Chapter 9
Crystallization and Melting Properties of Milk Fat

Christelle Lopez

1 Introduction

Fat is secreted in milk in the form of oil in water (O/W) emulsion droplets called the milk fat globules (size from 0.1 to 10 μm, mean diameter around 4 μm in bovine milk), that correspond to a core of triacylglycerols (TAGs) enveloped by a biological membrane rich in phospholipids, cholesterol and proteins (Lopez, 2011). Milk TAGs provide more than 50% of the dietary energy intake that is essential for the growth of newborns and also provide bioactive molecules involved in infant health. Milk fat is also consumed by infants and adults in various dairy products, i.e. milk, cream, whipped cream, cheeses, butter and as an ingredient in many bakery and confectionary applications in which it can be found in anhydrous state and as O/W or W/O emulsions.

Milk fat can be in a semi-solid state (i.e. a mixture of crystal network and liquid oil) over a wide range of temperatures, including the temperature of storage (4–7 °C), consumption and digestion (37 °C) of food products. This crystallization behaviour of milk fat results from its complex composition with a high amount of saturated fatty acids (FAs) and polymorphism of TAGs. The crystallization properties of milk fat can be affected by many parameters such as the cooling rates and thermal history, the shear, the presence of minor lipid components, the dispersion state (anhydrous *versus* emulsified), the changes in the FA and TAG composition. Milk TAG crystals are involved in the rheological and sensorial properties of products (e.g. butter, cream, cheese). Besides solid fat content, the functionality of a fat is also affected by the crystal structure and networks formed by TAG molecules. Understanding the crystallization and melting behaviours of milk TAGs, as well as

C. Lopez (✉)
INRAE, UMR STLO, Rennes, France

INRAE, UR BIA, Nantes, France
e-mail: christelle.lopez@inrae.fr

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the polymorphism of milk TAGs, is of great importance from a scientific point of view and with regard to the economic impact of milk fat, especially in fat-rich products.

2 A Wide Diversity of Fatty Acids and Triacylglycerols

Milk fat consists of 97–98% TAGs, i.e. molecules composed of a glycerol backbone with three fatty acid moieties esterified onto it. Bovine milk fat is the most complex fat found in the nature, with more than 400 different fatty acids (FAs) identified of which 12 are present in proportions greater than 1% (Fig. 9.1a). Milk fat contains short-chain (C4–C8), medium-chain (C10–C12) and long-chain (C14–C18) length FAs. About 70% of milk fat corresponds to saturated FAs and 25% to monounsaturated FAs mainly oleic acid (C18:1c9), with variations as a function of season and diet (Fig. 9.1A). Bovine milk fat contains a low amount of natural *trans* FAs produced by biohydrogenation in the cows. About 200 different TAGs have been identified (Fig. 9.1B). Milk fat contains many asymmetrical TAGs, i.e. TAGs of the SSU or UUS type, in which the single unsaturated (U) or saturated (S) FA resides in either the *sn*-1 or *sn*-3 position, or TAGs with differences between the FA chain length larger than two atoms of carbon, i.e. BPP (B: butyric acid C4:0, P: palmitic acid C16:0). This diversity of FAs and TAGs confers to milk fat specific physical properties, in particular, the presence of a solid TAG phase over a wide range of temperatures. The TAG composition induces a complex crystallization behaviour and a wide melting range with multiple melting points. Furthermore, individual TAG molecules are characterised by a complex thermal behaviour in relation to their polymorphism of monotropic type.

3 Crystallization and Melting Properties of Bovine Anhydrous Milk Fat

3.1 Thermal Properties

Bovine anhydrous milk fat (AMF), which is the fat isolated from butter, has a broad melting range from −40 to +40 °C (Fig. 9.2). AMF must be warmed at least to +40 °C to ensure complete melting of TAGs and is not completely solid until it reaches a temperature below −40 °C. Many authors have studied crystallization and melting behaviour of AMF by differential scanning calorimetry (DSC) and showed that it crystallises and melts in several steps (Lopez, Bourgaux, Lesieur, & Ollivon, 2007; Ten Grotenhuis, van Aken, van Malssen, & Schenk, 1999; Timms, 1980). A typical crystallization curve of AMF shows two main exotherms in the cooling DSC measurement, corresponding to groups of TAG molecules with high crystallization

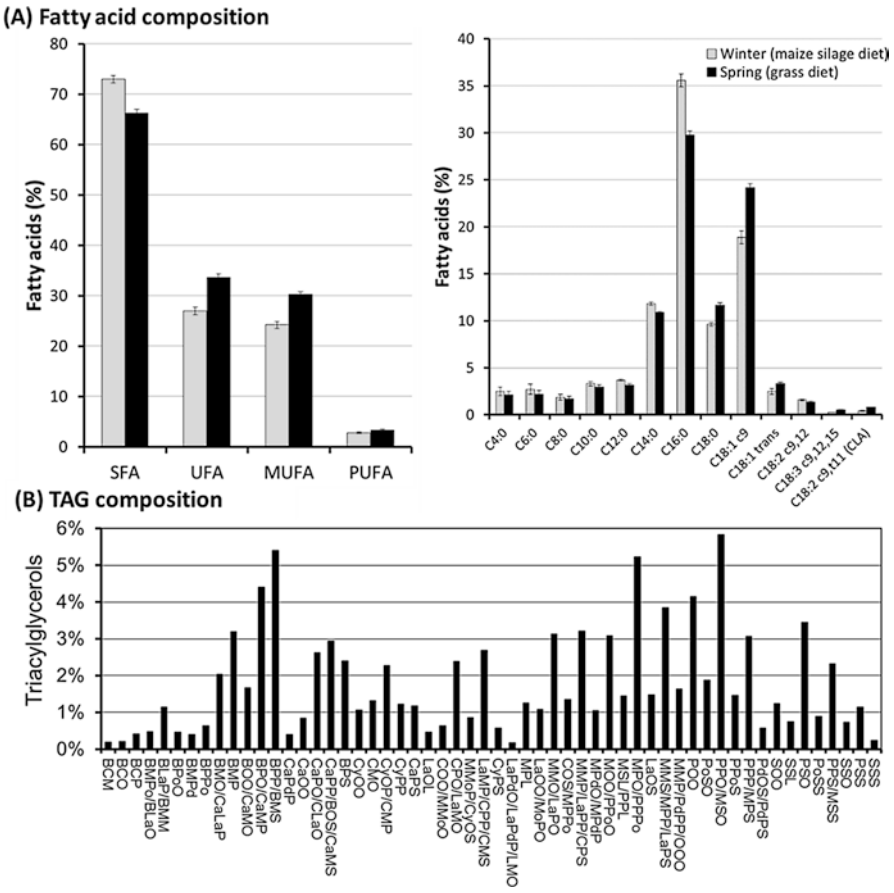

Fig. 9.1 Milk fat composition. (**A**) fatty acid (FA) composition with variations as a function of cow diet and season (**B**) triacylglycerol composition. Abreviations: *SFA* = saturated FA, *UFA* = unsaturated FA, *MUFA* = monounsaturated FA, *PUFA* = polyunsaturated FA, *B* = butyric acid (C4:0); *Ca* = caproic acid (C6:0), *Cy* = caprylic acid (C8:0), *C* = capric acid (C10:0), *La* = lauric acid (C12:0), *M* = myristic acid (C14:0), *Pd* = pentadecanoic acid (C15:0), *P* = palmitic acid (C16:0), *Po* = palmitoleic acid (C16:1), *S* = stearic acid (C18:0), *O* = oleic acid (C18:1c9). Adapted from Lopez, Bourgaux, et al. (2006) and Lopez et al. (2014)

temperature (HCT) and low crystallization temperature (LCT) segregated on cooling. A typical melting curve of AMF shows three overlapped endothermic peaks that have been called on the basis of their melting point, i.e. low melting point (LMP), medium melting point (MMP) and high melting point (HMP) (Fig. 9.2). The melting of TAGs in the MMP fraction is highly important for the sensory properties of milk fat in the mouth. The three melting peaks correspond to large groups of TAGs that melt separately and behave as solid solutions. The LMP endotherm corresponds to the melting of TAGs with a high content of long-chain unsaturated FAs and short-chain saturated FAs such as BOO, BPO and PPO (B: butyric acid

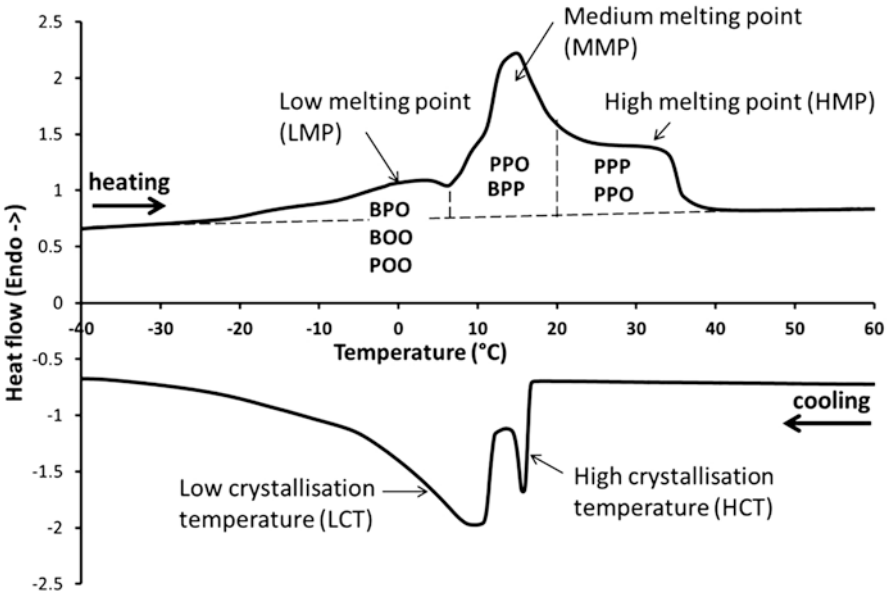

Fig. 9.2 DSC thermograms recorded on cooling and heating of anhydrous milk fat at a rate of 2 °C/min, and main triacylglycerols melting in low, medium and high melting point fractions. B = C4:0, P = C16:0, O = C18:1c9

C4:0; O: oleic acid C18:1c9; P: palmitic acid C16:0). The LMP fraction is liquid at room temperature. The main TAGs that melt in the MMP fraction contain one short-chain FA or one unsaturated FA such as BPP and PPO. The HMP fraction is rich in long-chain saturated FAs, such as PPP. The TAG PPO was found to melt under the three endotherms.

Since 1950, DSC was used to demonstrate the presence of polymorphism in AMF and X-ray diffraction (XRD) was used to identify the polymorphic forms obtained after rapid or slow cooling. The number of thermal transitions in DSC thermograms, the partial overlapping of the melting peaks, their respective enthalpies and transition temperatures depend strongly on the thermal treatments (e.g. heating and cooling rates, tempering) and on the entire thermal history of the sample. The wide melting range of AMF results in a wide range of plasticity where both solid and liquid TAGs are present in various proportions, i.e. increase in the solid fat content as a function of decrease in temperature.

3.2 Effect of Cooling Rate

Characterization of milk fat crystals at different length scales is important in the description and understanding of their functions in food products. The structure of TAG crystals networks is observed at the microscale level using polarized light

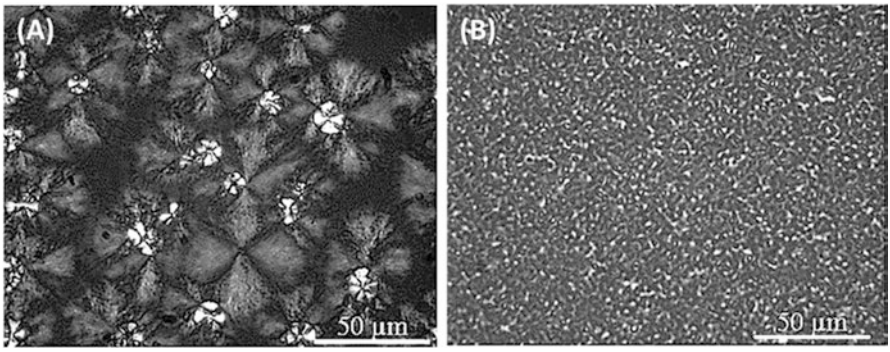

Fig. 9.3 Polarized light microscopy images taken at −8 °C after (**A**) slow cooling at 0.2 °C/min and (**B**) quenching anhydrous milk fat from 60 °C

microscopy because they are birefringent, i.e. TAG crystals appear bright whereas the liquid TAGs appear black. Milk fat crystals and crystal networks have been described at the microstructural level using polarised light microscopy (Campos, Narine, & Marangoni, 2002; Lopez, Lesieur, Bourgaux, & Ollivon, 2005). Milk fat crystals organize as needles or spherulites, depending on the cooling rate (Fig. 9.3). Cooling AMF below 5 °C at low cooling rate leads to crystals with a spherulitic microstructure, due to extensive crystal growth (Campos et al., 2002; Lopez, Lesieur, et al., 2005). When the same milk fat is cooled rapidly from the melt, a more granular microstructure is observed (Campos et al., 2002; Lopez, Lesieur, et al., 2005). Crystallization proceeds more quickly, and nucleation events predominate over crystal growth processes. The result is a large number of small microstructural features distributed in a less orderly fashion than in the case of slow cooling. Ramel, Peyronel, and Marangoni (2016) explored the structural features of high melting point milk TAG at the nanoscale, using the combination of ultra-small angle X-ray scattering and cryo-TEM, and described smooth crystalline nano-platelets.

