Comparison of Ultrasonic and Pulsed NMR Techniques for Determination of Solid Fat Content

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ABSTRACT: In this work an ultrasonic velocity technique was compared to direct pulsed NMR (pNMR) spectroscopy for the determination of the solid fat content (SFC) of anhydrous milk fat (AMF), cocoa butter (CB), and blends of AMF and CB with canola oil (CO) in the range 100 to 70% (w/w). *In situ* measurements of ultrasonic velocity were carried out during cooling (50–5°C) and heating (5–50°C) of the fat samples, and SFC values were calculated. The SFC were also determined simultaneously by pNMR. Peak melting temperatures determined by DSC were used as an indicator of the polymorphic state of the different fats and fat blends. Estimates of SFC obtained using pNMR and ultrasonic velocimetry did not agree. Our results suggested that ultrasonic velocity was highly dependent on the polymorphic state of the solid fat. Ultrasonic velocity in fat that contained crystals in a more stable polymorphic form was consistently higher than in fat that contained crystals in a less stable polymorphic modification. A high attenuation of the signal was observed in milkfat and CB at lower temperatures, particularly after sitting for 24 h. This high attenuation could be a product of scattering by crystallites or by microscopic air pockets formed upon solidification of the material, or it could be due to high ultrasonic absorption associated with phase transitions. This research highlights some of the problems associated with applying ultrasonics to the determination of SFC.

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KEY WORDS: Cocoa butter, crystallization, melting, microstructure, milk fat, polymorphism, SFC, ultrasonic velocity.

The bulk physiochemical properties and sensory attributes of many fatty foods are determined by the fraction of the fat phase that is solidified at a particular temperature. It is therefore important to develop analytical techniques that can measure the variation in solid fat content (SFC) of a food with temperature. In the food industry, SFC values measured at different temperatures can be used to help predict important attributes such as mouthfeel and hardness. Traditionally, techniques based on dilatometry were used to measure the temperature dependence of the SFC of fatty foods; however, these have largely been replaced by pulsed NMR (pNMR) techniques. NMR instruments are capable of accurate, precise, rapid, and nondestructive measurements of SFC; however, on-line measurements are somewhat difficult to implement. It would therefore be advantageous to have an alternative analytical technique that could measure the SFC of fats on-line so that the properties of fat-containing foods could be monitored during processing.

Analytical techniques based on measurements of the interaction of ultrasonic waves with food materials have been shown to be particularly suitable for studying the properties of fatty foods. The ultrasonic velocity of liquid oils has been correlated to the average molecular structure of the FA present (e.g., chain length and degree of unsaturation); hence, ultrasonic velocimetry has been proposed as a means of assessing the origin or quality of edible oils (1–7). The ultrasonic absorption spectra of liquid oils depend on their high-frequency shear and compression rheology (8,9). Ultrasonic attenuation measurements can therefore be used to provide information about high-frequency molecular relaxation mechanisms and to characterize oil type and properties. The ultrasonic velocity of a fatty material increases as its SFC increases; hence, ultrasonic velocity measurements can be used to determine the SFC of emulsions and bulk fats (10–15). Ultrasonic velocimetry techniques are capable of rapid, accurate, and nondestructive measurements and are particularly suitable for on-line measurements.

In this study, we compared ultrasonic velocimetry to the widely used pNMR spectroscopy technique for measuring the temperature dependence of SFC in cocoa butter (CB) and anhydrous milk fat (AMF) during crystallization and melting.

MATERIALS AND METHODS

Materials. Blends of AMF and CB with canola oil were prepared in 10% (w/w) increments, from 100 to 70% (w/w) fat/canola oil. The edible fats were melted at 80°C for 15 min to destroy any crystal memory, mixed by vortexing with canola oil, and stirred until thoroughly mixed.

pNMR measurements. The SFC was obtained by pNMR using a Bruker PC/20 series NMR analyzer (Bruker, Missis-

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sauga, Ontario, Canada). The melted fats were introduced into glass NMR tubes, placed in a thermostated water bath, and cooled from 50 to 5°C at an average rate of 1°C/min. SFC was monitored as the temperature changed from 50 to 5°C upon cooling. The tubes were left to equilibrate overnight at 5°C, and changes in SFC were monitored from 5 to 50°C upon heating at a rate of 1°C/min as well. Two replicates of each sample were equilibrated for 15 min at each temperature prior to measurement.

