

CRYSTALLIZATION BEHAVIOR OF BLENDS OF COCOA BUTTER AND MILK FAT OR MILK FAT FRACTIONS

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Abstract

Mixtures of milk fat or milk fat fractions, produced by melt crystallization, and cocoa butter were studied using isothermal calorimetry. Crystallization of cocoa butter (at 15, 20 and 25°C) was observed, and induction time for nucleation, peak time and amount of heat produced were recorded. Melting profiles and X-ray spectra were also obtained, yielding information about extent of crystallization and type of polymorph obtained. Induction time for nucleation generally increased with increasing temperature. Peak time increased at 15°C, but decreased at 20°C. Amount of crystallized fat decreased with increasing level of milk fat.

Keywords: cocoa butter, fat blends, fat crystallization, milk fat

Introduction

The solidification properties of the fat phase in chocolate (cocoa butter, CB) are of special interest in the chocolate industry. The desired quality of chocolate (hardness, snap, gloss and desirable melting properties) can be achieved by proper crystallization of CB during tempering of chocolate. Cocoa butter is mainly composed of only a few triacylglycerols, such as POP, POS and SOS (P: Palmitic acid, O: Oleic acid, S: Stearic acid) [1]. The melting temperature range of CB is typically between 30–35°C [2, 3].

Milk fat (MF) is one of the main components of milk chocolate and is often added to dark chocolate to control hardness. MF is also desired as a natural product that has excellent organoleptic properties in many foods. More than 400 fatty acids have been identified in MF [4]. It has been found that seasonal, geographical and environmental variations affect the composition of milk fat [5–7]. As a result of the diversity of fatty acids, MF has a very wide melting tempera-

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ture range from -40 to 40°C [8, 4]. The physicochemical properties of MF are very complex, and its functional incompatibilities with other food ingredients restrict its use in the food industry.

Utilization of MF can be increased through modifications, such that its functionality in food products, including chocolate, is improved. Modification of MF by chemical processes such as hydrogenation or interesterification has not been popular, since the natural state of the fat is altered by these processes. However, MF, with a broad melting range due to diversity in fatty acid structure, is a very suitable material for physical fractionation, which relies primarily on the melting points of different triacylglycerols [9]. The absence of organic solvents, the use of low cost, relatively simple instrumentation and minimal loss of aroma components compared with other methods have made melt-fractionation the most popular method in the industry [10, 7].

CB and MF are compatible up to a limit of about 30% milk fat [11]. MF does not affect the polymorphism of CB; however, the melting point of each polymorph is lower in blends due to the complex lipid structure of MF [11, 12]. In addition, MF decreases the rate of cocoa butter crystallization and causes softening of chocolate and confectionery products [13]. Jordan [14] showed that about 3% addition of a high melting fraction of MF into milk chocolate did not result in additional softening. Higher levels, however, resulted in increased softening. Moreover, hard milk fat fractions inhibit fat bloom in chocolate [15–18], while soft milk fat fractions sometimes enhance bloom [18].

In order to increase and optimize the use of milk fat fractions in chocolate, it is important to understand the compatibility/incompatibility between fats due to crystallization kinetic effects. The overall objective of this study was to study the crystallization behavior of cocoa butter with blends of milk fat or its fractions. Induction time for nucleation, peak time of the crystallization exotherm, and relative amount of crystalline material formed during isothermal crystallization were determined.

Material and methods

Materials

Ivory Coast cocoa butter was supplied from Guittard Chocolate Company, Burlingame CA. Anhydrous milk fat (AMF) from the winter feeding season, used for dry fractionation, was obtained from Level Valley Dairy, West Bend, WI.

Milk fat fractionation

AMF was held for one hour at 60°C , with stirring, in a 2 liter jacketed tempering vessel equipped with two internal baffles to ensure complete melting prior to fractionation. The temperature of AMF was changed to the crystal-

lization temperature, using a circulating refrigerated water bath, after which the AMF was held at constant temperature and agitated (100 rpm) for 24 h to obtain equilibrium crystallization. Once equilibrium was obtained, the AMF slurry was vacuum-filtered through Whatman #1 filter paper, while the crystallization temperature was maintained.

AMF was fractionated at three crystallization temperatures: 28, 30 and 32°C. The liquid components of these three fractionations were combined and crystallized at 23°C. The liquid fraction of the 23°C crystallization was crystallized at 17°C, and solid and liquid fractions were separated. A super-stearin fraction was obtained in two steps. First, AMF was fractionated at 27°C, and the solid fraction was separated from the liquid fraction. The solid fraction was then melted in the second step and crystallized at 32°C. The solid fraction of this second crystallization was filtered from the liquid fraction. In total, seven milk fat fractions (6 solid and 1 liquid) were produced from the AMF.

Melting point

The capillary-melting points (clear point) of milk fat, milk fat fractions and cocoa butter were determined using AOCS method Cc-125 [19].

Fat samples were melted at 60°C and placed in open-ended capillary tubes about 10 mm in height. The capillary tubes were fused at one end and held in a refrigerator at 5°C for 16 h. The tubes, attached to an AOCS-specification H 7-45 thermometer, were immersed in a water-filled beaker. The agitated water bath was slowly heated. The temperature at which the fat became completely clear was reported as the melting or clear point.