Most of the studies dedicated to AMF crystallization properties have focused on the description of TAG crystals at a molecular level and investigated the impact of different factors such as cooling rate, TAG and FA composition, and shear (Bugeat et al., 2015; Lopez, Lesieur, et al., 2005; Lopez & Ollivon, 2009; Mazzanti, Marangoni, & Idziak, 2009; Ten Grotenhuis et al., 1999). The identification of the crystalline structures formed by TAG molecules is possible by using XRD recorded at both small and wide angles to have information on the longitudinal organization of TAG molecules, e.g., double chain length (2*L*) or triple chain length (3*L*) and on the packing of the FA chains (polymorphic forms: α, β′, β), respectively. The high energy flux of synchrotron radiation XRD (SR-XRD) allows characterization of the solid TAG phase as a function of temperature or time. Detailed information about the crystallization properties of milk fat has been provided by Michel Ollivon's group (CNRS, France) using the coupling of DSC and XRD as a function of temperature (Ollivon et al., 2006). The AMF samples were heated to 60 °C for at least

5 min to ensure that all crystals and nuclei were melted and to erase the thermal history of TAGs. Then, the crystallization properties of AMF were investigated with cooling rates in the range of 0.1 to ~1000 °C/min. The melting behaviour of AMF was characterized on heating at 2 °C/min. The main results are presented below.

Slow Cooling (0.1 °C/min) of AMF On slow cooling (dT/dt = 0.1 °C/min) of AMF from the melt, XRD patterns revealed the successive formation and the coexistence of four different TAG crystals (Lopez, Lavigne, Lesieur, Keller, & Ollivon, 2001a; Fig. 9.4). The first TAG crystals formed at 24 °C correspond to lamellar structures with a two-chain length organization 2*L* (41.5 Å) and a packing of β′ type. Then, the formation of β′-2*L* (48 Å) crystals was characterised. From 13 °C, the formation of a three-chain length organization 3*L* (62 Å) of α type (α-3*L*) was observed. From 5.5 °C, β′-2*L* (39 Å) crystals were formed. WAXS patterns also showed the formation of traces of β crystals at low temperature (Fig. 9.4). The DSC thermogram recorded simultaneously to XRD experiments during the decrease in temperature showed three main exothermic events that correspond to the successive formation of

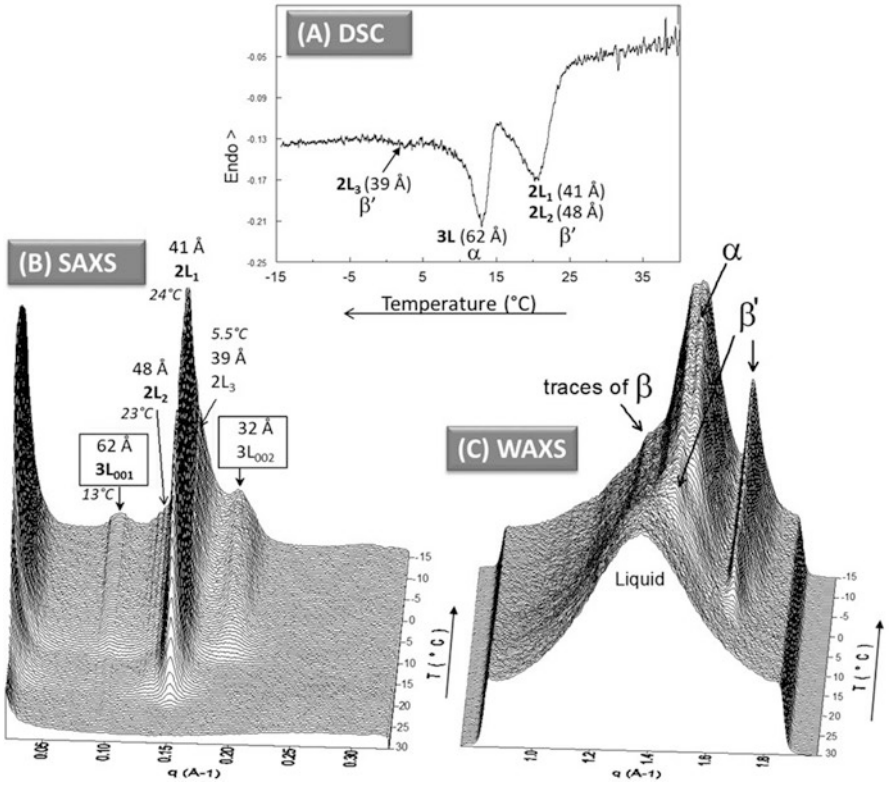

Fig. 9.4 Crystallization of anhydrous milk fat characterized on slow cooling at 0.1 °C/min. (**A**) DSC thermogram, and synchrotron radiation X-ray diffraction patterns recorded (**B**) at small angles (SAXS) and (**C**) at wide angles (WAXS). Adapted from Lopez, Lavigne, et al. (2001a)

TAG crystals present in AMF (Fig. 9.4A). On subsequent heating of the AMF sample, the α-3*L* crystals melt first around 13 °C, then the three different β′-2*L* crystals successively melt without any changes in the structural parameters. The high melting point TAG crystals correspond to a β′-2*L* (41.5 Å) organization that disappears around 40 °C. The absence of polymorphic transformation on heating of AMF is the signature of stable TAG crystals close to equilibrium. This study showed that a TAG molecular segregation occurs in milk fat on slow cooling from the melt. AMF crystallizes and melts in several independent steps corresponding to the phase separation of several groups of TAGs. Each TAG fraction acts as an independent solid solution. The ability of dry fractionation to separate such TAG crystal species into so-called olein and stearin fractions after slow cooling rates confirms that they correspond to different TAG compositions (Kaylegian & Lindsay, 1995).

Cooling of AMF at 1–3 °C/min On cooling of AMF from the melt at the rates of 1–3 °C/min (Fig. 9.5A), TAG molecules sequentially crystallize in α form under three different lamellar structures (Lopez, Lesieur, et al., 2005). From about 17 °C, the successive formation of the α-2*L* (46 Å) and α-2*L* (38 Å) crystals has been characterized and from 14 °C TAG molecules crystallize in a α-3*L* (72 Å) organization. A time-dependent sub-α ↔ α reversible transition was observed at −10 °C. DSC recordings show two successive exotherms that have been attributed to the successive crystallization of TAG in α-2*L* crystals and α-3*L* crystals (Fig. 9.5C). Subsequent heating at 2 °C/min has shown numerous structural rearrangements of the α-2*L* and α-3*L* crystals and the formation of β′-3*L* (67 Å) and β′-2*L* (40–41.5 Å) crystals that takes advantage of the melting of the other crystalline structures (Fig. 9.5B). The recording of an exotherm in the DSC thermogram confirms α to β′ polymorphic transition occurring on heating of AMF (Fig. 9.5D). For temperatures above 20 °C, the remaining β′-2*L* (40–41.5 Å) crystals progressively melt until their disappearance at about 39 °C (Fig. 9.5D). Such rearrangements of TAG molecules into a β′ lamellar organization are facilitated by the presence of the liquid TAG phase. The complex melting behaviour confirms that the TAG crystals formed on cooling at 1–3 °C/min are not thermodynamically stable forms.

Rapid Cooling of AMF The crystallization properties of milk TAGs were studied after quenching from the melt (cooling at ~1000 °C/min), to characterize the most unstable crystalline structures and their reorganization as a function of time (Lopez, Bourgaux, Lesieur, & Ollivon, 2002; Lopez, Lavigne, Lesieur, Keller, & Ollivon, 2001b). The samples were cooled rapidly from 60 to 4 °C or −8 °C to ensure crystallization of TAG molecules.

In the set of experiments performed after quenching of AMF to −8 °C, it was possible to identify the formation of α-2*L* (47 Å) and α-3*L* (70 Å) crystals thanks to the brightness of synchrotron X-ray beam which allows fast recordings (Fig. 9.6A and B). The α-2*L* (47 Å) crystals were very unstable since they disappeared during 20 min isothermal recording and progressively converted into 3*L* crystals (Fig. 9.6C; Lopez, Lavigne, et al., 2001b). On subsequent heating of the AMF sample, the α-3*L*

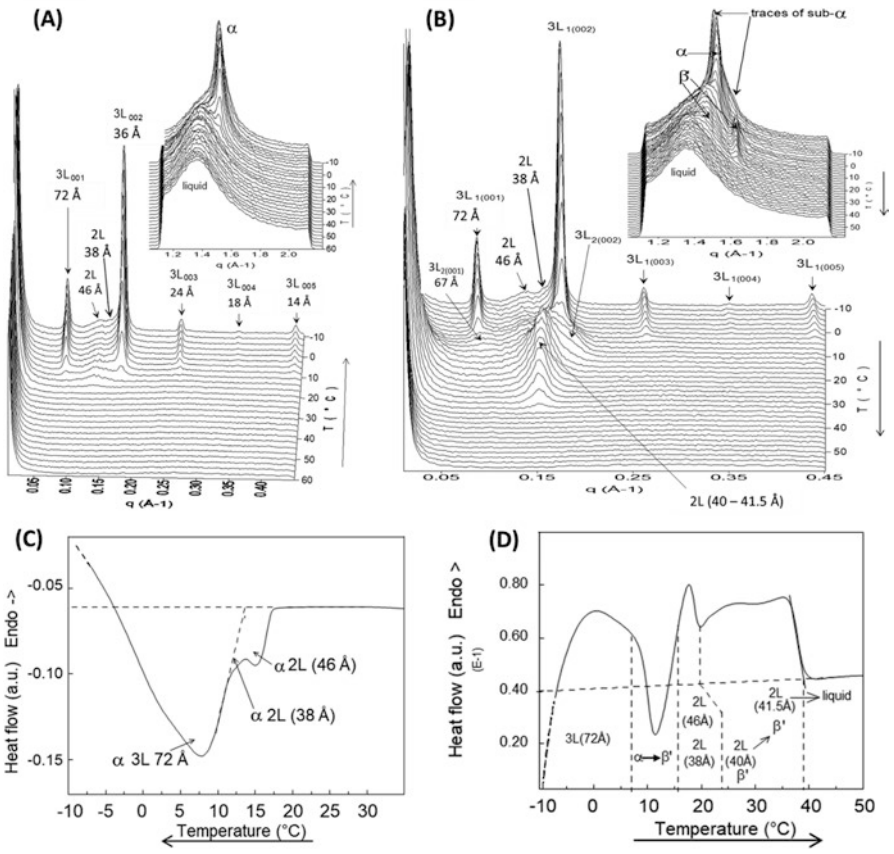

Fig. 9.5 Crystallization properties of anhydrous milk fat. SR-XRD patterns recorded at small and wide (insert) angles (**A**) on cooling at 3 °C/min from 60 to −10 °C, and (**B**) subsequent heating at 2 °C/min. DSC thermograms recorded (**C**) on cooling and (**D**) on heating. DSC thermograms and SR-XRD patterns were recorded simultaneously as a function of temperature. Adapted from Lopez, Lesieur, et al. (2005)

(70 Å) crystals melted. From about 11 °C, TAG molecules in the solid phase formed β′-2*L* (37 Å) crystals (Fig. 9.7). The exotherm recorded at around 11 °C in the DSC thermogram confirms the α to β′ polymorphic transition that occurs on heating of AMF (Fig. 9.7B). The thickness of this β′-2*L* lamellar structure increased up to 41 Å until its final melting, showing structural reorganizations as a function of the increase in temperature.