Ultrasonic measurements. The ultrasonic velocity of samples was measured using an ultrasonic spectrometer (16,17). The experimental arrangement for measurements consisted of a custom-designed cell, a broadband ultrasonic transducer (3.5 MHz, 0.5″ diameter crystal, Model V682; Panametrics, Waltham, MA), a pulser/receiver (200 MHz Computer Controlled Ultrasonic Pulser-Receiver, Model 5900PR; Panametrics), a digital storage oscilloscope (Lecroy 9300; Lecroy Instruments, Chestnut Ridge, NY), and a personal computer (PC) with appropriate software (LabVIEW for Windows; National Instruments, Austin, TX). The cell consisted of a Plexiglas (perspex) delay line and a brass reflector plate separated by a distance of about 16 mm, where a sample was placed. The cell containing the sample was placed in a water bath, where it was allowed to equilibrate at each measurement temperature for 15 min prior to analysis. Measurement times and temperatures were as for the pNMR experiment.

The pulser/receiver generated a broadband electrical pulse, which was converted into an ultrasonic pulse by the transducer. This pulse propagated along the delay-line until it reached the boundary between the delay-line and the sample, where it was partly reflected and partly transmitted. The reflected pulse returned directly to the ultrasonic transducer, where it was converted back into an electrical signal, amplified, and displayed on an oscilloscope. The transmitted pulse traveled across the sample, was reflected by the brass reflector plate, and then traveled back across the sample and through the delay-line, where it was detected by the transducer and also displayed on the oscilloscope. The resultant signal was averaged 200 times and then sent from the oscilloscope to a PC *via* a GPIB card (AT-GPIB/TNT; National Instruments) where it was stored and analyzed.

The ultrasonic group velocity of the sample was determined by analyzing the received signal. The time difference between the echo reflected from the delay-line/sample interface and that reflected from the sample/reflector plate interface is the time t taken for the pulse to travel twice the length of the sample. Thus the ultrasonic velocity could be calculated, $c = 2d/t$, where d is the path length. The path length is fixed and was measured accurately by calibrating the device with distilled water, a material whose ultrasonic velocity is accurately known: $2d = c_{water}t_{water}$. Values are reported as the average of three measurements on the same sample. This device is capable of measuring the ultrasonic velocity to ±0.5 m/s.

In this study, we use a simple theoretical equation to establish a relationship between the ultrasonic velocity of a partially crystalline fat and its SFC. To a first approximation, the ultrasonic properties of a multiphase material can be described by (18):

$$\frac{1}{c^2} = \sum_{j=1}^{n} \phi_j \rho_j \sum_{j=1}^{n} \phi_j \kappa_j \quad [1]$$

where ρ_j, κ_j, and ϕ_j are, respectively, the density, adiabatic compressibility, and volume fraction of phase j. The adiabatic compressibility of a material can be calculated from its density and ultrasonic velocity ($\kappa = 1/c^2\rho$). Equation 1 can be simplified if one assumes that the densities of the various components are approximately similar:

$$\frac{1}{c^2} = \sum_{j=1}^{n} \frac{\phi_j}{c_j^2} \quad [2]$$

This simple relationship gives a good description of the ultrasonic properties of food materials when the densities of the various phases are similar and the scattering of ultrasound is not appreciable (10,14,19,20). For partially crystalline fats, Equation 2 can be expressed in the following manner:

$$\frac{1}{c^2} = \frac{\text{SFC}/100}{c_s^2} + \frac{(1-\text{SFC}/100)}{c_L^2} \quad [3]$$

where SFC is the percentage of the fatty material that is crystalline, and c_s and c_L are the ultrasonic velocities of 100% solid fat and of 100% liquid oil at the measurement temperature, respectively. The SFC can be determined by rearranging Equation 3 (10) to

$$\text{SFC} = 100 \times \left(\frac{1/c^2 - 1/c_L^2}{1/c_s^2 - 1/c_L^2} \right) \quad [4]$$

Thus, the SFC of a partially crystalline fat can be determined by measuring its ultrasonic velocity, provided that the ultrasonic velocities of 100% liquid oil and 100% solid fat are known at the same temperature. These values can be obtained by extrapolating ultrasonic velocity measurements made at low or high temperatures to the region of interest (14). The temperature dependence of c_L was determined by extrapolating ultrasonic velocity measurements from the region where the fat was completely liquid using the following expression to fit the data (7):

$$c_L = A \exp(-BT) \quad [5]$$

where A and B are empirically determined constants. The temperature dependence of the ultrasonic velocity (m/s) in the 100% solid fat phase (c_S) was taken from the literature (10):

$$c_s = \{24.66 \cdot 10^{-8} [1 + 5.54 \cdot 10^{-3} (T + 30.87)]\}^{-1/2} \quad [6]$$