Fatty acid analysis

Fatty acid analysis was conducted on a Hewlett Packard Series II Plus 5890 GC (Hewlett Packard, Brookfield, WI), equipped with a flame-ionization detector (FID) and on-column injector. The column was a Supelco 30 m X 0.32 mm AT-WAX, Heliflex, fused-silica capillary column (Altech, Deerfield, IL), and the carrier gas was helium with a flow rate of 2 ml min⁻¹. The detector temperature was 300°C. The butyl esters were separated using the following temperature profile: initial hold at 55°C for 2 min, increase to 160°C at 30°C min⁻¹, increase to 190°C at 3°C min⁻¹, then increase to 227°C at 2°C min⁻¹ followed by a 2 min hold once temperature is reached. The data was collected and manipulated using ChemStation Chromatography software on a Hewlett Packard Vectra VL2 4150 computer interfaced to the GC by a Hewlett Packard 7673 controller (Hewlett Packard, Brookfield, WI).

The fatty acids in the fat samples were converted to butyl esters using a modified method of Iverson and Sheppard [20]. 75 mg of fat and 2 ml of 0.5M NaOH in butanol were placed into a screw-top test tube. 1 ml of nonanoic

acid (C9) internal standard (10 mg C9/1 ml pentane) was added to the mixture. The mixture was capped, vortexed and boiled for 10 min, and cooled to room temperature. 2.5 ml of 14% boron trifluoride in butanol were added and the tubes once again capped, vortexed and boiled for 10 min. After cooling to room temperature, 7.5 ml of pentane and 7.5 ml of methanol in water (6:39 v/v) wash solution were added and the mixture was centrifuged for 10 min. The aqueous phase was removed. This washing procedure was repeated two more times. Anhydrous sodium sulfate was then added to the remaining organic phase to absorb residual water. 0.5 ml of the sample was injected into the GC and analyzed for fatty acid butyl esters on a composition % spare basis.

Triacylglycerol analysis

Acyl carbon analysis was conducted on a Varian Model 3700 GC (Varian Association, Palo Alto, CA), equipped with a flame-ionization detector (FID) and on-column injector. The WCOT fused-silica stainless-steel capillary column (2.5 m×0.25 mm) was used, with helium gas at a flow rate of 2 ml min⁻¹ as a carrier gas. Hydrogen gas was supplied to the FID at all times. The detector temperature was regulated to 370°C, and the following column temperature profile was used: initial hold at 220°C for one minute, then increase to 355°C at 3°C min⁻¹.

The samples were prepared using a modified method of Lund [21]. 6 mg of fat were dissolved in 1 ml isooctane, and an internal standard of trinonanoïn (164 mg trinonanoïn/ml isooctane) was added. 0.3–0.5 ml of this solution was then injected into the GC, and the acyl carbon number profiles were calculated on a composition % basis.

Differential scanning calorimeter (DSC)

An automatic-cooling Differential Scanning Calorimeter (SSC/5200, Seiko Instruments, Inc.), attached to a disk station (55C5200H), was employed in isothermal mode. The DSC was calibrated with mercury, indium, lead and tin standards prior to analyses. Dry nitrogen gas was used to purge the thermal analysis system and liquid nitrogen was used to cool the system. 5–12 mg of fat were sealed into an aluminum sample holder (TA Instruments, New Castle). Changes in heat flow during isothermal DSC operation were recorded. The following temperature protocol was used: hold at 80°C for 5 min, cool to 50°C at a rate of 100°C min⁻¹ and hold for 3 min, cool at 100°C min⁻¹ to the isothermal temperature studied (15, 20 or 25°C), hold for 180 min. Following 3 h isothermal hold, samples were heated to 80°C at a rate of 20°C min⁻¹ to record melting profiles.

Each of the seven milk fat fractions and AMF were incorporated into cocoa butter at 5% and 10% levels. Induction time for nucleation and peak time for

the crystallization exotherm were determined from the isothermal exotherm. Enthalpy of melting, as found from the melting scan, was used as a direct indicator of the amount of crystallized fat.

X-ray diffractometer

X-Ray Diffraction (XRD) analyses were made on the pure fats and the mixtures to determine crystal form. A Nicolet I₂v diffractometer, using CuK_α radiation with a wavelength of 0.514 nm, was used to perform powder-XRD measurements. The diffraction measurements were made between 5 and 32° (2θ), with a counting time of 2 seconds and step width of 0.05°.

Prior to measurements, samples were melted at 80°C and poured into the sample holders. The X-ray diffraction patterns of the fat samples were recorded at 17±1.0°C after 3 h of solidification at the same temperature, to provide verification of the type of polymorph formed during isothermal crystallization.

Solid fat content

The solid fat content (SFC) at a given temperature was determined using a Bruker 120 Minispec NMR (Bruker, Ontario, Canada). The IUPAC method (2.150) was used to temper all samples prior to SFC determination [22]. The temperature profile was: 60°C for 30 min, 0°C (in ice) for 90 min, 26°C for 40 h, 0°C for 90 min, prior to holds of 60 min at each temperature studied. SFC was determined at 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 and 55°C.

Results and discussion

General designation of fats

Milk fat fractions were defined according to their melting properties and SFC values. The principal designation defines a general melting property based on capillary melting point (*m.p.*). These were defined as: VH=very high melting (*m.p.* > 45°C); H=high melting (35°C < *m.p.* < 45°C); M=medium melting (25°C < *m.p.* < 35°C); L=low melting (10°C < *m.p.* < 25°C).