In a second set of experiments, AFM was quenched at 4 °C and the thermal and structural changes were characterized as a function of time in isothermal conditions, as shown Fig. 9.8A (Lopez, Bourgaux, Lesieur, & Ollivon, 2002). During the 30 min following quenching, structural changes occurred, the α-3*L* (70 Å) structure melted and the 3*L* (66 Å) and 2*L* (39 Å) structures corresponding to β′ and β polymorphs were formed. Isothermal DSC recorded simultaneously to XRD experiments

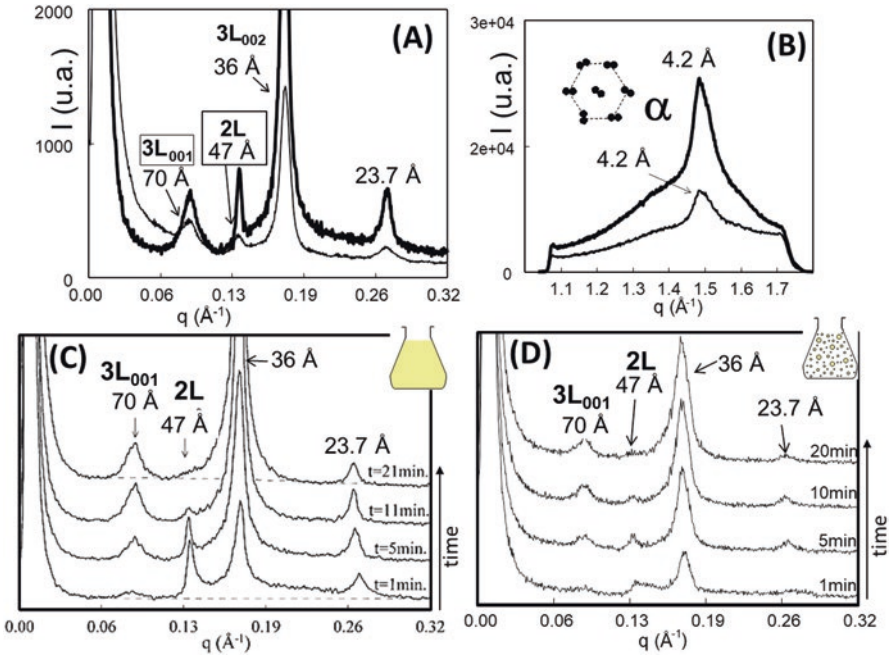

Fig. 9.6 Crystalline structures formed at −8°C by milk triacylglycerols after quenching from 60 °C and evolutions as a function of time in isothermal conditions. SR-XRD patterns recorded (**A**) at small angles and (**B**) at wide angles for anhydrous milk fat (thick lines) and cream (thin lines). Selected SR-XRD patterns recorded at small angles as a function of time at −8 °C just after quenching from 60 °C of (**C**) anhydrous milk fat and (**D**) cream samples. Adapted from Lopez, Lesieur, Keller, & Ollivon (2000) and Lopez, Lavigne, et al. (2001b)

showed exothermic signals with a release of the heat of crystallization as a function of time, corresponding to polymorphic transition from α to β′ and β of milk TAGs (Fig. 9.8C). The nucleation time (the time at which a peak starts forming), time of maximum crystallization rate (the time of peak maximum) and heat of crystallization (proportional to peak area) can all be determined from the DSC thermogram. After 4 days storage of AMF at 4 °C, the crystals were organized in 2*L* (40 Å) and 3*L* (54 Å) lamellar structures with the coexistence of α, β'_1, β'_2 and β polymorphic forms, as shown Fig. 9.8D (Lopez, Bourgaux, Lesieur, & Ollivon, 2002).

As a summary, milk TAGs segregate as a function of the decrease in temperature and display a complex polymorphism, as revealed by DSC and XRD investigations. Depending on the cooling rate, six different types of crystals were identified, several of them in coexistence, and their time- and temperature-dependent evolutions were quantitatively monitored. They correspond to lamellar structures with 2*L* (40–48 Å) and 3*L* (54–72 Å) organizations of TAGs (Fig. 9.9A). At least five crystalline subcell species were observed at wide angles: α and sub-α, two β′ and one β (Fig. 9.9B). All these crystalline structures coexist with a liquid phase even at low temperature, i.e. 4 °C. The comparison of the small number of crystal type formed to the large

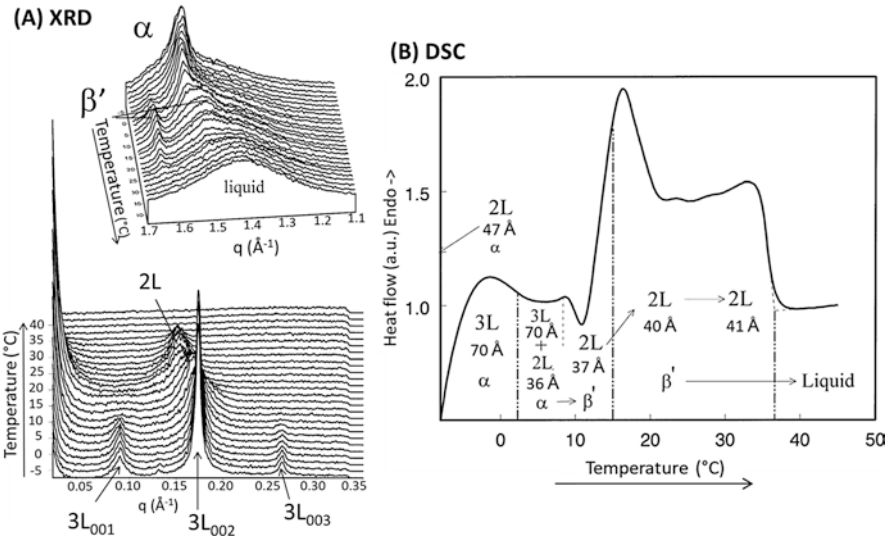

Fig. 9.7 Melting behavior of anhydrous milk fat during heating after quenching from 60 to −8 °C. (**A**) SR-XRD patterns recorded at small and wide (insert) angles on heating at 2 °C/min from −8 to 60 °C. (**B**) DSC thermogram recorded simultaneously to XRD experiments on heating. Adapted from Lopez, Lavigne, et al. (2001b)

number of TAGs present in milk fat (Fig. 9.1B) provides evidence that mixed crystals are formed in AMF (i.e. co-crystallization of different TAG molecules). On cooling, the first longitudinal organizations of TAG molecules correspond to 2*L* structures with long spacings of about 38–48 Å. These crystalline varieties may correspond to crystallization of HMP fractions of TAGs. These 2*L* crystals are generally formed by TAGs with saturated and similar chain length FAs, such as MPP and PPP (Fig. 9.9C). Then, crystallization of 3*L* structures (about 62–72 Å) occur. Triple chain length 3L stackings may correspond to crystallization of unsaturated TAG molecules (e.g. PPO) or to that of TAGs with FA chains of different lengths, like BPP (Fig. 9.9C). On heating, the crystals formed on cooling (2*L*, 3*L*) melt and recrystallization take place with the formation of a β′-2*L* (40–41.5 Å) structure. The increase in the thickness of the β′-2*L* crystals on heating is attributed to progressive and selective melting of the TAGs with the shortest FA chains so that the solid phase composition enriches with longer FA chains and higher melting points TAGs. Recrystallization also occurs during isothermal storage. The stable crystalline structures take advantages of the melting of the metastable crystals which melt first or Ostwald ripening occurring thanks to the liquid phase that coexists with the solid phases. The mixed TAG crystals formed in milk fat organize in the less polymorphic forms β′ and α because molecular packing is not very dense. Low amount of β crystals have been identified in milk fat, even after long time storage at low temperature. It has been reported on milk fats from individual cows with large differences in their

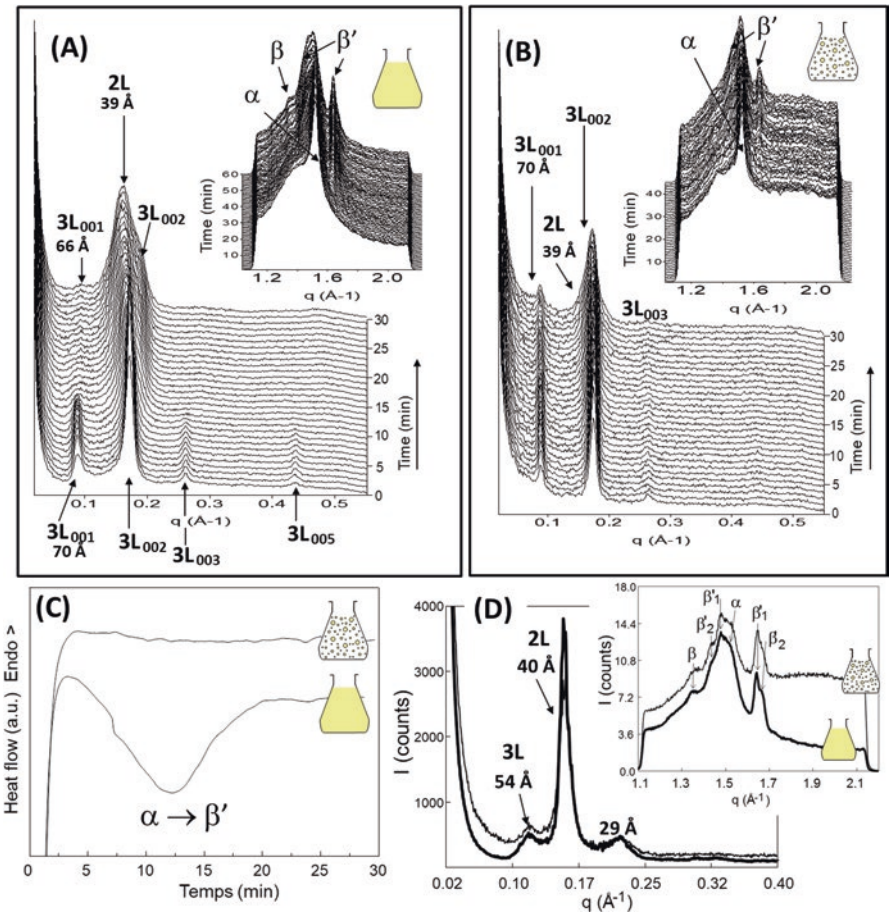

Fig. 9.8 Isothermal evolutions of SR-XRD patterns recorded at small and wide angles at 4 °C after rapid quenching from 60 °C of (**A**) anhydrous milk fat and (**B**) cream samples. (**C**) DSC recordings of AMF and cream samples obtained simultaneously with XRD experiments. The signal jumps observed on the left side of the DSC recordings correspond to the equilibration of the microcalorimeter after sample introduction. (**D**) Small and wide (insert) angle SR-XRD patterns recorded at 4 °C after storage of cream (thin line) and anhydrous milk fat (thick line) samples at this temperature for 135 and 105 h, respectively, following a rapid quenching from 60 °C. Adapted from Lopez, Bourgaux, Lesieur, and Ollivon (2002)

TAG profiles that the presence of β polymorphs is dependent on TAG composition and that cooling rate and tempering are not critical factors in the formation of β polymorphs (Tzompa-Sosa, Ramel, van Valenberg, and van Aken (2016). The high concentration of unsaturated TAGs with a carbon number 52–54 and the presence of a substantial amount of liquid TAGs may be equally important for the formation of β polymorphs.