Polarized light microscopy (PLM). The microstructures of samples of 100 and 70% AMF and CB were imaged upon cooling and heating using PLM. Slides were prepared by melting the fats and their blends at 80°C for 15 min. A preheated Pasteur pipette was used to deposit a small droplet of

fat onto a glass slide preheated to the temperature of the molten fat. A similarly heated glass cover slip was then placed on the surface of the droplet. Samples were then cooled on a Linkam LTS-350 hot/cold stage (Linkam Instruments, Tadworth, Surrey, United Kingdom) from 50 to 5°C at 1°C/min, as per the pNMR and ultrasonic velocimetry experiments, and stored at 5°C for 24 h. Slides were then transferred to the hot/cold stage and melted at 1°C/min. PLM images of AMF and CB were obtained at 20, 15, 10, and 5°C while cooling, and at 5, 10, 15, 20, 25, and 30°C while melting.

DSC. Approximately 10 mg of melted fat (80°C for 15 min) as placed in preheated aluminum pans and hermetically sealed. The pans were introduced into a Dupont 2090 differential scanning calorimeter (TA Instruments, Mississauga, Ontario, Canada) at 80°C, and cooled at 1°C/min from 50 to 5°C. A cooling thermogram was collected for each sample. Melting thermograms (1°C/min) were also collected after 15 min and 24 h of storage at 5°C.

RESULTS AND DISCUSSION

The velocity of ultrasonic waves traveling through AMF and CB diluted with different amounts of canola oil was determined during cooling (crystallization) and heating (melting). As the fats were cooled from 50 to 30°C, ultrasonic velocity increased gradually in the absence of visible crystallization (Fig. 1). In some samples, a marked relative increase in ultrasonic velocity as a function of temperature below approximately 20°C was indicative of the onset of crystallization. For example, ultrasonic velocity increased dramatically below 20°C for 100% AMF. Polarized light micrographs of AMF during cooling demonstrated the initial appearance of fat crystals around this temperature (Fig. 2). As expected, as more material crystallized, ultrasonic velocity increased. This increase in ultrasonic velocity upon appearance of the first fat crystals was not obvious in 70% AMF (Fig. 3). An increase in ultrasonic velocity was not observed in this sample at the onset of crystallization. Even though crystals appeared between 20 and 15°C, the change in ultrasonic velocity below this temperature was not as pronounced as for the case of 100% AMF. The same effects were evident in 100% CB, where even though crystals appeared above 20°C (Fig. 4), ultrasonic velocity did not increase dramatically at this temperature. For 70% CB, a dramatic increase in ultrasonic velocity was observed at 5°C (Fig. 1); however, the onset of crystallization was between 17 and 15°C, as determined by PLM (Fig. 5) and pNMR (Fig. 6). These results suggest that ultrasonic velocimetry is not a reliable indicator of the onset of crystallization of a fat and is rather system-dependent. The end of melt temperature could not be predicted from ultrasonic velocimetry measurements either. For the case of 100% AMF, for example, the ultrasonic velocity vs. temperature curve reached a plateau at ~25°C. However, fat crystals were clearly evident at 25°C as well as 30°C (Fig. 2). The same problem was observed in 70% AMF in canola oil (Fig. 3),