The fat was then described by the following designation: X - a, b, c, where X is the general melting property, *a* is SFC at 5°C, *b* is SFC at 25°C, and *c* is melting point. For example, the highest melting fraction had an SFC at 5°C of 73%, SFC at 25°C of 58%, and *m.p.* of 49°C, and was designated as VH - 73, 58, 49. The fats used in this study are tabulated, according to this general designation in Table 1.

Fatty acid analysis

The fatty acid profiles for CB, AMF and its fractions are given in Table 2. CB consisted of five fatty acids, of which almost 96% were C16:0, C18:0 and

Table 1 General designations of fats

Fat type	Remarks
CB(M) - 84, 61, 32 ^a	Ivory Coast cocoa butter
AMF(H) - 51, 18, 37	Anhydrous milk fat from winter feeding season
VH - 73, 58, 49	Solid fraction crystallized first at 27°C, then at 32°C
VH - 69, 53, 48	Solid fraction crystallized at 32°C
VH - 67, 48, 46	Solid fraction crystallized at 30°C
H - 61, 38, 44	Solid fraction crystallized at 28°C
H - 71, 43, 41	Solid fraction crystallized at 23°C from pooled liquid fractions of 32, 30 and 28°C solid fraction
L - 59, 9, 23	Solid fraction crystallized at 17°C from the liquid fractions of 23°C solid fraction
L - 23, 0, 12	Liquid fraction of 17°C crystallization

^a X - a, b, c, where X is the general melting property (CB=cocoa butter, AMF=anhydrous milk fat, VH=very high melting, H=high melting, M=medium melting, L=low melting), a is SFC at 5°C, b is SFC at 25°C and c is melting point

C18:1. AMF and milk fat fractions had more diverse fatty acid profiles, ranging from C4:0 to C18:3. Fatty acid analysis showed some significant compositional differences between the milk fat fractions. In general, very high and high melting fractions were enriched in longer-chain, saturated fatty acids (C16:0 and C18:0). The fraction crystallized at 23°C (H - 71, 43, 41) showed compositional similarities to the fraction crystallized at 28°C (H - 61, 38, 44). The H - 71, 43, 41 fraction had a slightly lower concentration of short-chain fatty acids (C4:0-C8:0), but had a higher amount of C18:2 than did the H - 61, 38, 44 fraction. The H - 71, 43, 41 fraction also showed the highest concentration of C14:0 among AMF and its fractions. The higher SFC values of the H - 71, 43, 41 fraction, as compared to the H - 61, 38, 44 fraction, can be attributed to the slightly lower level of short-chain fatty acids and slightly higher level of long-chain, saturated fatty acids. The low melting fractions (L - 59, 9, 23 and L - 53, 0, 12) exhibited the opposite trend, with the highest levels of short-chain fatty acids and long-chain, unsaturated fatty acids.

Triacylglycerol analysis

Table 3 shows the acyl carbon number profiles for CB, AMF and milk fat fractions. CB was composed of the high molecular weight TAGs, namely C50, C52 and C54. The AMF had more diverse TAGs with high concentrations of C34, C36, C38 and C40. In general, the milk fat fractions showed a trend of decreasing high molecular weight TAGs and increasing middle molecular weight TAGs with decreasing fractionation temperature. The very high melting fractions (VH - 73, 58, 49, VH - 69, 53, 48 and VH - 67, 48, 46) had higher

Table 2 Fatty acid profiles of fats

Fatty acid	Composition ^a /%										
	CB ^b - 84, 61, 32	AMF - 51, 18, 37	VH - 73, 58, 49	VH - 69, 53, 48	VH - 67, 48, 46	H - 61, 38, 44	H - 71, 43, 41	L - 59, 9, 23	L - 53, 0, 12		
C4:0	-	5.8	3.5	5.0	4.1	6.4	3.9	7.1	7.9		
C6:0	-	3.1	1.8	2.3	2.4	3.1	2.2	3.6	4.3		
C8:0	-	1.7	1.0	1.2	1.3	1.6	1.3	1.7	2.2		
C10:0	-	3.5	2.3	2.7	2.7	3.2	3.1	3.2	4.2		
C12:0	-	3.6	2.9	3.2	3.2	3.6	3.9	3.0	4.1		
C14:0	-	11.2	11.8	12.1	12.0	12.3	13.3	10.0	11.4		
C14:1	-	0.8	0.5	1.0	0.6	0.7	0.6	0.8	1.0		
C15:0	-	1.5	1.7	1.6	1.7	1.6	1.7	1.5	1.4		
C16:0	27.2	30.6	38.5	36.8	36.3	34.2	35.6	32.7	24.7		
C16:1	-	1.3	0.7	0.8	0.9	1.0	0.9	1.1	1.6		
C18:0	35.4	13.6	21.8	19.2	19.1	16.3	17.0	14.5	9.6		
C18:1	33.3	19.9	11.6	11.5	13.6	14.8	14.4	17.9	23.8		
C18:2	3.1	3.1	1.5	2.5	1.8	1.3	1.8	2.4	3.8		
C18:3	-	0.5	0.3	-	0.4	-	0.4	0.5	-		
C20:0	1.0	-	-	-	-	-	-	-	-		

