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Polymorphic Transformation in Mixtures of High- and Low-Melting Fractions of Milk Fat

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The kinetics of crystallization of high-melting fraction (HMF) and a mixture of 40% HMF and 60% low-melting fraction (LMF) of milk fat were studied at 5 °C by time-resolved in-situ synchrotron X-ray diffraction. HMF crystallized in the α polymorph, had a longer lifetime than the ones previously reported in pure milk fat, and was almost completely solid. The HMF/LMF mixture crystallized initially in the α form and transformed into the β′ polymorph, with a solid fat content much lower than that of HMF. The polymorphic change was therefore attributed to a delayed sudden formation of β′ mixed crystals from the uncrystallized melt. These findings are important for the food industry and as fundamental knowledge to improve our understanding of the origin of the macroscopic physical properties of solid milk fat fractions used in many manufacturing processes.

KEYWORDS: Milk fat crystallization; lipid polymorphism; lipid phase transitions; synchrotron X-ray diffraction

INTRODUCTION

The phase behavior of milk fat fractions (*1*, *2*) as well as the thermal and structural behavior of milk fat has been thoroughly studied (*3*−*6*); however, very little information about the structural dynamics of milk fat fractions in the early stage of crystallization has been reported. This early stage of crystallization is very important since it determines the later evolution of the system. The interest in studying the structural behavior of the milk fractions reported here arose from the observation of a sudden retarded crystallization event after a first plateau in solid fat content (SFC) during the crystallization of binary mixtures of milk fat fractions by pulsed nuclear magnetic resonance (pNMR). This behavior had been observed in other work (*7*) with milk fat, but its explanation remained only speculative in terms of structural behavior. With the aim of better understanding this dynamics, the kinetics of crystallization of a high-melting fraction (HMF) and a mixture of 40% HMF and 60% low-melting fraction (LMF) were studied by time-resolved in-situ synchrotron X-ray diffraction. The advantage of using a synchrotron source is that the high radiation flux allows diffraction patterns to be acquired during real-time crystallization.

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MATERIALS AND METHODS

The milk fat fractions were obtained by a multistep solvent fractionation described by Marangoni and Lencki (*1*) and Marangoni (*8*) with yields of 12, 34, and 54% for HMF, medium-melting fraction (MMF), and LMF, respectively. The chemical composition and phase behavior of these milk fat fractions have been reported elsewhere (*1*, *8*). The crystallization evolution of HMF and a mixture of 40% HMF and 60% LMF (w/w) was studied by measuring SFC as a function of time. Approximately 3 g of fat was weighed in glass NMR tubes (10 mm diameter) and heated at 80 °C for 30 min to destroy any crystal memory. The NMR tubes were immediately plunged into a water bath equilibrated at 5 °C. The SFC was measured by pNMR with a Bruker PC/20 series NMR analyzer (Bruker) at time intervals until the crystallization curves reached a plateau. For the X-ray experiments, the samples were melted at 80 °C for 30 min and introduced into capillary tubes (1 mm thin glass), which were hermetically sealed with a flame. The capillary tubes were heated at 80 °C for 15 min and immediately transferred to a capillary holder held isothermally at 5 °C. The temperature holder was controlled with LabView (National Instruments Co.). X-ray diffraction experiments were conducted at ExxonMobil beamline X10A at the National Synchrotron Light Source at Brookhaven National Laboratory (Upton, NY). Diffraction patterns were collected continuously with a Bruker Smart 1500 two-dimensional CCD detector, with an exposure time of 50 s. Tricaprin (Sigma) was used for calibration of the detector. The X-ray radiation had a wavelength λ of 1.602 Å with the detector located 734 mm from the capillary, for the small angle experiments. The wide angle experiments were carried out with a wavelength λ of 1.075 Å with the detector located 158 mm from the sample. The beam size was 0.5 mm × 0.5 mm in both cases. Radial averages of the diffraction patterns were obtained using a custom plugin for Image J, which allows the

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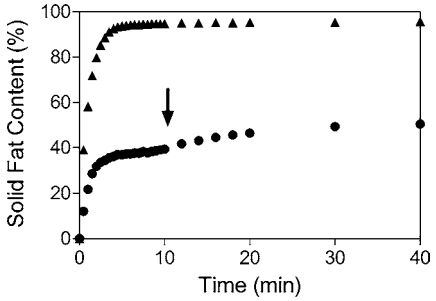

Figure 1. Solid fat content (%) vs time for (▲) HMF and (●) a 40% HMF/60% LMF mixture at 5 °C. The arrow indicates the end of the plateau.