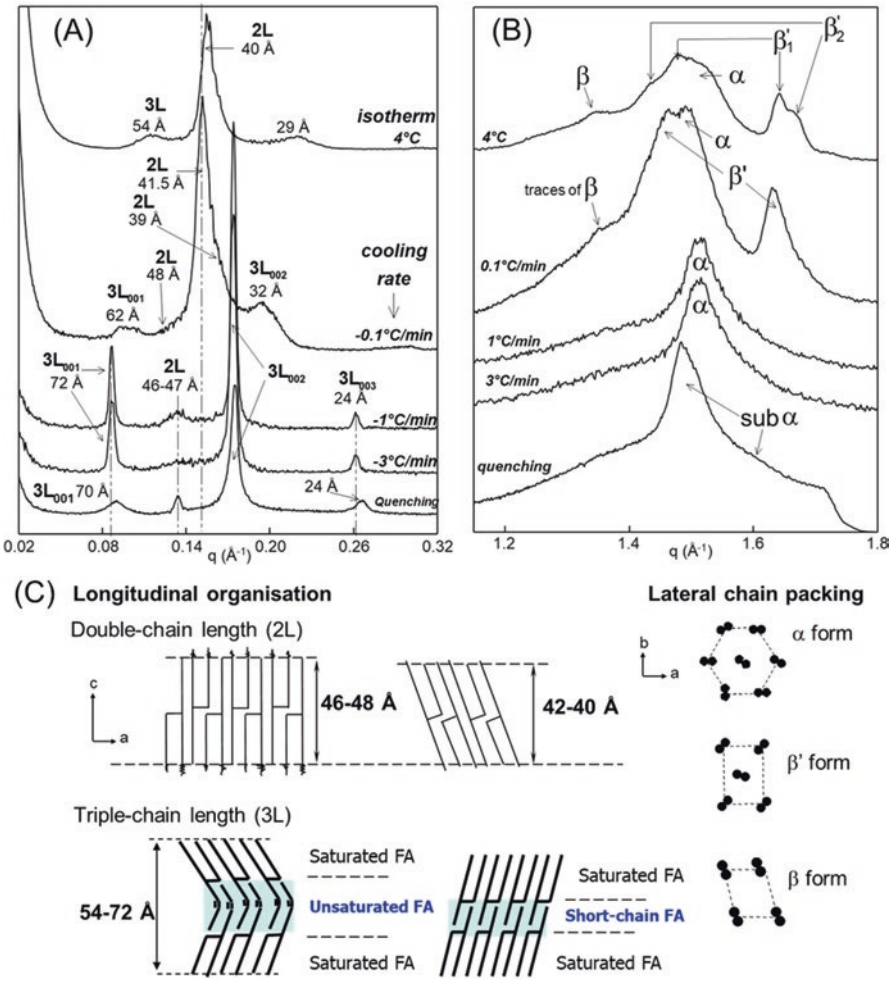

Fig. 9.9 SR-XRD patterns recorded at −8 °C (**A**) at small angles and (**B**) at wide angles after either cooling of anhydrous milk fat at different rates as indicated on the figure or isothermal conditioning at 4 °C during 5 days. (**C**) Lamellar structures formed by anhydrous milk fat molecules in the solid state (fatty acids are drawn as straight lines) and acyl chain lateral packings corresponding to α, β′ and β polymorphic forms. Adapted from Lopez, Lesieur, et al. (2005)

3.3 Effect of Minor Lipid Compounds

TAGs represent generally 97–98% of milk fat. The balance is composed mainly by minor lipids such as free fatty acids (FFAs), monoacylglycerols (MAGs), diacylglycerols (DAGs) and phospholipids that can influence the mechanisms of TAG crystallization in AMF i.e. the nucleation stage, the crystal growth and/or the polymorphic behaviour. Most of the experiments investing the effect of minor components on

milk fat crystallization are performed under isothermal conditions and the crystallization behaviour is monitored by DSC and pulsed nuclear magnetic resonance (pNMR). The crystallization process is described by the Avrami and the Gometz models which are fitted by non-linear regression.

It has been shown that removal of minor lipid components from milk fat has no effect on melting point, equilibrium solid fat content, polymorphic forms, microstructural crystal network and mechanical properties (Wright, Hartel, Narine, & Marangoni, 2000; Wright & Marangoni, 2003). However, the minor components affect the crystallization kinetics of milk fat and delay the onset of crystallization at low degrees of supercooling (Wright et al., 2000). Milk fat DAGs have been reported to have an inhibitory effect on the crystallization of milk TAGs without modifying the microstructure of crystals. It was suggested that structural complementary between DAGs and crystallizing TAGs allowed the TAGs to co-crystallize within early seed crystals and subsequent further delay TAG crystallization (Wright et al., 2000). Other studies showed that the effect of DAGs and MAGs on the crystallization behaviour of milk fat depends on temperature and concentration. Moreover, the type of esterified FAs and the polar head of the amphiphilic molecules determine to what extent the partial glycerides MAGs and DAGs influence the nucleation and crystal growth of TAGs (Foubert, Vanhoutte, & Dewettinck, 2004). For example, stearic acid (C18:0) based MAGs and DAGs enhance heterogeneous nucleation at low temperatures, while at higher temperatures an interaction with the crystal growth predominates. Oleic acid (C18:1c9) based MAGs and DAGs have an effect on the nucleation process while no interaction with the crystal growth was observed (Foubert et al., 2004). The effects of MAGs on milk TAG crystallization in recombined cream revealed differences as a function of the FA esterified (Fredrick et al., 2013). MAGs with C18:0 formed upon cooling a two-dimensional crystal at the surface of the emulsion droplet which induced interfacial heterogeneous nucleation and an acceleration of TAG crystal growth and α to β′ polymorphic transition. On the contrary, MAGs with C18:1c9 did not affect the crystallization behaviour while MAGs with C12:0 showed an intermediate behaviour. None of the MAGs influenced the solid fat content after storage for 5 days at 5 °C. The observed differences in nucleation mechanisms and crystallization kinetics may influence the microstructural arrangement of the milk TAG inside the emulsion droplets and consequently the partial coalescence rate and hence the whipping properties of recombined creams. Phospholipids were shown to delay the onset time of AMF crystallization under isothermal conditions at 25 °C by their fast adsorption on the growth sites of crystals inducing steric hindrance (Vanhoutte, Dewettinck, Foubert, Vanlerberghe, & Huyghebaert, 2002). The effect of FFAs on milk fat crystallization has been demonstrated under isothermal conditions at 25 °C. The addition of short-chain FFAs increased the induction time of milk fat crystallization while the addition on saturated long-chain FFAs accelerated the crystallization kinetics with consequences on the microstructure of milk fat crystals (Bayard, Leal-Calderon, & Cansell, 2017). As a conclusion, the molecular interactions between the minor lipids and milk TAGs able to affect milk fat crystallization depend on the temperature, on their concentration, and on the FA composition (mainly the chain length similarity) of the phospholipids, FFAs, MAGs and DAGs.

3.4 Effect of Shear on AMF Crystals

AMF crystallization studies carried out under shear are of particular interest (Grall & Hartel, 1992; Mazzanti et al., 2009; Van Aken & Visser, 2000; Vanhoutte et al., 2002). Shear affects the crystallization process as a whole by enhancing molecular diffusion and favouring the rearrangement of TAG molecules in the melt, which helps to overcome the kinetic barriers for nucleation and growth. The studies on shear effects demonstrated the formation of smaller TAG crystals and narrower distribution sizes at higher shear rates and attributed this to higher nucleation rates and breakdown of milk fat crystals. Under very slow cooling rates and mixing speeds, little effect was found from the agitation speed on the kinetics of milk fat crystallization (Vanhoutte et al., 2002). A detailed synchrotron XRD study on the kinetics of crystallization of AMF performed in a Couette cell showed a shear-induced acceleration of the α to β′ form transition and the presence of crystalline orientation (Mazzanti et al., 2009). Shear, i.e. agitation of milk fat, affects the crystallization kinetics and the microstructure with consequences on the mechanical properties of the fat crystal network obtained. Agitation enhances nucleation, which leads to the formation of numerous small crystals with a softening of the material.

4 Crystallization of TAGs in Bovine Milk Fat Globules and Emulsion Droplets

Milk and many dairy products are O/W emulsions (e.g. cream, cheeses). Studying the crystallization properties of milk TAGs in milk fat globules and processed emulsion droplets is of prime importance because it can affect many properties, such as: (1) the resistance of fat globules to disruption and then to coalescence, (2) the susceptibility of fat globules to churning for the manufacture of butter, (3) the partial coalescence and stability of whipped cream, and (4) the texture, rheological properties and mouth feel of high-fat content food products, e.g. cream, butter, cheeses products. It is therefore important to understand better the physical properties of TAGs in milk fat globules, e.g. their thermal and crystallographic properties, for industrial applications and to improve the quality of food products. Moreover, it is interesting to compare crystallization of milk TAGs dispersed in emulsion, such as milk or cream (which is the high concentration of fat globules from milk to reach at least 30% fat) in which fat globules are surrounded by a biological membrane rich in phospholipids, with crystallization of bulk anhydrous milk fat (AMF). It is also important to know the crystallization properties of TAG dispersed in processed O/W emulsion droplets of various sizes to better control the quality of food products.

The crystallization properties of milk TAGs in O/W emulsions were studied at different scale levels by using polarized light microscopy and transmission electron microscopy (TEM), DSC and XRD. Microscopy techniques showed that the

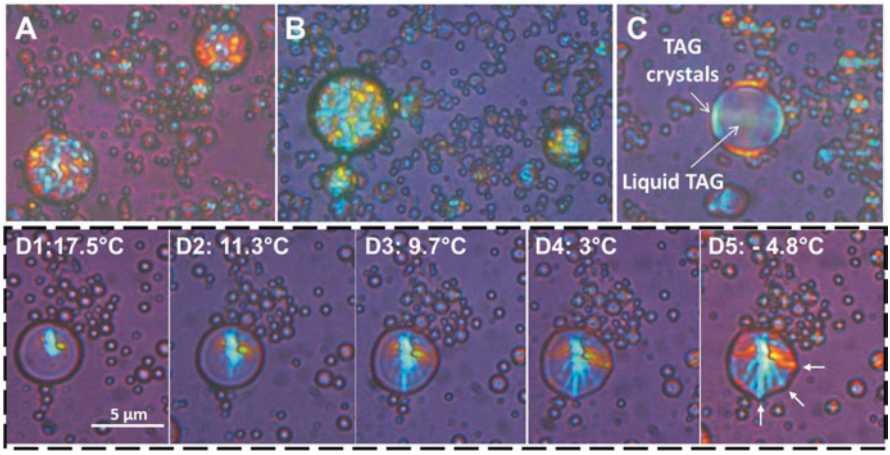

Fig. 9.10 Polarized light microscopy images of TAG crystals within milk fat globules formed after cooling at different rates from 60 to −5 °C, (**A**) cooling at 5 °C/min, (**B**) quenching, (**C**) cooling at 0.5 °C/min. (**D**) Images taken during cooling of milk at 1 °C/min, the intermediate temperatures are indicated in the figure. TAG crystals can deform the milk fat globule membrane as indicated by arrows. Adapted from Lopez (2011)

morphology and the location of TAG crystals within milk fat globules are affected by the cooling rates and tempering. After rapid cooling of milk, numerous small crystals of needle type are formed with no preferred orientation. At slow cooling rate, fat globules display needle-shaped crystals that can deform the biological membrane surrounding milk fat globules (Fig. 9.10). After long storage at low temperature (4–7 °C) or after tempering, TAG crystals are of needle type with a radially organized crystallization. Using cryo-TEM, Prof. Bhesh Bhandari's group showed the stacking of individual lamellar layers with 38–42 Å thickness formed by milk TAG molecules at the periphery of droplets in nano-emulsions upon cooling at 4 °C (Fig. 9.11; Truong et al., 2015).

The examination of the crystallization behaviour of TAGs and TAG polymorphism in emulsion by using XRD is much more challenging than for AMF and especially difficult since (1) both small and wide angle XRD should be considered at the same time and compared to determine the evolution of each of the species as a function of time, (2) the X-ray intensity diffracted by each of the crystalline structures is proportional to the fraction of particular crystal in the structure, (3) the whole XRD signal is largely absorbed by the surrounding water and its solutes (e.g. casein micelles, minerals, lactose) and (4) the small-angle XRD peak broadening results from the crystallization constraints in dispersed systems and to the lower size of the crystals. The use of DSC coupled to synchrotron radiation XRD is a suitable way allowing identification of the crystalline structures formed by TAG molecules as a function of temperature and time in dispersed systems such as milk fat globules (Lopez et al., 2000; Lopez et al., 2002; Lopez, Bourgaux, Lesieur, & Ollivon, 2002;

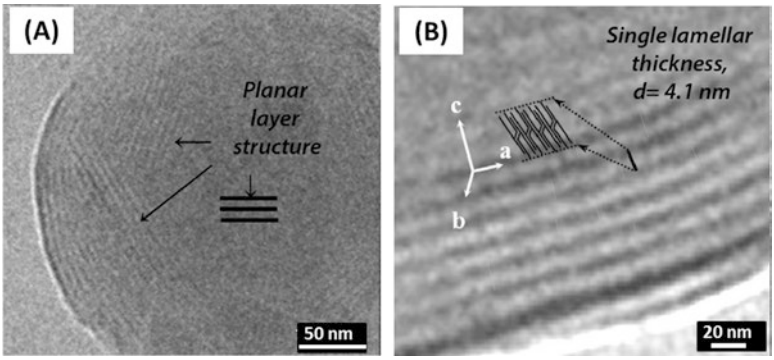

Fig. 9.11 Cryo-TEM micrographs of nano-emulsions containing crystalline particles of high melting point fraction of milk fat called stearin, upon crystallization at 4 °C. (**A**) stacking of individual TAG lamellar layers formed in the stearin nano-emulsions after cooling at 1 °C/min. (**B**) Thickness of single lamellae including light and dark layers in the stearin nano-emulsions. Adapted from Truong, Morgan, Bansal, Palmer, and Bhandari (2015)

Lopez, Lesieur, Bourgaux, Keller, & Ollivon, 2001) and emulsion droplets of various sizes (Bugeat et al., 2011; Lopez, Bourgaux, Lesieur, Bernadou, et al., 2002; Michalski, Ollivon, Briard, Leconte, & Lopez, 2004). The investigations of milk TAG crystallization within emulsion droplets is not performed below about −10 °C to avoid the formation of ice crystals that could alter the physical stability of the emulsion.