^b Composition % = (fatty acid peak area)/(total peak area)*100

^a X - a, b, c, where X is the general melting property (CB = cocoa butter, AMF = anhydrous milk fat, VH = very high melting, H = high melting, M = medium melting, L = low melting), a is SFC at 5°C, b is SFC at 25°C and c is melting point

Table 3 Acyl carbon number profiles of fats

Acyl carbon number	Composition ^a /%											
	CB ^b - 84, 61, 32	AMF - 51, 18, 37	VH - 73, 58, 49	VH - 69, 53, 48	VH - 67, 48, 46	H - 61, 38, 44	H - 71, 43, 41	L - 59, 9, 23	L - 53, 0, 12			
C26	-	1.4	0.2	0.3	0.9	0.6	0.1	0.6	0.7			
C28	-	1.4	0.5	0.4	0.9	0.7	0.3	0.9	1.1			
C30	-	2.6	0.9	0.7	1.4	1.6	0.6	2.1	2.2			
C32	-	5.3	2.2	1.6	2.3	3.2	1.7	4.2	3.8			
C34	-	10.4	4.6	3.5	5.6	6.7	4.3	10.4	8.4			
C36	-	16.7	7.4	6.8	10.0	10.8	8.0	20.5	14.8			
C38	-	17.1	8.0	8.3	11.0	11.8	9.5	21.0	15.2			
C40	-	11.3	6.3	6.7	8.0	8.4	8.0	12.9	11.0			
C42	-	6.4	4.9	5.3	6.0	5.9	7.5	7.0	6.9			
C44	-	4.9	6.3	6.6	6.9	6.5	9.2	4.9	5.8			
C46	-	4.7	10.0	9.8	9.2	8.1	10.7	3.8	6.0			
C48	-	5.1	14.2	13.8	11.4	9.9	12.1	4.0	6.8			
C50	24.6	6.0	17.3	17.5	13.5	12.3	13.6	4.0	7.3			
C52	44.2	5.0	12.9	13.3	10.0	10.0	10.3	2.8	6.6			
C54	31.3	1.9	4.4	5.6	3.1	3.4	4.1	1.0	3.7			

^a Composition % = (triacylglycerol peak area)/(total peak area)*100

^b X - a, b, c, where X is the general melting property (CB=cocoa butter, AMF=anhydrous milk fat, VH=very high melting, H=high melting, M=medium melting, L=low melting), a is SFC at 5°C, b is SFC at 25°C and c is melting point

concentrations of C46–C52 and lower concentrations of C34–C40 than did AMF. The 23°C-solidified fraction (H – 71, 43, 41) had higher concentrations of C42 and C44 than did AMF, very high melting fractions and the other high melting fraction (H – 61, 38, 44). The H – 71, 43, 41 fraction also contained higher concentrations of C46 and C52. This may be due to a large proportion of higher molecular weight TAGs leftover in the liquid from 32, 30 and 28°C solid fractions, which was used to produce the 23°C solidified fraction. The low melting fractions (L – 59, 9, 23 and L – 53, 0, 12) had higher concentrations of C36–C40 and lower concentrations of C46–C52.

Isothermal DSC

A typical exothermal heat-flow curve resulting from crystallization during the isothermal hold in the DSC is given in Fig. 1 for the blend of 5% VH – 73, 58, 49 with 95% CB crystallizing at 20°C. Crystallization is noticeable by the small exotherm that occurs after a certain induction time (t_i), determined as the point of initial deviation from the baseline. Heat flow increased until the peak-maximum time (t_{pm}), at the maximum crystallization heat flow, was reached, and then decreased to the baseline [23].

After isothermal crystallization, the fat samples were melted in the DSC to record their melting curves. A representative melting curve, for the blend of 5% VH – 73, 58, 49 with 95% CB, is shown in Fig. 2.

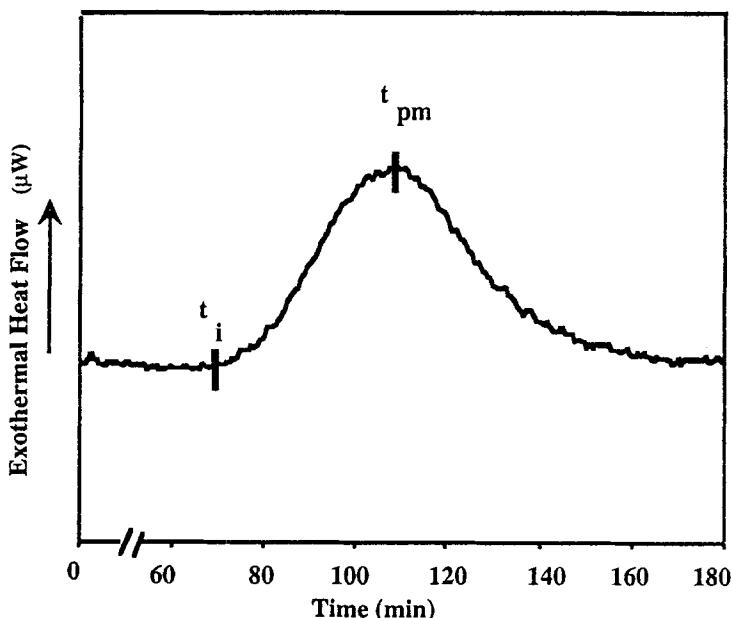


Fig. 1 DSC isothermal crystallization curve for the blend of 5% very high melting milk fat fraction (VH – 73, 58, 49) with 95% cocoa butter (CB – 82, 59, 32), where t_i = induction time and t_{pm} = peak-maximum time. Designation of fats is as given in Table 1

