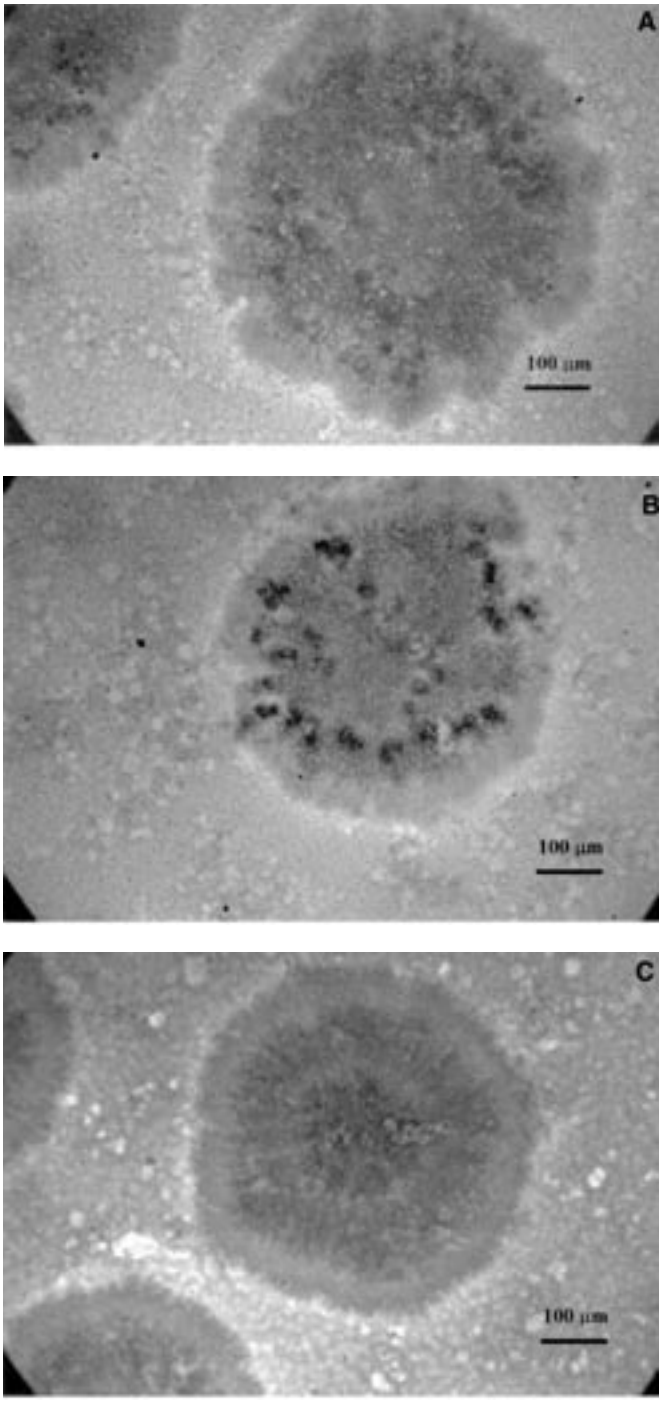

FIG. 4. Confocal laser scanning micrographs of Ivory Coast cocoa butter with (A) 10% VHM, (B) 10% purified TAG of VHM, and (C) 10% VHM with twice the levels of minor lipids. Samples were stained with Nile red and Nile blue used in a ratio of 0.5:100. Magnification = 100×. Sample crystallized at 25°C for 1 h, placed on microscope slide, and then further crystallized at 10°C for 16 h. For abbreviations see Figure 1.

were more irregular than those found in pure CB or even with 10% WAMF. In this case, primary crystals had numerous concentric rings, or haloes, perhaps indicative of significant cocrystallization between the high-melting component of milk fat and the CB. Removal of the ML from the VHM resulted in even more irregular primary crystals in the CB blend (Fig. 4B). The secondary crystals were also more random in size and morphology. Addition of twice the level of ML in the VHM fraction resulted in even greater disruption of the CB crystal structure (Fig. 4C). Here, the primary crystals contained several strong concentric rings as well as many inclusions. The secondary crystals also were more random in size and morphology, even than those found upon removal of ML.