4.1 Effect of Cooling Rate and Tempering

The crystallization behaviour of milk fats in emulsion is influenced by changing the cooling rate, more remarkably than that in a bulk phase. It is also affected by tempering (i.e. successive cooling and heating). Therefore, we discuss here the effects of cooling rate on the crystallization properties of milk fat dispersed in milk fat globules or lipid droplets, which are crystallized at the rates of cooling in the range of 0.1–1000 °C/min from 60 °C and subjected to subsequent heating at the rate of 2 °C/min.

Slow Cooling of Milk Fat Globules Slow cooling of cream (0.1–0.15 °C/min) leads to the DSC recording of a single broad exotherm corresponding to crystallization of TAG in fat globules (Fig. 9.12B). However, precise XRD study allowed isolating four polymorphic forms that are successively formed during the cooling process (Fig. 9.12A, Lopez, Lesieur, et al., 2001). On cooling from the melt, the 2*L* (47 Å) crystals were first formed from 22 °C, then the 2*L* (40 Å) crystals were observed from 20 °C. From 16 °C, the formation of 3*L* (71 Å) and 3*L* (65 Å) organizations were reported. On slow cooling of cream (0.1–0.15 °C/min), nucleation

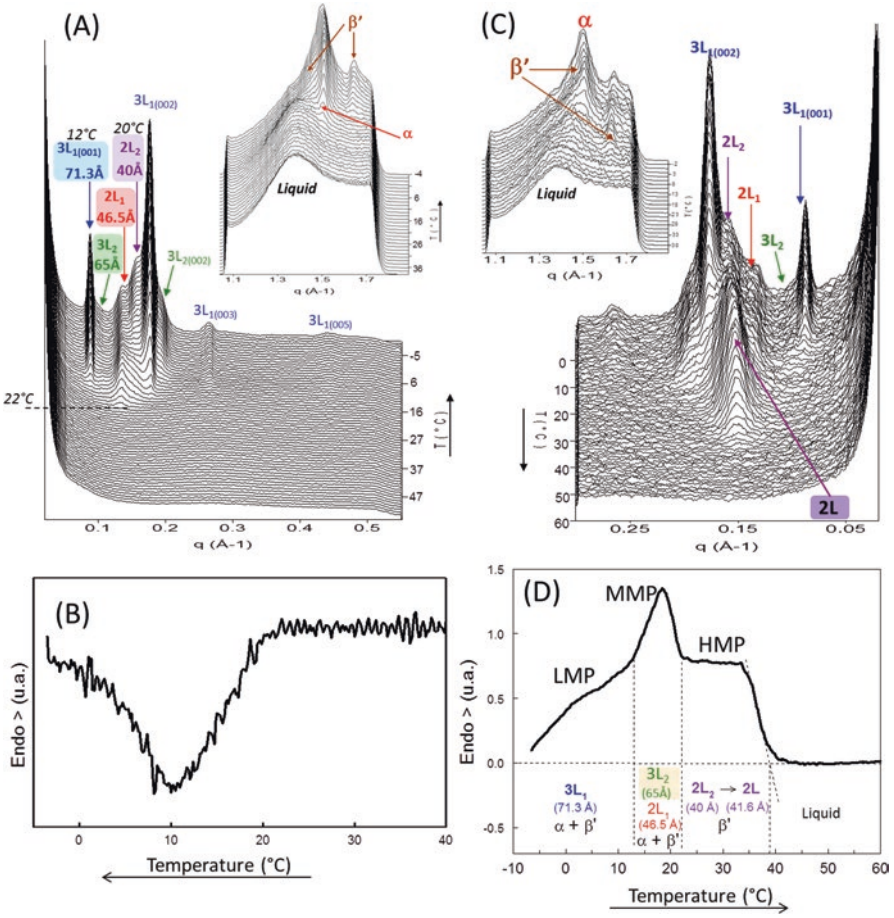

Fig. 9.12 Crystallization of triacylglycerols within milk fat globules characterized on cooling at 0.1 °C/min. SR-XRD patterns recorded (**A**) on cooling and (**C**) on subsequent heating at 2 °C/min. DSC thermograms recorded (**B**) on cooling and (**D**) on heating. DSC and SR-XRD were measured simultaneously. LMP: low melting point, MMP: medium melting point, HMP: high melting point. Adapted from Lopez, Lesieur, et al. (2001)

occurs in the α form, then both the α and β′ polymorphic forms coexist until the end of cooling. On subsequent heating of the cream, the successive melting of the 3*L* (71 Å), 3*L* (65 Å) and 2*L* (47 Å) organizations occurred (Fig. 9.12C). The β′-2*L* (40 Å) predominantly occurred during the cooling and heating processes, and its lamellar thickness increased to 416 Å on heating. The DSC curve recorded on heating of the cream showed three endotherms (Fig. 9.12D). The LMP endotherm corresponds to the melting of the 3*L* (71 Å) crystals, the MMP endotherm was related to the melting of both the 3*L* (65 Å) and the 2*L* (47 Å) crystals, the HMP endotherm was attributed to the melting of the β′-2*L* crystals (Fig. 9.12D).

The comparison of the crystallization properties of milk TAGs dispersed within fat globules and in anhydrous state revealed the following differences:

- The initial crystallization temperature of TAGs within milk fat globules was depressed compared to bulk TAGs (T_{onset} = 20.4 versus 25.7 °C), showing that cream requires a much higher supercooling than does AMF. The differences in supercooling can largely be explained by the theory of nucleation for TAG crystallization. In cream, milk TAGs are divided into numerous fat globules that are isolated from the others by the aqueous phase and by the biological membrane, stabilizing the fat globule surface (Lopez, 2011). This means that, if TAG crystallization occurs in one fat globule, it will not easily spread to the TAGs in the surrounding fat globules, at least when no shear is applied to the system. In AMF, once TAG crystallization begins, it rapidly spreads throughout the whole system because of the processes of secondary nucleation and crystal growth.
- Different TAG crystals were formed (nucleation in α form in cream vs. in β′ form in AMF; Lopez, Lesieur, et al., 2001), revealing the high role played by the dispersion of milk TAGs on their crystallization properties.

Cooling of Milk Fat Globules at 1–3 °C/min On cooling of cream from the melt at the rates of 1–3 °C/min (Fig. 9.13A and B), milk TAGs sequentially crystallize within milk fat globules in three different lamellar structures (Lopez, Bourgaux, Lesieur, Bernadou, et al., 2002). From about 19 °C, α-2*L* (47 Å) and α-2*L* (42 Å) crystals were formed and below 15 °C the crystallization of TAGs in the α-3*L* (71 Å) organization was recorded (Fig. 9.13A). On subsequent heating from −10 to 60 °C at 2 °C/min (Fig. 9.13C and D), the α-3*L* (71 Å) crystals melt at about 16 °C and some TAGs are involved in the formation of α-3*L* (65 Å) organization before its melting at about 20 °C. The α-2*L* (47 Å) and α-2*L* (42 Å) structures also melt around 20 °C. Between about 17 and 20 °C, crystallization of a new lamellar structure β′-2*L* (39 Å) occurred with structural reorganization leading to an increase of its thickness up to 41.7 Å. This high-melting point organization of TAGs in their solid state, likely formed by the reorganization of the TAGs initially incorporated in the crystals, progressively melted from about 21 °C and disappeared over 38 °C. On cooling at 1–3 °C/min, similar crystalline structures are formed by TAGs within milk fat globules of cream (Fig. 9.13) and in AMF (Fig. 9.5). However, the thickness of the α-3*L* organization is slightly thicker in AMF (72.5 *versus* 72.1 Å) and the small-angle XRD peak widths were larger in cream, showing defects of longitudinal stacking in α-3*L* crystals within milk fat globules. DSC curves recorded on cooling showed differences between cream and AMF (Fig. 9.13B, Fig. 9.5C). Crystallization of TAGs in AMF is induced at higher temperature with a sharp exotherm at about 18 °C, related to crystal growth of α-2*L* crystals.

Tempering of milk fat globules and emulsion droplets (i.e. successive cooling and heating) at controlled temperatures allows tailoring TAG crystals. In the previous paragraph, we discussed the formation of α-3*L* (71 and 65 Å) and α-2*L* (47 and 42 Å) crystals within milk fat globules after cooling at 1–3 °C/min from the melt. The subsequent heating of the cream to about 17–20 °C leads to the melting of these

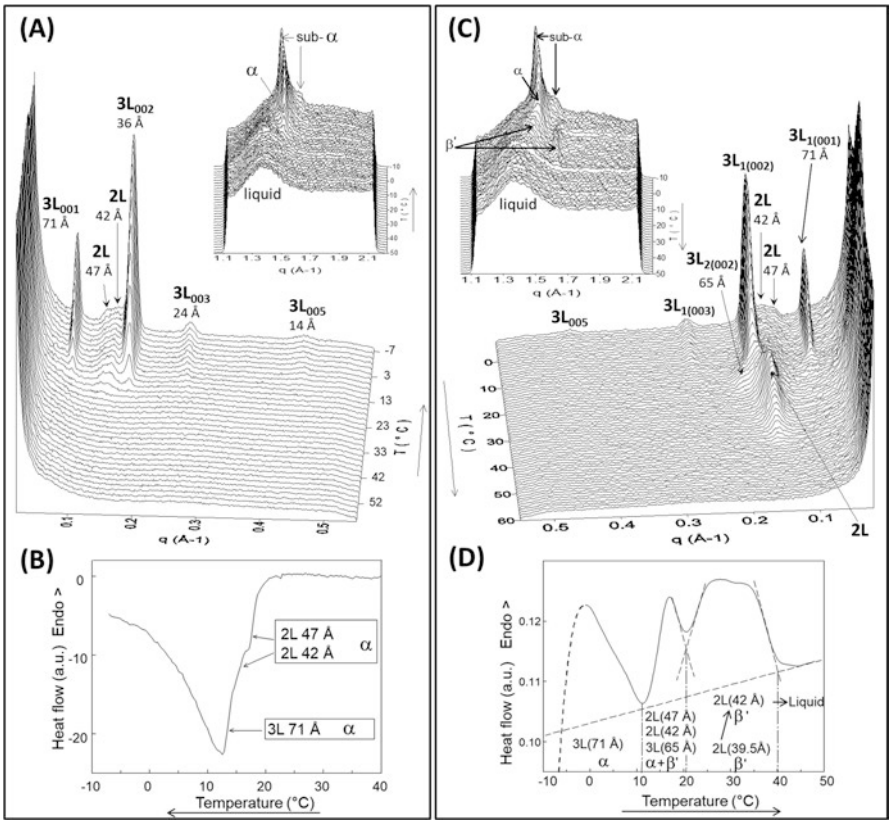

Fig. 9.13 Crystallization of triacylglycerols within milk fat globules characterized on cooling at 1 °C/min. SR-XRD patterns recorded at small and wide (insert) angles (**A**) on cooling at 1 °C/min from 60 to −10 °C, and (**C**) on subsequent heating at 2 °C/min. DSC thermograms recorded (**B**) on cooling and (**D**) on heating. DSC and SR-XRD were recorded simultaneously. Adapted from Lopez, Lesieur, et al. (2005)

TAG crystals and to crystallization of a new lamellar structure β′-2*L* (39 Å). The stabilization of temperature at about 20 °C and then cooling of the cream to 4 °C favours the growth of β′-2*L* (39 Å) crystals. Tempering of cream leads to reorganization of TAGs within emulsion droplets and can have implications on the physical stability and functional properties of milk fat globules (e.g. in the manufacture of butter, whipped cream).