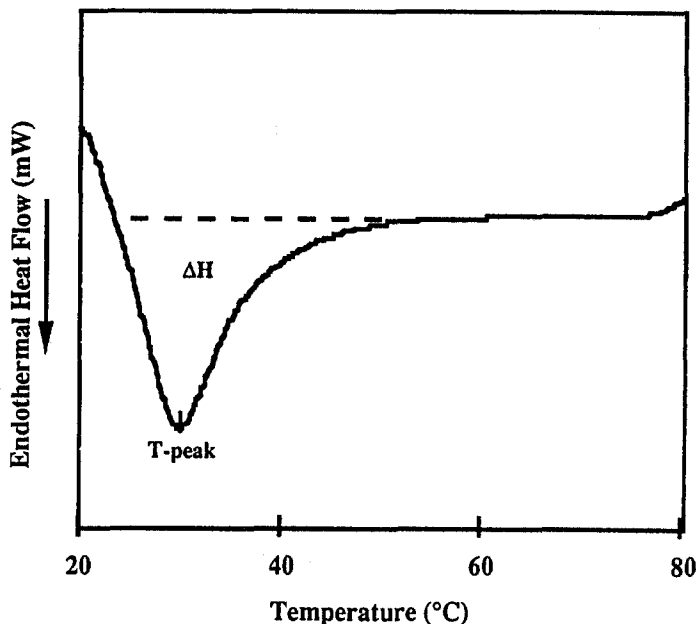


Fig. 2 DSC melting curve for the blend of 5% very high melting milk fat fraction (VH - 73, 58, 49) with 95% cocoa butter (CB - 82, 59, 32), after isothermal crystallization at 20°C for 3 h. ΔH is the enthalpy of melting and T -peak is the melting peak temperature. Designation of fats is as given in Table 1

The isothermal crystallization and melting curves of the fat samples showed similar behavior. CB had a single crystallization peak and a single melting peak. All pure fats showed single crystallization and melting peaks varying only in width and height. The addition of AMF at a 5% level to CB did not change the shape of the crystallization curve, whereas 10% addition of AMF caused splitting of the crystallization peak. This may be due to a physical separation of TAGs in the mixture, since the melting curve of the blend had only one peak. The crystallization curves of blends of milk fat fractions with CB showed a single peak, but broader in shape than those of the pure components alone.

X-ray powder diffractometer (XRD) analyses showed that all pure fats, except low melting ones, crystallized in the β' -form, based on analysis of short spacings at 4.35 and 4.17 Å [24], after isothermal crystallization for 3 h. Low melting milk fat fractions crystallized in a form resembling the α -polymorph, with a single short spacing at 4.27 Å [11, 24]. However, the blends of AMF or milk fat fractions, even the low melting milk fat fractions, with CB crystallized in the β' -form. The presence of these additives at 5 or 10% levels did not cause a change in the crystallographic dimensions of CB. This means that the additive molecules were incorporated in the crystal lattice, without interfering with the

crystal packing of CB. Melting-peak temperatures of the fat mixtures confirmed the occurrence of β' crystals of CB [11, 24].

Induction time

Induction times for the pure fats and the mixtures of CB with AMF or its fractions are given in Table 4. AMF, very high melting and high melting milk fat fractions crystallized very rapidly (between 0.4 and 4.5 min) during the 3 h time period at the temperatures studied (15, 20 and 25°C). Statistical analysis using *t*-tests at the 95% confidence interval (*CI*) showed no significant differences either among the milk fat fractions or between the AMF and its fractions. Low melting milk fat fractions (L - 59, 9, 23 and L - 23, 0, 12) did not crystallize in 3 h at the temperatures studied. CB crystallized more slowly than did AMF and its fractions at 15 and 20°C, but did not crystallize in 3 h at 25°C. In addition, induction time for nucleation of CB was shorter at 15°C than at 20°C. However, no significant difference was observed in the induction times of AMF or the milk fat fractions at the three temperatures (*CI*=95%). It is generally believed that in a bulk fat, crystallization starts at the surface of extraneous substances. Therefore, nucleation is always heterogeneous [4]. The deeper the supercooling, the greater the possibility that any impurity present can catalyze nucleation. Consequently, nucleation rate is higher at lower temperatures, resulting in a shorter induction time for nucleation [4]. Cocoa butter shows this behaviour quite well, while AMF and its fractions do not.

5% addition of AMF or its fractions to CB generally caused a slight increase in induction time for nucleation of CB at 15 and 20°C. However, a statistically significant increase (*CI*=95%) in the induction time for nucleation at 15°C was only observed for 5% addition of H - 71, 43, 41 or L - 59, 9, 23 fractions to CB. These fractions were composed mainly of shorter chain fatty acids and TAGs. It has been stated that if the chain length of an additive is shorter than that of a macrocrystallizing substance, the additive is likely to hinder nucleation [25]. The crystallization of CB at 20°C was significantly retarded by addition of AMF or 46°C-melting point milk fat fraction (VH - 67, 48, 46). None of the other fractions caused a change (*CI*=95%) in induction time for nucleation of CB at 20°C. Note that addition of H - 71, 43, 41 prevented CB crystallization at 20°C, at least for the 3 h duration of this study. No crystal formation was observed in 3 h, either in pure CB or in any of the 5% mixtures at 25°C.