These results indicate that different milk-fat components result in solidification of different lipid crystalline networks in this system with CB. Both TAG composition and ML level influenced the solidification properties of the CB. The presence of the ML at low levels may direct the formation of uniform spherical crystals by serving as nucleation sites and filling lattice spaces in the CB crystals. Removal of the ML, as well as addition of twice the level of ML, generally led to more random crystal growth. These results may lead to different physical properties in lipid-based foods, like chocolate. For example, formation of fat bloom in chocolate may be, in part, related to migration of liquid fat through the chocolate structure. Different CB crystal structures result in different oil migration rates and may impact rate of bloom formation in chocolate. To test this hypothesis, chocolates were made with different levels of ML and tested for bloom stability.

Bloom formation. To understand the importance of chemical composition and microstructure on bloom formation in chocolate, the different levels of milk-fat ML (none, natural level, and twice the natural level) for each fraction were compared. Significant differences in bloom development were found for the different levels of ML contained in the milk-fat fractions added to chocolate. Figure 5 compares the effects of ML in WAMF on change in WI during temperature cycling of chocolate. Bloom development was best inhibited in these chocolates with the natural level of ML in WAMF. Chocolates made with increased levels of ML from milk fat or with

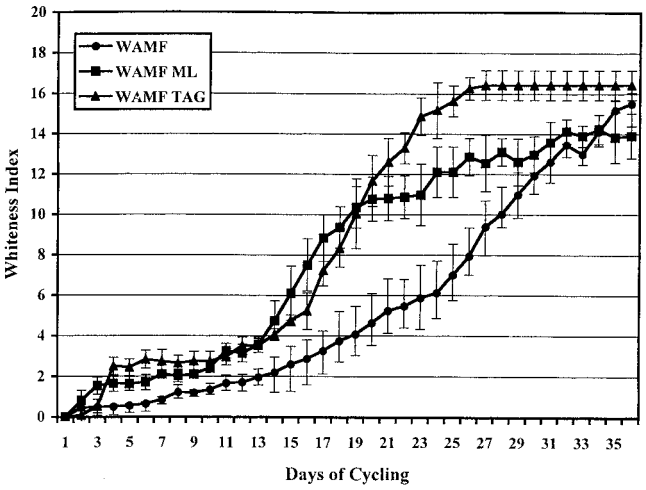

FIG. 5. Bloom development of chocolates made with 10% WAMF with varying levels of ML. No milk-fat ML (WAMF TAG), normal levels of ML (WAMF), and twice the level of ML (WAMF ML). For abbreviations see Figure 1.

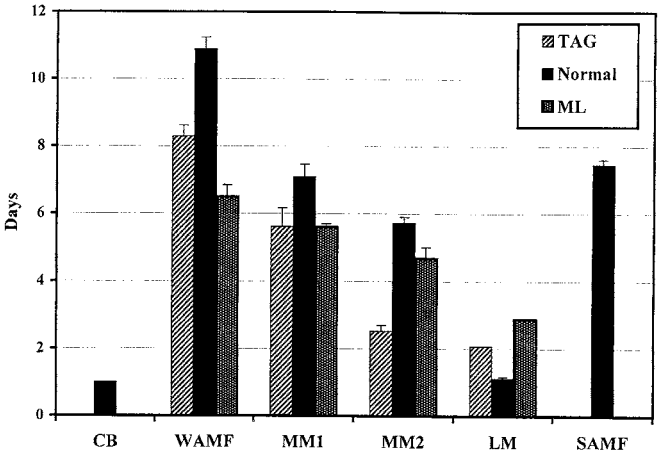

FIG. 6. Bloom induction time for chocolates made with 10% milk-fat fractions with varying levels of ML. No milk-fat ML (TAG), normal levels of ML (Normal), and twice the level of ML. Cocoa butter (CB), WAMF, summer anhydrous milk fat (SAMF), milk-fat fractions; MM1, MM2, and LM milk-fat fractions. VHM and HM fractions did not show visual bloom after 60 d and were not included. See Figure 1 for other abbreviations.

no milk-fat ML exhibited more rapid bloom formation. Figure 6 shows that, in general, the induction time for onset of bloom was longest for chocolates made with milk-fat components containing their natural level of ML. Again, either removing milk-fat ML or adding twice the level of ML generally resulted in faster onset of bloom formation. The rate of bloom formation (slope of linear increasing portion of WI) was less affected by the ML (data not shown), although rate of bloom formation was slightly lower for chocolates made with milk-fat components containing their natural level of ML.

The ML from milk fat clearly affected crystallization kinetics and the nature of the crystalline microstructure. The ML from milk fat also had an impact on bloom formation in chocolates, potentially through their effect on crystalline microstructure. This relation between crystalline structure and physical properties of lipid-based foods should be explored further.

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