Rapid Cooling of Milk Fat Globules In the dairy industry, milk and cream are often submitted to high-temperature thermal treatments (e.g. pasteurization) and rapidly cooled in a tank for storage before processing. Understanding the crystallization behaviour of milk TAGs within milk fat globules during this thermal history is therefore of industrial relevance. The most unstable crystalline structures of the TAGs formed within milk fat globules were studied after quenching from the melt

down to −8 or 4 °C (~1000 °C/min), as shown Fig. 9.6A. Their organization was characterized as a function of time (Fig. 9.6D) and on subsequent heating (Lopez et al., 2000; Lopez, Bourgaux, Lesieur, & Ollivon, 2002). The most unstable TAG crystals formed in milk fat globules correspond to α-2*L* (47 Å) and α-3*L* (70 Å) lamellar structures, as in AMF (Fig. 9.6A). Due to the rapid cooling, the crystallization starts in the metastable α-polymorph. The α-2*L* organization is very unstable and disappears during a 20 min conditioning in isothermal conditions (Fig. 9.6D).

In a first series of experiments, the cream was heated at 2 °C/min. On heating, the α-2*L* (47 Å) and α-3*L* (70 Å) lamellar structures progressively melted and from 13 °C a new lamellar organization was formed, β′-2*L* (38 Å), and was the single solid TAG organization until its final melting above 38 °C. The crystallization occurring in emulsion is similar to in anhydrous state, showing that the metastable TAG molecular packings obtained after quenching from the melt are not drastically affected by the dispersion state. However, the width of small-angle XRD peaks indicated that TAG crystallization is more disordered in emulsion, which has been attributed to the constraints due to the interface curvature in the emulsion droplets.

In a second series of experiments, the cream and AMF were quenched from 60 to 4 °C and stored in isothermal conditions for comparison, as shown Fig. 9.8B (Lopez, Bourgaux, Lesieur, & Ollivon, 2002). After quenching at 4 °C, similar liquid to solid TAG phase transition occurred as after quenching to −8 °C, but the α-2*L* (47 Å) structure remained less than 1 min since the higher liquid TAG phase amount favoured the α-2*L* (47 Å) to α-3*L* (70 Å) transition. During isothermal storage at 4 °C, crystallization and polymorphic evolutions occurred. The α-3*L* (70 Å) structure transformed as a function of time into 2*L* (39 Å) and 3*L* (66 Å) lamellar structures through $\alpha \rightarrow \alpha + \beta' + \beta$ secondary exothermic transitions. This secondary process is faster in AMF (within 30 min) than in cream (>1 h). The delayed polymorphic evolution observed in milk fat globules shows that the dispersion state of TAGs plays a role in the transition process and could be explained by a lack of stable nuclei in each fat globule at 4 °C as compared to AMF. Similar conclusions were drawn by comparing the isothermal crystallization behaviour at 5 °C of milk fat in bulk and emulsified state in natural and recombined creams (Fredrick et al., 2011). It is important to note that this exothermic transition leads to an increase in temperature during the storage of cream at the industrial level.

After 4 days storage at 4 °C, similar TAG crystals were characterized within milk fat globules and in AMF, i.e. coexistence of 2*L* (40 Å) and 3*L* (54 Å) lamellar structures corresponding to α, β'_1, β'_2 and β polymorphs (Fig. 9.8D). This is in line with Söderberg, Hernqvist, and Buchheim (1989) who reported that the main structure formed by the TAGs in milk fat globules after long-time storage at low temperature was a bilayer with a thickness of 3.9 nm. Also, these TAG crystals are similar to those characterized in butter (Buldo, Kirkensgaard, & Wiking, 2013; Ronholt, Kirkensgaard, Mortensen, & Knudsen, 2014). At 4 °C, the HMPF and the MMPF of milk fat will crystallize and constitute the solid TAG phase while the LMPF is present is the liquid TAG phase. Therefore, the solid TAG phase primarily consists of TAGs containing three long-chain saturated FAs and TAGs containing two

long-chain saturated FAs and a long chain unsaturated FA or a short-chain saturated FA (Fig. 9.2).

The existence of an isothermal polymorphic evolution both in milk fat globules and in AMF was demonstrated (Lopez, Bourgaux, Lesieur, & Ollivon, 2002). Density measurements were sensitive to the α to β′ and β′ to β polymorphic transitions occurring within milk fat globules following the quenching of cream at 4 °C (Lopez, Bourgaux, Lesieur, & Ollivon, 2002). The increase in density, that corresponds to an increase in the compactness of milk TAGs, is explained by a reduction of the sub-cell volume from about 25.5 A^3 (α form) to 23.5 A^3 (β′ form) and a possible increase in the amount of TAGs crystallised.

After 6 days of storage at 4 °C, the cream and AMF were heated to 60 °C at 2 °C/min. The 3L crystals corresponding to α, β'_2 and β polymorphs melted first, and from about 23 °C the solid TAG phase corresponded to a β'_1-2*L* (40 Å) organization until its final melting. The DSC curves recorded simultaneous on heating showed to main endotherms corresponding to the successive melting of 3*L* and 2*L* crystals.

As a summary concerning the crystallization behaviour of milk TAGs in cream (i.e. concentrated milk fat globules), DSC curves recorded on cooling show two overlapped exothermal events, i.e. a small event due to the crystallization of 2*L* forms and a broad event related to crystallization of 3*L* structures identified thanks to the coupling with XRD. Whatever the cooling rate of cream, the DSC curve recorded on subsequent heating exhibits three main endotherms more or less overlapped corresponding to the LMP, MMP and HMP fractions of TAGs (Lopez, Bourgaux, Lesieur, Bernadou, et al., 2002). An exothermic event corresponding to α → β′ polymorphic evolution can be recorded between the first two endotherms after fast cooling of milk fat globules. As for AMF, the LMP fraction corresponds to melting of 3L lamellar structures, the MMP fraction to the melting of 2*L* lamellar structures formed on cooling. The HMP fraction corresponds to the progressive melting of the β′ 2*L* lamellar structures formed during heating until final melting of TAGs dispersed within fat globules of cream. These studies dedicated to the crystallization properties of milk TAGs in emulsion showed that milk TAGs are partially crystallized and that the solid TAG phase displays a complex polymorphism which is temperature and time dependent. The polymorphic transitions that occur in the solid TAG phase are favoured by the liquid TAG phase.

Figure 9.14 shows the crystalline structures formed within milk fat globules at −8 °C after cooling at different rates and the crystals formed upon storage at 4 °C. As for AMF, the comparison of the small number of crystal type formed to the large number of TAGs present in milk fat provides evidence that mixed crystals are formed within milk fat globules (i.e. co-crystallization of different TAG molecules). On cooling, the first longitudinal organizations of TAG molecules dispersed within fat globules correspond to 2*L* structures with long spacings of about 40–42 Å and 46–48 Å. These crystalline varieties may correspond to crystallization of HMP fractions of milk TAGs. These 2*L*-type crystals are generally formed by TAGs with saturated and similar chain length FAs, such as MPP and PPP (Fig. 9.9C). Then, crystallization of 3*L* structures (about 70–72 Å) occurs. The formation of a 3*L* (65 Å) structure was only observed on slow cooling of milk fat globules (0.15 °C/

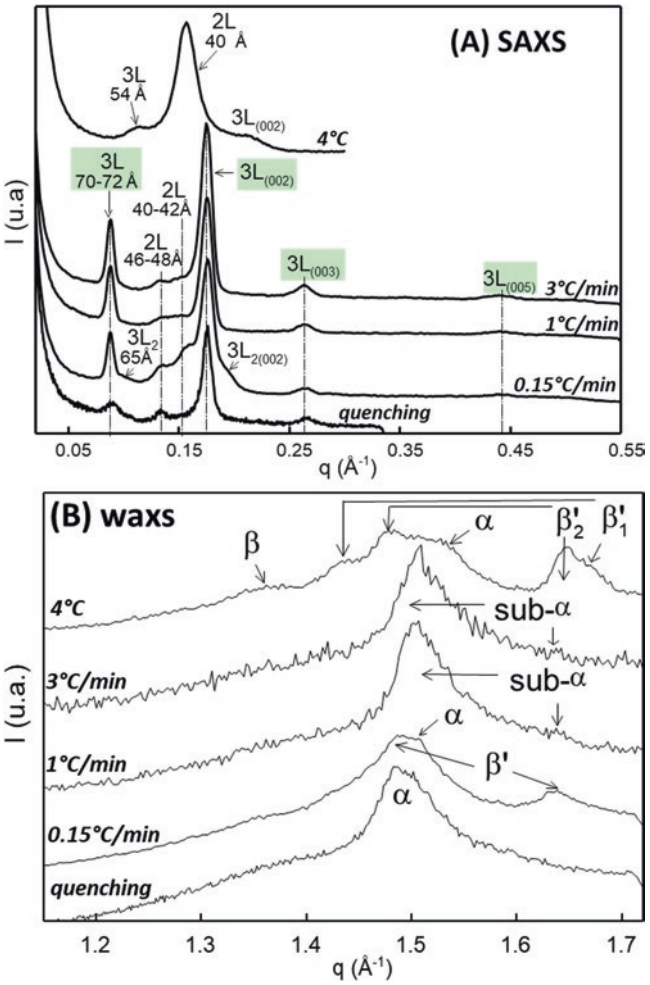

Fig. 9.14 SR-XRD patterns recorded at −8 °C: (**A**) at small angles (SAXS) and (**B**) at wide angles (WAXS) after either cooling of milk fat globules concentrated in cream at different rates as indicated on the figure or isothermal conditioning at 4 °C during 5 days. Adapted from Lopez, Bourgaux, Lesieur, Bernadou, et al. (2002)

min). Triple chain length (3*L*) stackings may correspond to crystallization of unsaturated TAG molecules or to that of TAGs with FA chains of different lengths, like BPP, OPP (Fig. 9.9C). Whatever the cooling rate, initial crystallization occurs in a hexagonal packing (α form). Then, as a function of the decrease in temperature, the formation of β′ form and the coexistence of α and β′ polymorphic forms was observed. Traces of β form crystals were only recorded on slow cooling of milk fat globules (0.15 °C/min) and after at least 3 days storage at 4 °C. According to Lopez, Bourgaux, Lesieur, and Ollivon (2002), stabilization of the TAG crystals is only

attained after at least 4 days storage at 4 °C. On heating, the crystals formed on cooling (α 2*L*, α 3*L*) melt and reorganizations of crystals take place within milk fat globules with the formation of a β′-2*L* (40 Å) structure, accompanied or not by α-3*L* (54 Å). Recrystallization also occurs during isothermal storage. The stable crystalline structures take advantages of the melting of the metastable crystals which melt first or of some kind of Ostwald ripening occurring within the milk fat globules thanks to the liquid TAG phase that coexists with the solid TAG phases. The β′-2L mixed crystals selectively melt starting with the TAGs with shorter chains as shown by a progressive increase on their thickness. As for AMF, low amount of β crystals have been identified in milk fat globules, even after long time storage at low temperature. The mixed TAG crystals formed in milk fat organise in the less polymorphic forms β′ and α because molecular packing is not very dense.

4.2 Effect of the Size of Milk Fat Globules and Lipid Droplets

Studies on milk fat globules and protein-coated lipid droplets showed that the temperature of the initiation of TAG crystallization is lowered with decreasing size (Fig. 9.15), due to increased supercooling (Lopez, Bourgaux, Lesieur, Bernadou,

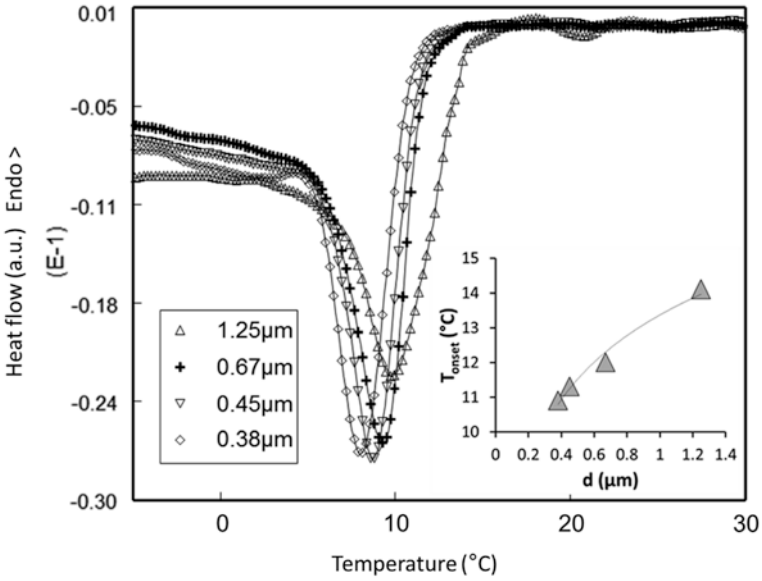

Fig. 9.15 Effect of the size of O/W emulsion droplets on the initial temperature of milk fat crystallization (T_{onset}). DSC thermograms recorded on cooling of protein-coated milk fat droplets of different sizes and changes in T_{onset} as a function of size (insert). Adapted from Lopez, Bourgaux, Lesieur, Bernadou, et al. (2002)

et al., 2002; Michalski et al., 2004; Truong, Bansal, Sharma, Palmer, & Bhandari, 2014). This can be explained by the theory of nucleation for crystallization. When the liquid TAGs are divided in droplets, as in an emulsion, not all the TAG droplets may contain the catalytic impurities required to start heterogeneous nucleation. The number of catalytic impurities per unit volume may be far too low to produce nuclei in every emulsion droplet, and considerable supercooling may occur. Then, it is accepted that, on cooling, TAG crystallization in the smaller globules is delayed compared to the larger ones. Assuming that there is no difference of composition from a milk fat globule to another, the major influence in TAG crystallization properties is that of fat droplet size.