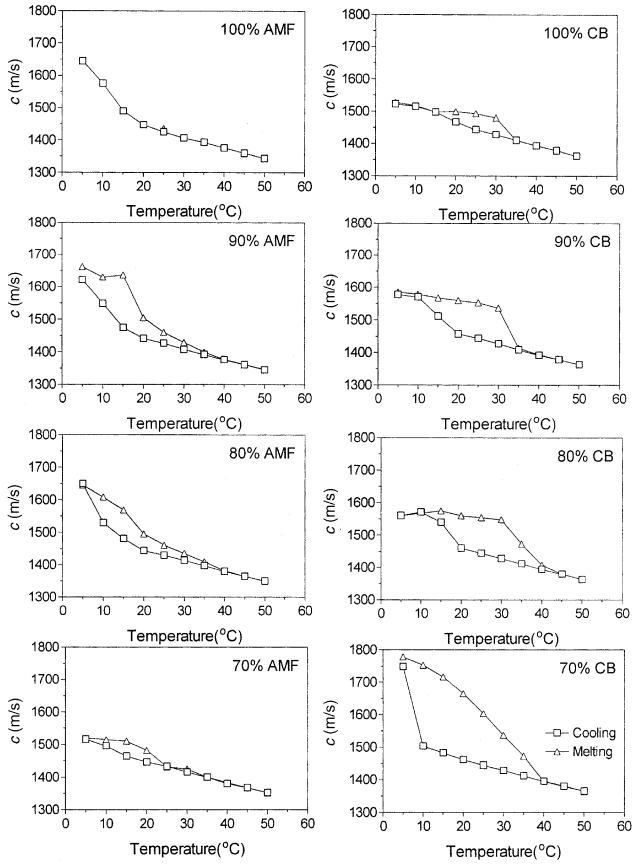

FIG. 1. Ultrasonic velocity vs. temperature profiles for different dilutions of anhydrous milk fat (AMF) and cocoa butter (CB) with canola oil during cooling (□) and melting (△).

where an apparent end of melt would have been predicted at ~25°C, but fat crystals were still evident at 30°C (Fig. 3). Similar lack of agreement was found in the CB samples as well.

During crystallization, a stage was reached where the monitoring of ultrasonic velocity was not possible owing to high attenuation of the signal within the crystallized fat. Thus, ultrasonic velocity measurements seem to be feasible in partially crystallized fats and in systems with low solids content. For the melting experiments on 100% AMF and CB after overnight storage at 5°C, the high degree of signal attenuation did not allow measurements to be obtained below 25°C. Again, this attenuation was system-dependent.

Figure 6 shows the temperature dependence of the SFC as determined by pNMR spectroscopy during cooling and melting of AMF and CB diluted to different extents with canola oil. SFC measurements correlated well with microscopy results. For both 100 and 70% AMF and CB in canola oil, pNMR proved to be an accurate and reproducible technique to determine the onset of crystallization and the end of melt.

SFC values of AMF and CB, as well as the 70% blends of these fats in canola oil, determined by pNMR and ultrasonic velocimetry are shown in Table 1. The missing SFC values for ultrasonic velocimetry-based SFC determinations correspond

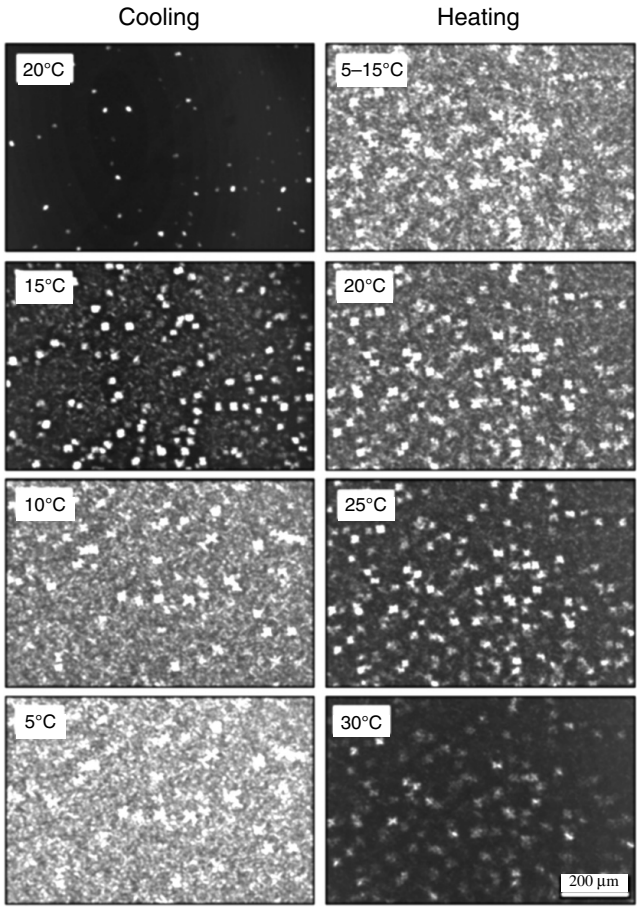

FIG. 2. Polarized light micrographs of 100% AMF during crystallization and melting. For abbreviation see Figure 1.