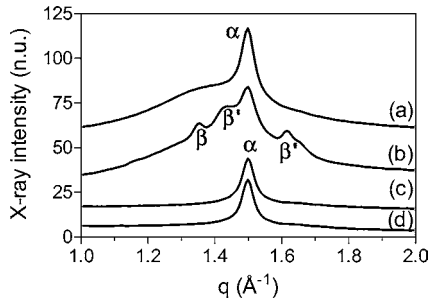

Figure 2. X-ray intensity, in normalized units (n.u.), of the wide angle radial-averaged diffraction patterns as a function of reciprocal lattice spacing q (in Å^{-1}): (**a**) mixture of 40% HMF and 60% LMF, after 3.5 min at 5 °C, (**b**) same mixture 55 min later, (**c**) HMF after 4 min at 5 °C, and (**d**) HMF 35 min later. The background has been subtracted, and plots **a**–**c** have been offset from their baseline to improve clarity.

normalization of the intensities with respect to the incident beam. The X-ray-diffracted intensity was plotted as a function of reciprocal lattice spacing q, where $q = 2\pi/d = 4\pi \sin(\theta)/\lambda$, where d is the interplanar spacing and 2θ is the Bragg angle. The one-dimensional diffraction profiles were fitted with Prism version 3.0 (Graph Pad, San Diego, CA) to a Lorentzian equation for the α polymorph peaks and a Gaussian equation for the β' polymorph peaks. Assignment of the subcell packing (α, β', or β polymorphs) was done on the basis of information from the literature (*3*, *6*).

RESULTS AND DISCUSSION

The crystallization kinetics of HMF and a mixture of 40% HMF and 60% LMF obtained by pNMR displayed a single crystallization event for the HMF, while for the HMF/LMF mixture, a sudden increase after a first plateau in solid fat content (SFC) was observed (**Figure 1**). With the aim of understanding the structural nature of the crystallization events, the crystallization kinetics of both samples were studied by time-resolved in-situ synchrotron X-ray diffraction.

The wide angle X-ray diffraction patterns (WAXD) of HMF, presented in panels **c** and **d** of **Figure 2**, displayed a clear single peak at $q \sim 1.5$ Å^{-1} during the whole experiment, the characteristic signature of phase α. An underlying broad liquid peak is almost entirely absent, since the sample would have been, according to the pNMR measurement, mostly in the crystalline state, with an SFC of ∼95%. The small angle diffraction patterns (SAXD) of HMF displayed a single peak with a d of 47.14 Å ($q = 0.1333$ Å^{-1}). According to the literature (*3*, *6*), these X-ray diffraction patterns are consistent with a bilayer (2L) lamellar packing arrangement and a hexagonal subcell or α polymorph. The intensity of the diffraction pattern of HMF remained essentially constant during the length of the study (80 min), indicating that no crystalline growth or polymorphic transformation took place. It has been