Induction time for nucleation of CB generally increased to a greater extent in the presence of 10% AMF or its fractions, as compared to 5% addition, indicating greater inhibition of nucleation of CB. At 10% addition level, milk fat fractions with the lowest melting points (41, 23 and 12°C) extended the induction time of CB at 15°C to a greater extent, compared to the other additives (*CI*=95%). In addition, these three fractions prevented crystallization of CB in

Table 4 Induction times for mixture of cocoa butter (CB) with milk fat (AMF) or its fractions

Fat type ^a	AMF or its fractions		Induction time/min		
	% in mixture	$T_{\text{isothermal}}/^{\circ}\text{C}$			
		15	20	25	
CB - 84, 61, 32	100	9.4±1.2	58.0±8.0	N ^b	
AMF - 51, 18, 37	5	13.2±0.8	72.6±4.5	N	
	10	15.3±0.9	72.9±4.4	N	
	100	2.6±0.4	2.1±0.5	4.5±0.4	
VH - 73, 58, 49	5	12.7±3.0	60.5±0.2	N	
	10	20.8±3.5	64.4±7.2	41.7±0.1	
	100	1.4±0.2	0.9±0.1	0.4±0.5	
VH - 69, 53, 48	5	11.9±0.8	46.2±1.2	N	
	10	10.1±6.1	57.6±13.0	N	
	100	0.8±0.4	1.2±0.1	0.7±0.1	
VH - 67, 48, 46	5	15.2±0.7	70.8±9.9	N	
	10	20.8±1.0	79.3±7.4	N	
	100	1.8±0.4	1.4±0.6	1.1±0.1	
H - 61, 38, 44	5	12.3±2.7	61.1±4.4	N	
	10	16.0±1.3	84.7±3.8	N	
	100	1.8±0.6	1.3±0.2	2.5±0.6	
H - 71, 43, 41	5	21.9±10.6	N	N	
	10	46.2±2.3	N	N	
	100	2.2±0.2	2.5±0.3	2.5±0.6	
L - 59, 9, 23	5	17.9±1.2	63.5±6.5	N	
	10	29.0±2.9	N	N	
	100	N	N	N	
L - 23, 0, 12	5	11.9±1.5	53.2±9.0	N	
	10	20.6±1.4	N	N	
	100	N	N	N	

^a X - a, b, c, where X is the general melting property (CB=cocoa butter, AMF=anhydrous milk fat, VH=very high melting, H=high melting, M=medium melting, L=low melting), a is SFC at 5°C, b is SFC at 25°C and c is melting point.

^b N: no crystallization was observed in 3 h.

3 h at 20°C. No crystal formation was observed at 25°C for any blend, except that of VH - 73, 58, 49 with CB. This fraction (VH - 73, 58, 49) caused crystallization at 25°C, even though CB by itself did not crystallize in 3 h at that temperature. This resulted from the fraction crystallizing separately from the CB, as determined from the melting curve following crystallization. This might be due to the differences in melting point of the glycerides in both fats. If the

melting points of the glycerides are far apart, mixed crystals may not form, and hence, the lower and higher melting component(s) may crystallize separately [4].

Crystallization peak time

Peak time was calculated by subtraction of induction time from peak-maximum time of the isothermal crystallization curves. Although peak time values have been used as a measure of crystallization rate [26, 27], other factors such as the type of polymorph formed and the amount of crystals also affect this measurement. Thus, peak crystallization times give, at best, a rough estimate of crystallization rate.

Peak times for pure fats and blends of CB and AMF or its fractions are given in Table 5. Among the pure fats, CB had the longest peak times at both 15 and 20°C. No significant differences were found in the peak times for AMF and its fractions at the three temperatures (15, 20 and 25°C). The very high melting milk fat fractions had shorter peak times than did other fats. In addition, no significant differences were found in the peak times for the very high melting fractions ($CI=95\%$). As mentioned previously, the low melting fractions (L - 59, 9, 23 and L - 23, 0, 12) did not crystallize in 3 h at the temperatures studied.

At 15°C, no significant difference was observed in peak times for CB and AMF, but all other pure fats had shorter peak times. A 5°C increase in temperature, to 20°C, caused a substantial increase in peak time for CB. In contrast, increasing temperature generally caused a slight reduction in peak time for AMF and its fractions. The exception was the H - 71, 43, 41 fraction, which showed a slight increase in peak time with increasing temperature.

Addition of AMF or its fractions to CB caused different effects on the crystallization peak time of CB, depending on the type of added fat, addition level and temperature. 5% addition of AMF or its fractions to CB did not affect the peak time of CB at 15°C ($CI=95\%$). However, the effects were dramatically different for crystallization at 20°C. 5% addition of AMF did not change the peak time of CB at 20°C. However, addition of all very high melting fractions significantly shortened the peak time of CB in the mixtures ($CI=95\%$). 5% addition of the H - 61, 38, 44 and the low melting fractions also decreased the peak time of CB, but the reduction was less pronounced.