It was reported that milk TAGs dispersed in small milk fat droplets have a lower final melting temperature compared to large droplets, in relation to the crystal structure (Bugeat et al., 2011). Furthermore, small droplets (0.18 μm) have a lower melting enthalpy than larger droplets (1.7 μm), which could be related to a lower solid TAG content in small droplets (Bugeat et al., 2011). Truong et al. (2014) also reported a strong tendency towards decreasing proportion of milk fat crystallinity with smaller droplet size stabilised by dairy proteins.

To elucidate the effects of O/W emulsion droplet size on the organization of TAG molecules, crystallization behaviour of protein-coated lipid droplets homogenized at different pressures were examined (Bugeat et al., 2011; Lopez, Bourgaux, Lesieur, Bernadou, et al., 2002; Truong et al., 2014). On cooling at 1 °C/min from 60 to −7 °C, similar lamellar structures were formed by milk TAG molecules in their solid state whatever the size of the emulsion droplets from 1.3 to 0.4 μm: i.e. α-3*L* (72 Å) crystals (Lopez, Bourgaux, Lesieur, Bernadou, et al., 2002). However, a decrease in diffraction intensities along with broaden of the SAXS peak width in smaller droplet size were observed, showing that crystallization in small milk TAG droplets is more disordered than in large droplets and in AMF and/or that the size of TAG crystals confined in lipid droplets is smaller (Lopez, Bourgaux, Lesieur, Bernadou, et al., 2002). After 48 h storage of dairy emulsions at 4 °C, Bugeat et al. (2011) identified the coexistence of up to four different types of TAG crystals within emulsion droplets whatever their size in the range 1.7–0.2 μm, i.e. 2*L* and 3*L* corresponding to β'_1, β'_2, β_1 and β_2 polymorphs. It was observed that the confinement of milk TAG molecules in small emulsion droplets enhanced the segregation of some types of TAGs and the formation of β polymorph, at the expense of β′ polymorphs (Bugeat et al., 2011). The crystallization properties of TAG within native milk fat globules selected as a function of their size, i.e. small (1 μm) and large (7 μm) milk fat globules, revealed differences (Michalski et al., 2004). XRD permitted the identification of different crystallization behaviour in natural milk fat globules with different sizes, which could be implicated in the manufacture of dairy products involving tempering periods in the technological process (e.g. butter, ice-cream, whipped products).

5 Crystallization Properties of Milk Fat in Dairy Products

The thermal properties and crystallization behaviour of milk fat in complex food products (e.g. butter, whipped cream, ice cream, cheeses) have been investigated since TAG crystals can impact the physical stability, texture, sensorial properties and acceptability by the consumer.

During manufacture of butter, milk fat globules concentrated in cream are first subjected to a specific time-temperature program to obtain partially crystallized fat globules and subsequently, the cream is exposed to a severe mechanical agitation in which fat globules destabilize through a mechanism known as partial coalescence for which TAG crystals are indispensable. Butter consists of a continuous fat phase in which water droplets, residual milk fat globules and a network of fat crystals are dispersed (Lopez, Cauty, & Guyomarc'h, 2015). The mechanical properties of butter (i.e. its consistency, spreadability, firmness), appearance and mouth-feel depend not only on the ratio of solid to liquid TAGs but also, to a large degree, on the size, shapes and spatial distribution of the TAG crystal network, that depend on the milk TAG composition and crystallization behaviour of TAG molecules. Both compositional and processing conditions influence crystallization of TAGs in butter.

The FA composition of butter, which can be affected by seasonal variations and cow diet (Fig. 9.1A), affects its crystallization and melting properties with consequences on the texture (Lopez et al., 2007). Butter produced during the period of the year when cows are fed a maize-silage based diet (i.e. during the winter) tends to have a higher level of palmitic acid C16:0 and less oleic acid C18:1c9 than butter produced when the cows are fed a grass-based diet (i.e. during spring; Fig. 9.1A). This results in a firmer consistency of butter in winter. Processing conditions (temperature, cooling rate, scale of operation, agitation, storage conditions) can impact crystallization and ultimately the rheology of butter (Ronholt et al., 2014). For example, rapid cooling of cream leads to the formation of many small TAG crystals, a higher solid fat content and a firmer texture of butter. It is well-known that thermal kinetics (i.e. tempering, cold-warm-cold processes also called physical ripening) are applied to cream in order to control the solid to liquid fat ratio and to govern the size and orientation of TAG crystals in milk fat globules before churning. Such treatment of cream prior to butter manufacturing largely determines the final textural characteristics of the butter. The rheological properties of milk fat and butter, in connection with the TAG crystal networks, are detailed elsewhere (Wright & Marangoni, 2006).

Among dairy products, cheeses have received special attention to better understand the role played by the physical properties of milk fat, especially the formation of TAG crystals. However, studies of the crystallization properties of milk fat in as such complex food products as cheeses remain scarce. It has been demonstrated that the liquid to solid milk TAG phase transition recorded by DSC on cooling of hard-type cheeses is sensitive to the microstructure of fat within the protein matrix, especially the destabilization of fat globules and the formation of non-emulsified fat during the manufacture of cheese (Lopez, Briard-Bion, Camier, & Gassi, 2006).

When cheese fat is dispersed in fat globules, the DSC profile is close to the recordings of cream, e.g. a single broad exotherm recorded on cooling from 60°C. When non-emulsified fat, also called free fat, is formed within the cheese matrix, the DSC profile evolves toward the behaviour of AMF, e.g. two successive exotherms recorded on cooling. Dairy products are stored at low temperature (e.g. 4–7 °C in the fridge), which raises questions about the crystallization of milk fat. Using synchrotron radiation XRD, Lopez, Briard-Bion, Beaucher, and Ollivon (2008) revealed the coexistence of several types of TAG crystals within hard-type cheese stored in the fridge and identified 2*L* (41 Å) and 3*L* (55 Å) longitudinal organizations of TAG molecules corresponding to the coexistence of α, β'_1, β'_2 and β polymorphic forms. These results obtained in cheese are in line with the TAG crystals formed upon long storage of AMF and cream at 4 °C. On heating of cheese previously stored at 4 °C, the DSC profile showed the three endotherms corresponding to the LMP, MMP and HMP fractions of milk fat (Fig. 9.2). Similar milk TAG crystals and melting behaviour have been characterized within the small size droplets of processed cheese (below 1 μm, Gliguem, Lopez, Michon, Lesieur, & Ollivon, 2011). The final melting point of milk fat within cheese, about 41 °C, is higher than the temperature of digestion in the gastro-intestinal tract of humans. The TAG that remain in their solid state above 37 °C, estimated to be about 3% of milk fat (Lopez, Briard-Bion, et al., 2006) and composed by long-chain saturated FAs such as palmitic acid (C16:0), could impact the digestibility of milk fat consumed in cheese.

The texture of processed cheese is a very important parameter affecting its acceptability by the consumer. Both microstructure and rheological properties of spreadable processed cheeses are strongly dependent on the properties of fat, mainly the amount and type of TAG crystals that can be formed at the temperature of storage and cheese consumption. A study combining DSC, XRD and rheology as a function of temperature demonstrated the influence of milk TAG crystallization, melting and polymorphism upon the viscoelastic properties of processed cheese (Gliguem et al., 2009). On cooling at 2 °C/min from 60 °C (Fig. 9.16), the crystals formed by milk TAG within processed cheese were observed from about 15 °C and corresponded to α-3*L* (72 Å) structures. These results were consistent with previous observations stating that crystallization in milk fat globules (4 μm in diameter) gives rise to a α-2*L* (42–47 Å) structure followed by α-3*L* (71 Å) structure, while in fat droplets ranging from 1.25 to 0.38 μm only the α-3*L* (72 Å) structure was observed (Lopez, Bourgaux, Lesieur, Bernadou, et al., 2002). The formation of α-3*L* (72 Å) crystals and the absence of α-2*L* crystals in processed cheese may be related to the small size of fat droplets (0.7 μm). The crystallization of milk TAG within the processed cheese matrix, characterized simultaneously by XRD and DSC (Fig. 9.16A and B), was related to an increase in the viscoelastic moduli G′ and G″ (Fig. 9.16C). These results showed that milk TAG crystals contribute to the firmness of processed cheese at low temperature, e.g. below 15 °C (Gliguem et al., 2009). On subsequent heating of the processed cheese, the α-3*L* (72 Å) crystals melted and from about 12 °C the formation of β′-2*L* (41 Å) crystals was characterized until its final melting over 38 °C. The successive melting of the 3*L* and 2*L* TAG crystals embedded in the

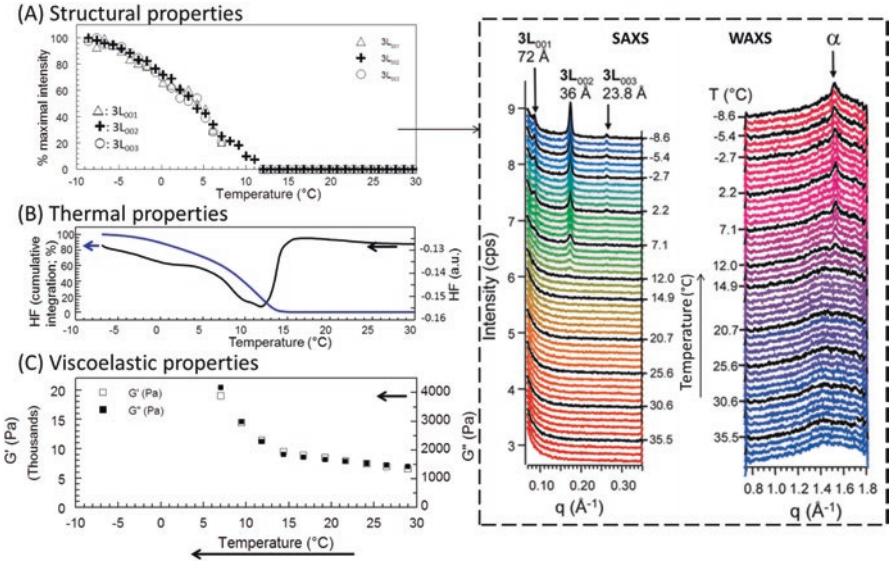

Fig. 9.16 Characterization of the crystallization of milk fat in processed cheese on cooling at 2 °C/min showing the consequences on the rheological properties. (**A**) Evolution as a function of temperature of the maximal intensities of the SR-XRD peaks recorded at small angles and corresponding to crystallization in α-3*L* (7.2 nm) structures as shown in the right part. (**B**) DSC curve recorded simultaneously on cooling and its cumulative integration as a function of temperature. (**C**) Changes in the viscoelastic moduli G′ and G″ of processed cheese on cooling. Adapted from Gliguem et al. (2009)

fat droplets was related to a successive decrease in the viscoelastic moduli recorded on heating of processed cheese.

These studies combining different biophysical techniques allowed the identification the milk TAG crystals formed within complex dairy products (i.e. butter, cheeses) and revealed polymorphic evolutions on heating. Moreover, the impact of milk fat crystals on the rheological properties fat-rich products was demonstrated.

6 The Crystallization Properties of Milk Fat Are Affected by the FA and TAG Compositions

The FA and TAG compositions of milk can be tailored for technological, nutritional and health reasons (e.g. increase in unsaturated FAs that are known to provide health benefits, and decrease in palmitic acid content that is involved in cardiovascular risk). In this respect, numerous techniques have been applied, including physical, chemical and dietary manipulation by means of feeding dairy animals. Technological treatments are often applied to have desired functionalities (e.g. improved cold spreadability of butter) and expend the use of milk fat in the food industry. Milk

TAGs also exhibit compositional differences between the mammal origin of the milk (e.g. bovine, goat, sheep, dromedary, human). The variations in TAG composition affect the crystallization and melting properties of milk fats.