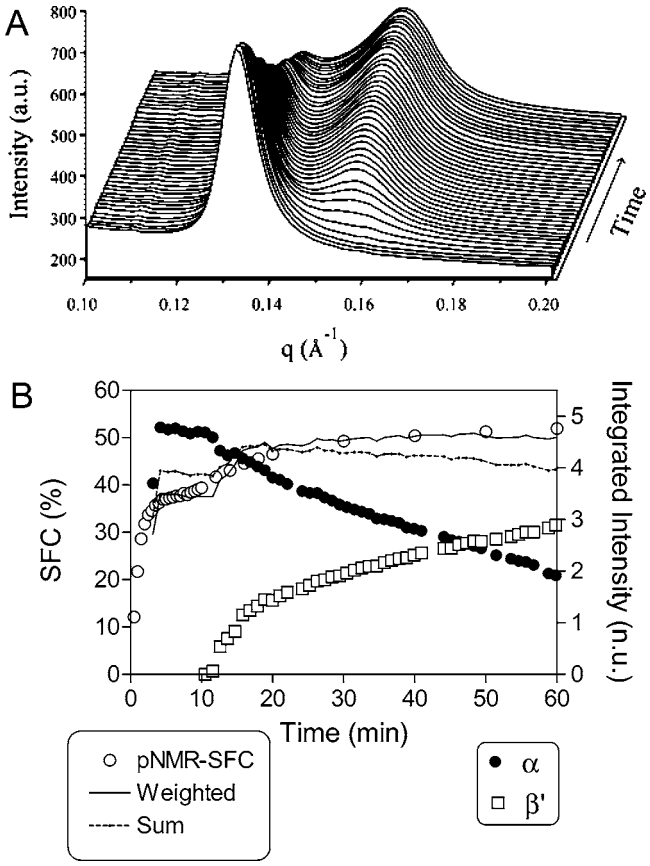

Figure 3. (**A**) Three-dimensional composite of the radial plots of SAXD (X-ray intensity vs reciprocal lattice spacing q in Å^{-1}) recorded as a function of time at 5 °C, after rapid cooling of the sample (40% HMF and 60% LMF) from 80 °C. Both SAXD peaks displayed 2L lamellar arrangements; the $q = 0.133$ Å^{-1} peak corresponds to the α polymorph, and the $q = 0.154$ Å^{-1} peak corresponds to β'. (**B**) Normalized integrated intensity (in arbitrary units) of the intensity peaks of the (●) α polymorph and (□) β' polymorph (both plotted on the right axis). On the left axis, the (○) pNMR SFC data are plotted together with (- - -) the sum of integrated intensities multiplied by the same response factor (9) and (—) the sum of the integrated intensities multiplied by different response factors (8 for α and 12 for β').

reported that the 2L packing crystalline structures of milk fat obtained during fast cooling are very unstable and therefore have a very short lifetime (*3*); however, HMF in this study displayed a lifetime of >1 h. A very small shoulder on the SAXD peak was observed to appear almost simultaneously with the phase α peak, corresponding to a small amount of another phase, likely β'. The intensity of this second phase was also constant throughout the experiment.

The early WAXD patterns of the HMF/LMF mixture displayed the single characteristic peak of phase α over a strong broad liquid peak, as seen in **Figure 2a**, consistent with the low SFC in **Figure 1** during the first stage of the crystallization. At the end of the experiment, three phases could be identified (α, β', and a small amount of β), as shown in **Figure 2b**. As indicated by Mazzanti et al. (*6*), in quickly cooled samples of milk fat the formation of phase α is often a precursor for the formation of small amounts of phase β that coexist with larger amounts of phase β'. The evolution of the SAXD patterns (**Figure 3A**) displayed an initial peak with a d of 47.60 Å ($q = 0.1320$ Å^{-1}), which corresponded to the 2L lamellar packing of the α polymorph (*3*, *6*). After isothermal crystallization for 10 min, a second SAXD peak began to grow while the initial

diffraction peak ($d \sim 47$ Å) began to decrease in intensity (**Figure 3B**, right axis). The second diffraction peak with a d of 40.6 Å ($q = 0.154$ Å^{-1}) corresponded to the 2L packing of the orthorhombic subcell (β' polymorph) (*3*, *6*). The WAXD showed the presence of phase β together with phase β', but it did not appear as a separate peak in the SAXD, probably due to the small amount of β present (*6*). Thus, we will refer hereafter to this phase simply as the β' polymorph. The β' SAXD peak position shifted during the growth from a d of 40.6 Å to a d of 41.1 Å ($q = 0.153$ Å^{-1}). This peak position shifting is a consequence of the changing composition of the material being crystallized from the melt as time goes by, given that this is a multicomponent system (*6*, *9*). The area under the SAXD peak, called the integrated intensity, is proportional to the crystallinity of the sample, via a response factor that is characteristic of each phase. The evolution of the integrated intensities of the SAXD peaks (**Figure 3B**) during the crystallization of the HMF/LMF mixture indicated that the polymorphic transformation from the α to β' polymorph started shortly after isothermal crystallization for 10 min at 5 °C. This polymorphic transformation of the HMF/LMF mixture took place concurrently with an increase in SFC during isothermal crystallization (**Figure 1**). By assuming response factors (SFC/integrated intensity) of 8 for phase α and 12 for phase β', we were able to estimate a curve of SFC versus time (**Figure 3B**, solid line) that is consistent with the data obtained by pNMR, shown in **Figure 3B** as circles. If the integrated intensities are simply summed and multiplied by a single response factor, the result is not consistent with the pNMR data, like the example with the dashed line in **Figure 3B**, where a single response factor of 9 was used for both phases. The fact that the response factors of the integrated intensities are different is analogous to what Mazzanti et al. (*9*) reported for palm oil. However, only a very precise knowledge of the crystal compositions and structures could allow for a calculation of these factors ab initio. In the case of natural fats and oils, this is further complicated by the multicomponent nature of the crystals.