10% addition of AMF or its fractions, except the L - 59, 9, 23 and VH - 69, 53, 48 fractions, to CB increased the peak time of CB at 15°C. The L - 59, 9, 23 and VH - 69, 53, 48 fractions did not change the peak time of CB at 15°C ($CI=95\%$). The softest fraction (L - 23, 0, 12) caused the greatest increase in the peak time of CB. At 20°C and 10% addition level, the effects of the additives were again dramatically different. The peak time of CB was decreased by addition of AMF at the 10% level, whereas 5% addition of AMF had no effect. Addition of the three very high melting fractions (VH - 73, 58, 49, VH - 69,

Table 5 Peak times for mixture of cocoa butter (CB) with milk fat (AMF) or its fractions

Fat type ^a	AMF or its fractions		Peak time/min		
	% in mixture	$T_{\text{isothermal}}/^{\circ}\text{C}$			
		15	20	25	
CB - 84, 61, 32	100	16.4±4.0	83.4±10.4	N ^b	
AMF - 51, 18, 37	5	21.5±1.3	83.4±24.6	N	
	10	28.4±1.6	48.6±4.6	N	
	100	13.2±0.8	10.8±1.3	9.9±1.0	
VH - 73, 58, 49	5	19.2±1.2	40.4±4.8	N	
	10	29.5±4.9	60.5±5.5	28.2±12	
	100	3.3±0.2	2.0±0.1	1.9±0.1	
VH - 69, 53, 48	5	10.6±0.5	31.4±2.6	N	
	10	10.4±1.3	42.7±4.2	N	
	100	3.0±0.1	3.0±0.2	2.3±0.1	
VH - 67, 48, 46	5	20.2±2.6	44.1±5.1	N	
	10	29.0±1.1	55.8±9.0	N	
	100	4.5±0.9	3.7±0.6	2.6±0.1	
H - 61, 38, 44	5	21.5±0.3	57.0±11.2	N	
	10	30.7±2.6	58.7±3.6	N	
	100	7.8±1.5	5.3±0.5	3.4±2.6	
H - 71, 43, 41	5	18.2±9.3	N	N	
	10	28.3±0.5	N	N	
	100	3.8±0.7	5.3±1.8	9.3±5.6	
L - 59, 9, 23	5	14.3±2.1	46.6±5.1	N	
	10	18.4±1.1	N	N	
	100	N	N	N	
L - 23, 0, 12	5	17.4±4.2	52.8±3.2	N	
	10	31.6±2.6	N	N	
	100	N	N	N	

^a X - a, b, c, where X is the general melting property (CB=cocoa butter, AMF=anhydrous milk fat, VH=very high melting, H=high melting, M=medium melting, L=low melting), a is SFC at 5°C, b is SFC at 25°C and c is melting point.

^b N: no crystallization was observed in 3 h.

53, 48 and VH - 67, 48, 46) at 10% caused an increase in peak time, as compared to 5% addition, although peak times were still shorter than that for the pure CB. 10% addition of H - 61, 38, 44 caused no change in peak time, as compared to 5% addition.

Enthalpy of crystallization

After nuclei have formed, crystal growth is generally slow in natural fats, due to their molecular diversity. Crystal growth rate depends on degree of supersaturation (subcooling), diffusion rate of molecules and time necessary for a molecule to fit into a vacant site on the crystal lattice [4].

Enthalpies of melting and crystallization can be readily obtained from DSC scans that show only one polymorphic form, because peak area directly reflects heat flow [1]. Enthalpy of crystallization (ΔH_c) and enthalpy of melting (ΔH_m) values are tabulated in Table 6. In general, each pure fat and fat mixture had higher ΔH_m values than ΔH_c values, since not all of the enthalpy associated with crystallization can be detected in the crystallization exotherms. Crystallization may have continued to take place, even after this peak apparently returned to the baseline. However, the rate of crystallization during this period was so low that no significant change in heat flow was detectable by DSC. Therefore, the enthalpy of melting values were used to evaluate the total amount of fat crystallized in 3 h [27, 28]. Melting peak temperature of CB did not change with addition of AMF or any of its fractions at either 5% or 10% levels. This indicates that AMF and its fractions did not alter the polymorphic form of CB. This was corroborated by XRD spectra, obtained at an intermediate temperature of 17°C.

As expected, the amount of crystallized material (as indicated by ΔH_c) in the individual fats generally decreased with decreasing SFC of the fats, for crystallization at either 15 or 20°C. CB had the largest ΔH_c value of the fats studied, and also had the largest difference between ΔH_c and ΔH_m . At 20°C, CB crystallization was not completed in 3 h, so ΔH_c and ΔH_m values were not reliable and should not be compared. For AMF and milk fat fractions, the amount of crystallized material decreased as temperature increased from 15 to 20°C, as expected from the decrease in SFC at higher temperature.