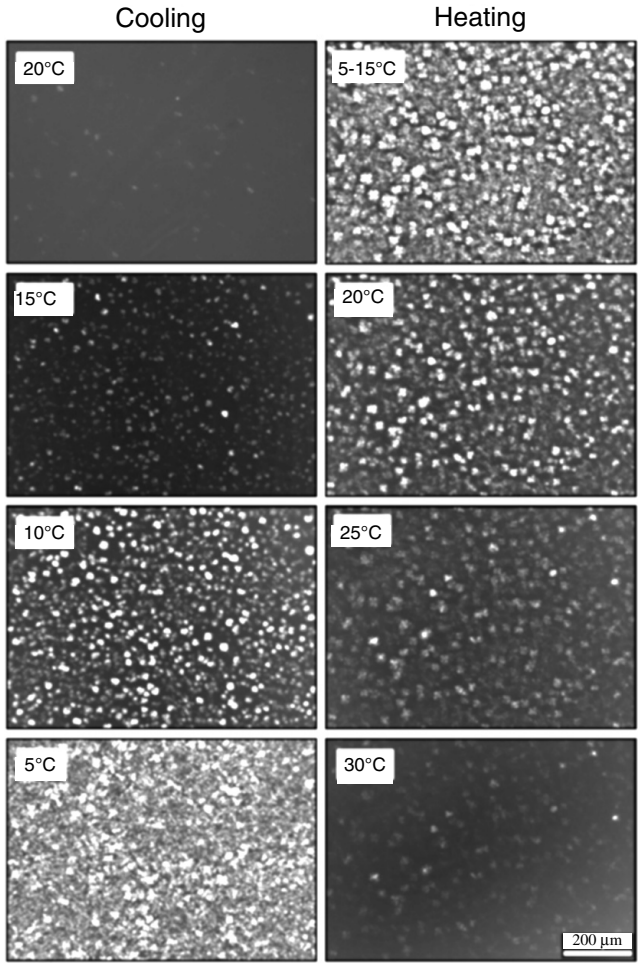

FIG. 3. Polarized light micrographs of 70% (w/w) AMF–canola oil during crystallization and melting. For abbreviation see Figure 1.

to samples for which the high degree of signal attenuation made detection of the reflected waves impossible with our experimental setup. As can be appreciated in Table 1, no signal was measurable in AMF during melting below 25°C. The agreement in the SFC determination by the two techniques was rather poor. For CB, the ultrasonic velocimetry-based SFC values did not agree with SFC values obtained by pNMR. In CB a high signal attenuation was commonly observed, possibly owing to the high solid content, as well as air pockets created upon solidification and contraction of the material, and/or owing to high absorption of ultrasonic energy associated with any phase transitions (14). There can be an enormous increase in ultrasonic attenuation and a large decrease in ultrasonic velocity when an ultrasonic wave has a frequency corresponding to a solid–liquid phase transition. This could potentially be one of the major applications of ultrasound for studying fat crystallization—to provide information about fast molecular processes. But it is a major problem for SFC determination in systems undergoing phase transitions.

To pinpoint the reasons for the observed differences in ultrasonic velocity in the different samples, we decided to monitor the polymorphism of the solids immediately upon crystallization and upon melting after an overnight storage period. Figures 7A and 7D show the crystallization curves for AMF and CB, respectively. The patterns observed for all dilutions were similar, indicative of a similar crystallization mechanism. The crystallization temperatures decreased as a function of increasing dilution, as expected from freezing-point depression arguments (i.e., colligative properties). Melting of the samples immediately upon completion of the crystallization process provides an estimate of the initial polymorphism of the fat crystals. As can be appreciated for AMF (Fig. 7B) and CB (Fig. 7E), all melting thermograms were similar, indicative of the presence of the same polymorph, initially, for all dilutions. After an overnight storage period, AMF also displayed similar polymorphism for all dilutions (Fig. 7C), which, however, differed from initial polymorphism. Some interesting trends were observed for CB (Fig. 7F). Upon dilution with canola oil, the peak melting temperature of CB increased, rather than decreased, indicative of the presence of a more stable polymorph. These results were corroborated by PLM evidence presented in Figures 2–5. Of particular interest is the drastic effect of this polymorphic transformation on microstrucure in 70% CB upon overnight storage (Fig. 5).