Van Aken and Visser (*10*) have reported a second increase in SFC in milk fat crystallized at 10, 14, and 17 °C, and it was attributed to a retarded crystallization of triglycerides. Wright et al. (*2*) have also reported crystal complex formation in HMF and LMF mixtures crystallized at 5 °C due to the low SFC in LMF at this temperature and its molecular complementarity with HMF. Breitschuh and Flöter (*7*) stated that the polymorphic transformation can be excluded as a cause of the intermediate constant solid content observed in some milk fat fractions. Our findings differ from theirs, but it is not surprising since their X-ray diffraction studies were not performed in real time. It is thus likely that the melt-mediated polymorphic transformation of the phase α into β' is the result of the heat released due to nucleation and initial growth of phase β' from the melt in the HMF/LMF mixture, that is, the so-called retarded crystallization event.

In conclusion, with the aid of synchrotron X-ray diffraction, it was possible to establish that the plateau and subsequent sudden growth in the SFC curve can be attributed to the polymorphic transformation that takes place during the crystallization of a 40% HMF/60% LMF mixture at 5 °C, likely due to a delayed formation of β' mixed crystals from the melt. The α polymorph of the isolated HMF of milk fat has a lifetime longer than the ones previously reported in pure milk fat crystallized at 5 °C (*3*), and its SFC was >95%. This supports the hypothesis that the presence of the liquid phase is necessary for the fast phase transition (*11*−*13*) to the β' polymorph, as observed in the mixture, which reaches only 35% SFC in the α phase before phase β' starts to form. The difference of 0.46 Å between the SAXD peaks of the α polymorphs of pure HMF and the HMF/LMF mixture may reflect a difference in the triglyceride composition of the α crystals in pure HMF and the α crystals in the HMF/LMF mixture.

The likelihood of forming a particular phase in a given triacylglycerol multicomponent system depends on the temperature−time profile that is applied, i.e., the combination of cooling rate, final temperature, and time. Since the material was brought well below the melting point of the three phases that are mentioned (α, β', and β), it is not surprising that there is evidence of the presence of all three. What is precisely interesting is that the kinetics of formation of the more stable phases seem to be impeded by the lack of mobility, as in the case of HMF, because there is virtually no liquid phase left, whereas in the case of the HMF/LMF mixture, there is still a considerable amount of liquid present.

It is difficult to know the quantitative extent to which the modification of the liquid composition due to the partial crystallization of the mixture (to produce α crystals) affects the tendency of the remaining liquid to crystallize in the β' form. Essentially, the remaining triacylglycerols will have an average lower "melting range" than the total original material. We can thus speculate that the driving force to form β' crystals in such materials has been reduced. This would add a delay to the already slower kinetics of crystallization of this polymorphic form, which requires more time to begin its self-assembling process, due to the less accommodating structure. However, there is no clear way to test this difference, since we cannot prevent α from appearing under these conditions.

These findings are important for the food industry and as fundamental knowledge to improve our understanding of the macroscopic physical properties of solid milk fat fractions used in the food industry through their microstructure and polymorphic state (*14*). If the addition of LMF to HMF promotes the formation of the β' polymorph which is the preferred polymorph for spreads, in terms of texture and stability (*15*), there is the possibility of adding small amounts of LMF to HMF to produce fats resistant to mechanical work due to their high melting points but in a stable polymorphic state.

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Supporting Information Available: Plots of q versus intensity. This material is available free of charge via the Internet at http://pubs.acs.org.

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