At 15°C, addition of AMF or its fractions generally had no effect on the measured exotherm (ΔH_c), but caused a reduction in the total amount of fat crystallized (as indicated by a decrease in ΔH_m). Also, higher addition levels resulted in a slightly lower ΔH_c for most of the fat mixtures. There was also a general trend where by lower melting point fractions caused a greater reduction in ΔH_m . However, some significant exceptions were noted. One very high melting fraction (VH - 69, 53, 48) caused a significantly greater reduction in ΔH_m than anticipated, while another fraction (H - 61, 38, 44) had less effect on ΔH_m than expected. The very high melting fraction, VH - 69, 53, 48, was also the only additive that caused a significant reduction in ΔH_c . Although the high melting fractions had similar chemical composition, significant differences were found in the amount of fat crystallized. This may not be due to differences in chemical compositions, but rather specific interactions between the TAGs

Table 6 Enthalpy of crystallization (ΔH_c), enthalpy of melting (ΔH_m) values and melting peak temperature (T_{mp}) for mixture of cocoa butter (CB) with milk fat (AMF) or its fractions

AMF or its fractions Fat type ^a	% in mixture	$T_{isothermal}/^{\circ}\text{C}$					
		15			20		
		$\Delta H_c/$ mJ mg ⁻¹	$\Delta H_m/$ mJ mg ⁻¹	$T_{mp}/$ °C	ΔH_c mJ mg ⁻¹	ΔH_m mJ mg ⁻¹	$T_{mp}/$ °C
CB - 84, 61, 32	100	17.7±2.6	75.0±1.4	27.7±0.4	NC ^b	27.7±6.2	29.1±0.5
AMF - 51, 18, 37	5	23.0±3.8	65.5±0.7	27.7±0.7	NC	20.6±5.2	29.5±0.6
	10	22.1±4.0	52.9±7.4	27.0±0.2	NC	29.7±2.8	27.7±0.3
	100	18.1±0.5	23.2±0.3	35.8±0.9	12.0±2.2	13.2±0.4	36.4±1.0
VH - 73, 58, 49	5	31.9±1.1	72.8±2.9	28.7±0.2	49.8±0.6	47.0±0.9	29.5±0.5
	10	30.8±0.7	72.5±3.2	28.0±0.3	NC	38.1±3.1	27.7±0.4
	100	28.6±3.0	71.3±10	48.6±0.8	23.4±2.1	56.1±5.7	49.1±0.6
VH - 69, 53, 48	5	8.5±1.8	48.5±3.6	29.6±1.1	35.8±3.2	35.0±1.2	30.5±1.1
	10	9.1±2.5	46.4±0.6	29.0±1.8	31.0±1.2	33.0±2.6	30.2±0.9
	100	18.4±2.5	53.8±3.4	48.7±0.2	21.1±0.8	50.0±1.3	48.7±0.2
VH - 67, 48, 46	5	25.3±8.8	70.8±8.0	27.4±0.8	NC	30.6±3.1	29.0±1.2
	10	27.3±7.3	66.6±4.1	27.9±0.3	NC	29.8±6.0	27.8±0.3
	100	33.3±6.8	61.0±1.7	46.2±0.7	31.3±4.2	52.9±5.2	46.1±0.8
H - 61, 38, 44	5	27.6±3.0	77.0±2.9	27.1±0.6	NC	34.9±11	28.2±0.9
	10	28.5±1.6	66.0±5.6	28.0±0.9	NC	29.1±0.7	28.2±0.9
	100	14.3±6.2	46.4±11	42.8±0.2	22.9±6.7	43.2±10	43.1±0.2
H - 71, 43, 41	5	37.6±28	56.4±3.0	27.7±1.5	N ^c	-	-
	10	42.4±7.7	53.5±0.5	27.0±0.8	N	-	-
	100	33.8±6.4	54.0±4.2	41.0±1.1	37.6±0.4	40.8±1.7	41.1±1.1
L - 59, 9, 23	5	24.4±3.1	48.0±4.0	27.0±0.7	NC	19.6±8.3	29.4±0.6
	10	18.4±2.2	40.1±1.8	27.1±0.2	N	-	-
	100	N	-	-	N	-	-
L - 23, 0, 12	5	20.4±0.8	48.0±1.5	29.0±0.3	NC	28.0±0.1	30.8±1.1
	10	26.4±5.5	41.8±6.4	27.9±0.9	N	-	-
	100	N	-	-	N	-	-

^a X - a, b, c, where X is the general melting property (CB=cocoa butter, AMF=anhydrous milk fat, VH=very high melting, H=high melting, M=medium melting, L=low melting), a is SFC at 5°C, b is SFC at 25°C and c is melting point;

^b crystallization was not completed in 3 h;

^c No crystallization was observed in 3 h

may be responsible. Similar arguments were made by Herrera [29] with regard to isothermal crystallization of sunflower seed oil.

Summary

The effects of milk fat and its fractions on CB crystallization were studied using an isothermal DSC technique. AMF and its fractions crystallized faster than CB. However, addition of these fats to CB generally retarded the induction time for nucleation of CB, with 10% addition having a greater effect than 5%. In general, higher melting point milk fat fractions produced only a slight retardation effect on induction time, whereas low melting point fractions caused substantial inhibition of isothermal crystallization of CB. Peak times of the crystallization exotherms for CB in mixtures were also affected by type and level of additives. For crystallization at 15°C, addition of AMF or its fractions caused an increase in peak time for CB crystallization, whereas at 20°C, peak time was decreased by addition of these fats. Furthermore, the amount of fat crystallized at 15°C generally decreased as a consequence of the addition of AMF or its fractions. In summary, temperature of crystallization, level of additive and physical and chemical characteristics of the additive significantly affected crystallization of CB.

* * *

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