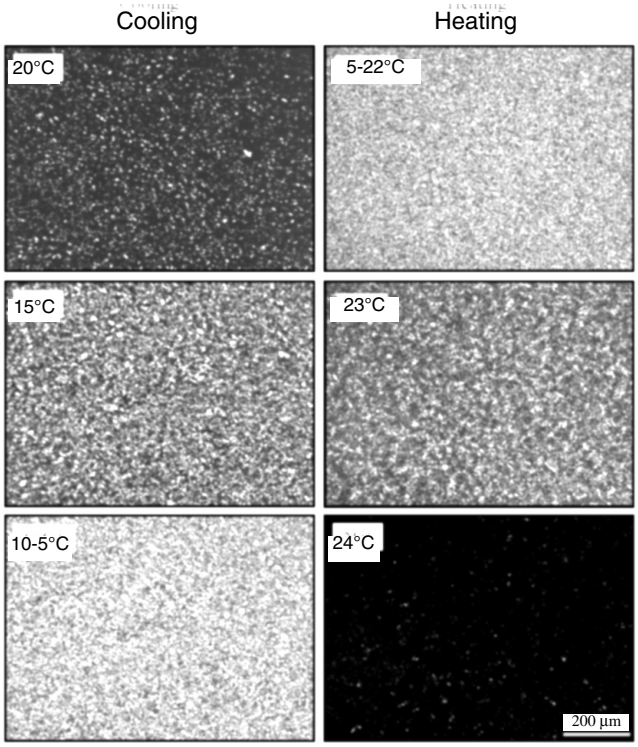

FIG. 4. Polarized light micrographs of 100% CB during crystallization and melting. See Figure 1 for abbreviation.

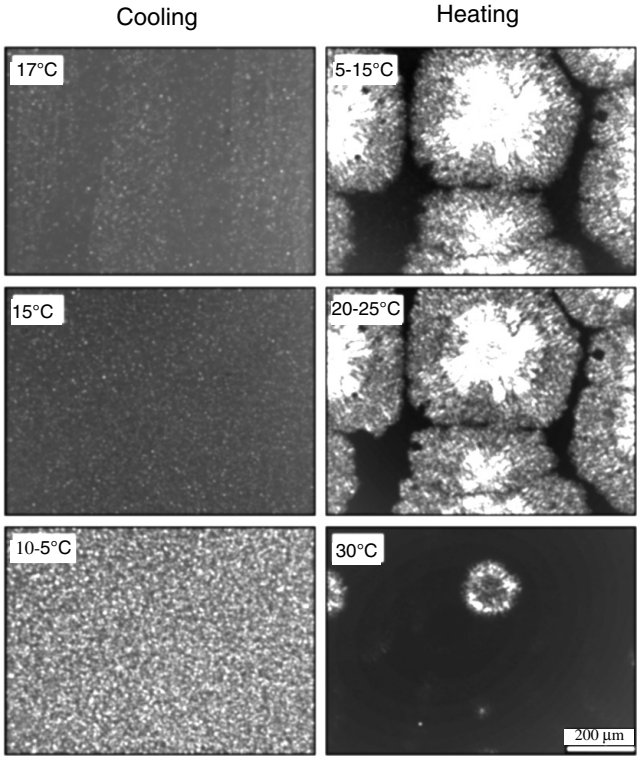

FIG. 5. Polarized light micrographs of 70% (w/w) CB–canola oil during crystallization and melting. See Figure 1 for abbreviation.

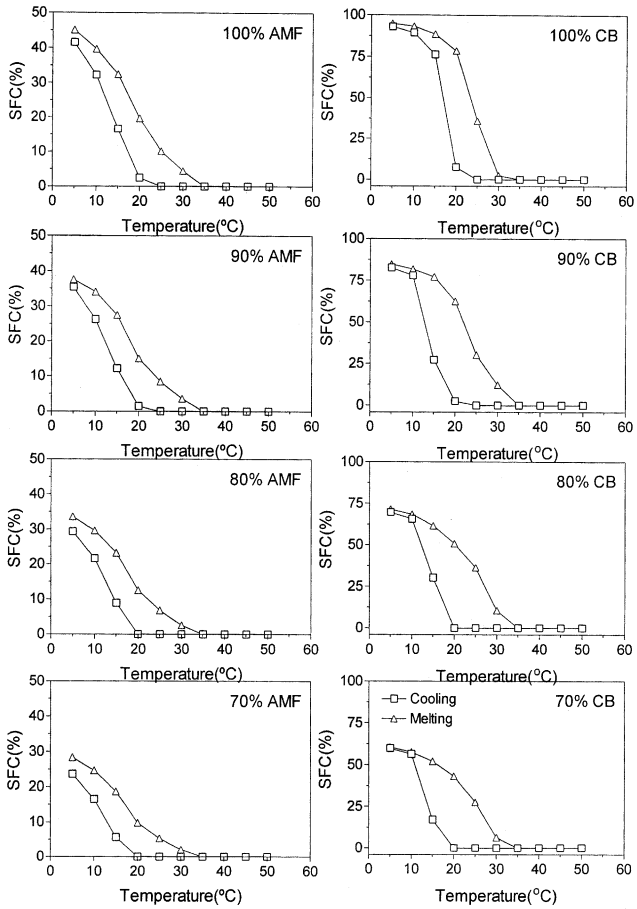

FIG. 6. Solid fat content (SFC) vs. temperature profiles as determined by pulsed NMR for different dilutions of AMF and CB during cooling (□) and melting (△). See Figure 1 for abbreviations

It is interesting to notice how the ultrasonic velocity in fat samples after overnight storage is higher than upon crystallization, even though SFC can be similar. For example, upon immediate crystallization or melting, 70% CB has an SFC of ~52% at 10°C (Fig. 6). Even though the SFC is the same, the ultrasonic velocity is much higher after overnight storage. This effect is due to the large polymorphic transformation that takes place in this sample upon storage. The same argument applies to the other CB and AMF samples. In general, it would seem that ultrasonic velocity through a solid in a less stable polymorphic modification is lower than through a solid in a more stable polymorphic modification, which implies that the more stable polymorph had a higher bulk modulus. This effect was first described by Kloek *et al.* (21) in emulsified mixtures of hydrogenated palm oil in sunflower oil. These authors suggested that ultrasonic velocity through an α crystal would be lower than through a more stable β or β′ crystal. Our results agree very well with their findings.

An added element to be considered is whether changing the solid content leads to a polymorphic change (as in the case of CB upon storage). One would expect that as the solid content increases, so would the ultrasonic velocity. However, as

TABLE 1
Solid Fat Content (SFC) of 100 and 70% Anhydrous Milk Fat and Cocoa Butter at Various Temperatures in a Cooling and a Melting Profile Determined by Pulsed NMR Spectroscopy and from Ultrasonic Velocity Measurements

	Anhydrous milk fat[a]				Cocoa butter[b]			
	100%		70%		100%		70%	
Temperature (°C)	NMR	Ultrasonic	NMR	Ultrasonic	NMR	Ultrasonic	NMR	Ultrasonic
Cooling								
5	41.6	51.7	23.7	5.8	93.0	3.9	59.8	77.7
10	32.3	36.1	16.5	4.9	89.5	8.0	56.4	3.2
15	16.7	12.1	5.7	0	76.3	7.9	17.2	1.6
20	2.4	2.1	0	0	7.4	2.5	0	0
25	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0
Melting								
5	45.1		28.4	7.3	95.0	5.9	60.5	85.6
10	39.7		24.7	12.1	93.4	8.9	57.6	84.3
15	32.5		18.8	17.2	88.7	7.9	52.2	79.7
20	19.7		9.8	13.1	78.6	15.4	43.3	70.8
25	10.2	4.4	5.4	0	36.0	19.8	27.7	57.8
30	4.5	0	2.1	0	2.6	21.2	6.4	41.6
35	0	0	0	0	0	0	0	24.7
40	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0

[a]Either 100% anhydrous milk fat or 70% anhydrous milk fat + 30% canola oil.
[b]Either 100% cocoa butter or 70% cocoa butter + 30% canola oil

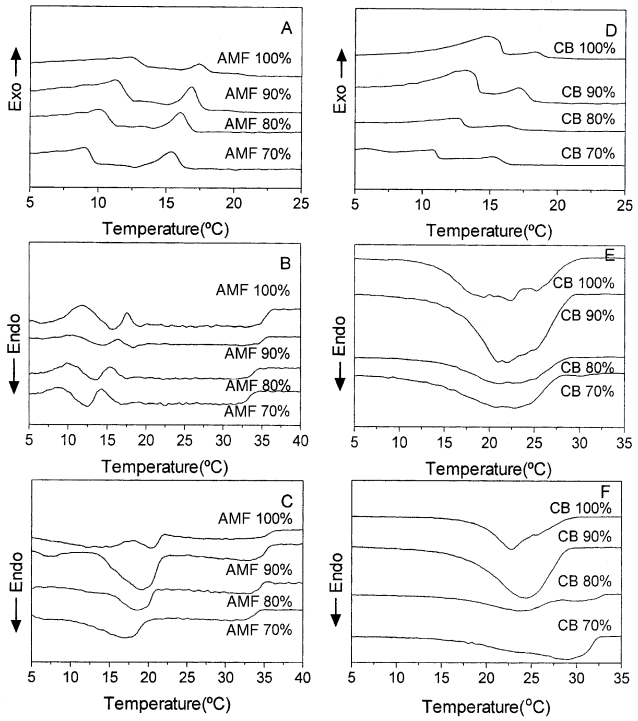

FIG. 7. DSC traces for the crystallization of (A) AMF and (D) CB and their dilutions with canola oil at 1°C/min. DSC traces for the melting of (B) AMF and (E) CB and their dilutions with canola oil immediately upon completion of the crystallization process. DSC traces for the melting of (C) AMF and (F) CB and their dilutions with canola oil after overnight storage at 5°C. For abbreviations see Figure 1.

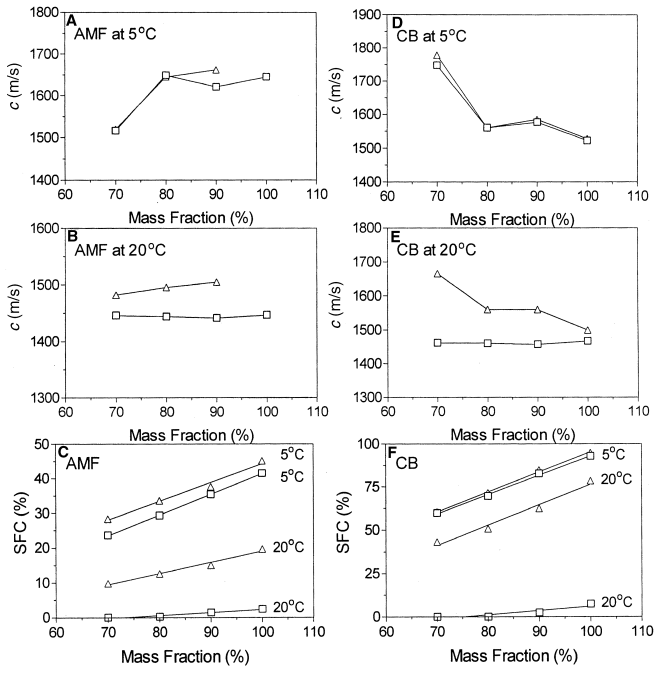

FIG. 8. Changes in ultrasonic velocity (A,B,D,E) in AMF and CB as a function of dilution with canola oil at selected temperatures during cooling (□) and melting (△). Changes in pulsed NMR-determined SFC (C,F) for AMF and CB as a function of dilution with canola oil at selected temperatures during cooling (□) and melting (△). For abbreviations see Figures 1 and 6.

can be appreciated in Figure 8, ultrasonic velocity increases as a function of increasing amounts of solids for AMF, but *decreases* for CB. Shown are the trends for cooling and melting of the different dilutions at both 5 and 20°C. As discussed before, dilution of CB with canola oil allows a polymorphic transformation to a more stable crystal form to take place (i.e., more solid-like). Ultrasonic velocity is higher through solids in more stable polymorphic modifications. This explains the observed increase in ultrasonic velocity as a function of increasing dilution of CB with canola oil. No such polymorphic transformation was observed in AMF, and therefore, ultrasonic velocity decreased as a function of dilution with canola oil, since a higher amount of solids in a fat should translate into a higher ultrasonic velocity.

Even though not performed in this study, stirring the samples during measurement could affect the ultrasonic velocity and attenuation measurements because it could change the dimensions and spatial orientation of the fat crystals. If this was the case, this would constitute indirect evidence that microstructure is an important factor in determining ultrasonic velocity in semicrystalline fats.

A final point that should be stressed is the fact that this study concentrated on pure fat systems at fairly high SFC. Previous studies have generally concentrated on emulsified fat systems at low SFC. Obviously, these two types of systems behave very differently.

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