6.1 Technological Process: Dry Fractionation

Among the technological processes, hydrogenation, interesterification or blending of milk fat with fats from other origin (e.g. vegetable oil) can be used to tailor the FA and TAG composition but will not be discussed in this chapter.

Dry fractionation, i.e. the crystallization of milk fat from the melt and the subsequent filtration of the slurry, is a common industrial process to obtain milk fat fractions with different TAG compositions and physical properties (Kaylegian & Lindsay, 1995). Dry fractionation is based on the different thermal (crystallization and melting) properties of TAGs resulting from their different FA compositions. At a fixed temperature during the fractionation process, the solid fraction is called stearin while the liquid fraction is called olein. Fractionation of milk fat and recombination of the fractions in various proportions allow to control and to improve the thermal and physical properties, e.g. its consistency and the development of cold spreadable butter (Kaylegian & Lindsay, 1995). Milk fat fractions are employed for pastry-making, as chocolate bloom inhibitor, butter flavour-rich concentrates and for improving the rheology of reduced fat cheese curds.

Many studies have focussed on the milk fat fractionation process, on the chemical and thermal characteristics of the separated fractions and on the phase behaviour of milk fat and its fractions (Marangoni & Lencki, 1998; Timms, 1980; Van Aken, ten Grotenhuis, van Langevelde, & Schenck, 1999). So far only a few studies have been reported about the structural characteristics of milk fat fractions investigated using time-resolved synchrotron radiation XRD as a function of temperature. The chemical composition, crystallization properties and melting behaviour of milk fat and its primary fractions, stearin and olein fractions, obtained by dry fractionation at 21 °C were characterized (Lopez, Bourgaux, Lesieur, Riaublanc, & Ollivon, 2006; Lopez & Ollivon, 2009). Compared to whole milk fat, the stearin fraction was enriched in TAGs with (1) three saturated long-chain FAs (SSS, PSS, PPS, PPP, MSS, MPS, MPP, SSL), (2) one or two saturated medium-chain FAs and a saturated long-chain FA (MMS, MMP, LaPS, LaPP, LaMP, CPS, CPP, CMS) and (3) one monounsaturated long-chain FA and two saturated long-chain FAs (PoSS, PPoS, LaOS, PSO). The olein fraction was enriched in TAGs with (1) two monounsaturated long-chain FA (SOO, PoSO, POO, MOO, PPoO), (2) one monounsaturated and two medium-chain FAs (CMO, CPO, LaMO, MMO, LaPO), (3) a short-chain FA and two saturated long-chain FAs (BMP, BPS, BPP, BMS, CaMP, CaPP, CaMS) or one monounsaturated FA (BPO, BMO) or two monounsaturated long-chain FAs (BOO). On cooling from the melt at 1 °C/min, milk fat showed the formation of two α-2*L* (47 and 42 Å) and one α-3*L* (72 Å) lamellar structures, as previously reported and discussed (Lopez, Lavigne, et al., 2001a; Fig. 9.5A). In similar experimental

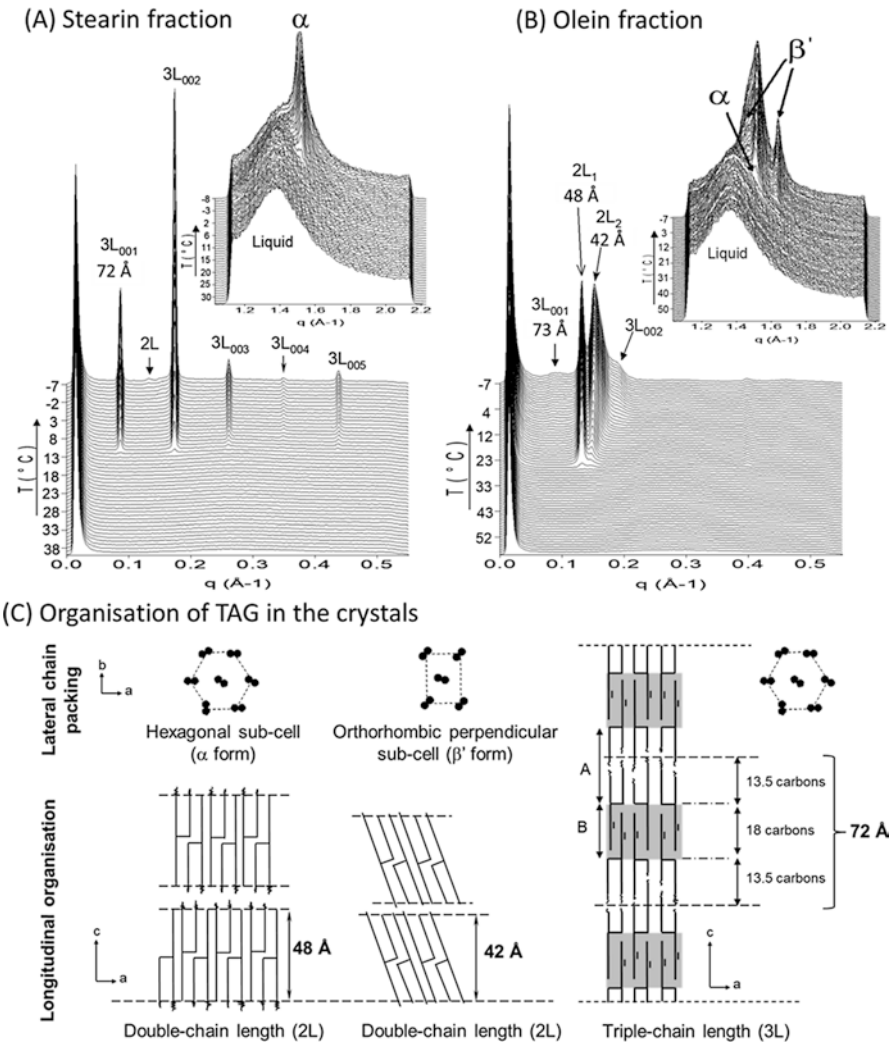

Fig. 9.17 TAG crystals formed in stearin fraction and olein fraction on cooling at 1 °C/min from 60 to −7 °C. SR-XRD patterns recorded at small and wide (insert) angles as a function of temperature (**A**) for stearin fraction and (**B**) for olein fraction. (**C**) Proposed TAG packing in the main crystals formed on cooling. The α-3L (7.2 nm) crystals in olein fraction correspond to the packing of short and medium-chain length fatty acids in layers A with unsaturated and saturated long-chain fatty acids in layers B. Adapted from Lopez, Bourgaux, et al. (2006)

conditions, the stearin fraction started to crystallize at 26 °C with the formation of two main lamellar structures, α-2*L* (48 Å; molecules arranged perpendicular to the methyl end group plane) and β′-2*L* (42 Å; tilt of the chains) (Fig. 9.17A). Then, from about 13 °C, a low amount of 3*L* (68 Å) crystals that may correspond either to α or β′ polymorphs were formed. In the olein fraction cooled in similar experimental

conditions, α-3*L* (72 Å) lamellar structures started to crystallize from 13 °C (Fig. 9.17B). The thickness of this α-3*L* structure corresponds to the packing of short and medium-chain FAs with a mean number of atoms of carbon of about 13.5 in two layers, and to the packing of unsaturated and saturated long-chain FAs with a mean number of atoms of carbon of 18 in the third layer (Fig. 9.17C; Lopez, Bourgaux, et al., 2006). The different type of TAG crystals formed in stearin and olein fractions, as compared to whole milk fat, result from their different TAG composition. On subsequent heating at 2 °C/min, the final temperature of melting recorded for stearin fraction, milk fat and olein fraction were 44, 37.5 and 22 °C, respectively (Lopez & Ollivon, 2009).

The structure of TAG crystals networks observed at the microscale level using polarized light microscopy, after cooling from the melt at 1 °C/min, corresponded to spherulitic organizations in milk fat and stearin fraction while needle-shape crystals were formed in the olein fraction (Lopez & Ollivon, 2009). The microstructure and crystallization kinetics of binary and ternary mixtures of milk fat fractions during isothermal crystallization at 5, 15, and 20 °C were characterized using polarized light microscopy and the Avrami model (Ramel & Marangoni, 2016). Results showed that for both binary and ternary mixtures, high concentrations of the high-melting fraction result in the formation of rod or needle-like crystals (i.e., one-dimensional growth and low values of Avrami index, *n*) while at relatively higher concentrations of the middle-melting and low-melting fractions, multi-dimensional crystal growth is favored (i.e., higher *n* values). On the effect of temperature, for binary mixtures, it was found that at high undercooling conditions (5 °C) one dimensional growth is favored while for ternary mixtures, increasing the crystallization temperature (i.e., decreasing supersaturation) from 15 to 20 °C results in large differences in crystal structure. Ramel and Marangoni (2016) were, therefore, able to propose a concentration—temperature map for different fat crystal structures in milk fat.

6.2 Dietary Manipulations

Seasonal variations in the diet of cows naturally occurring in some countries, e.g. maize based diet in winter vs. fresh grass based diet in spring, affect the FA and TAG composition of milk (Fig. 9.1) and have consequences on the crystallization properties of milk fat and final texture of fat-rich products. Thus, the control of fat-rich product quality, e.g. butter, in different seasons is a real challenge for the industrials. The composition of milk fat can also be modified by specific feeding strategies and alter consequently the physical and functional properties of high-fat content dairy products. For example, feeding cows with highly unsaturated oils or whole oilseeds can reduce the level of saturated FAs while simultaneously increasing the unsaturated fatty acid (UFA) content. Several studies reported the improvement in the spreadability and softer texture of winter butter and milk fat in general through

changes in the feed of the cow by adding unsaturated oils or fresh grass (Couvreur, Hurtaud, Lopez, Delaby, & Peyraud, 2006; Wright & Marangoni, 2006). Yoghurt, ice cream and cheeses made from milk enriched in UFAs have been reported to show a softer texture than the products made from control milk. The relationship between the FA composition of milk fat and the texture of dairy products has been demonstrated. However, few authors studied the effect of cow diet on the crystallization properties of milk fat enriched in UFAs (Bugeat et al., 2011; Bugeat et al., 2015; Smet et al., 2010). An increased amount of UFAs in milk TAGs was reported to decrease the solid fat content at 5 °C from 60% (control TAGs; 28% UFAs) down to 46% (UFA-enriched TAGs; 39% UFAs) (Smet et al., 2010). Upon isothermal crystallization monitored by pulsed NMR, higher content of the UFAs resulted in a slower nucleation, a longer induction time to crystallization and a lower solid fat content at the end of crystallization, although crystallization occurred according to similar α to β′ polymorphic transition (Smet et al., 2010).

Bugeat et al. (2015) compared the crystallization properties of control milk TAGs (29% UFAs) and UFA-enriched TAGs (51% UFAs obtained with a linseed oil rich diet) using the coupling of DSC with synchrotron radiation XRD (Fig. 9.18). On cooling from the melt at 3 °C/min, both milk TAG mixtures started to crystallize from about 16 °C in α-2*L* (45–49 Å) structures then formation of α-3*L* structures occurred with a higher thickness (75.5 versus 71.5 Å) and a delay (T_{onset} = 8.5 versus 12.1 °C) for UFA-enriched TAGs that result from a higher amount of C18:1c9. Groups of TAG molecules with high crystallization temperature (HCT; α-2*L* crystals) and low crystallization temperature (LCT; α-3*L* crystals) segregated on cooling. On subsequent heating, melting of TAG crystals and formation of a new 3*L* (65–80 Å) and β′-2*L* (40–44 Å) crystals associated with polymorphic reorganizations have been characterized. Increase in thickness of the lamellar structures was characterized for UFA-enriched TAGs as compared to control TAGs, demonstrating differences in the FA composition of the crystals. Interestingly, the melting profile of the UFA-enriched TAGs was mainly altered in the range 11–21 °C, corresponding to the MMP fraction, and not in the HMP fraction since the final melting temperatures of both the control and the UFA-enriched TAGs were similar.

It has also been demonstrated with the same milk fats that the enrichment of UFAs in TAGs decreases the solid fat content and affects the type of crystalline structures that are formed within O/W emulsion droplets upon storage at 4 °C (Bugeat et al., 2011). Control TAGs were crystallized in 2*L* (39.5 nm) and 3*L* (56.6 nm) lamellar structures with four polymorphic forms (β_1, β_2, β'_1, β'_2) while UFA-enriched TAGs were crystallized in 2*L* (41.8 nm) lamellar structures displaying three polymorphic forms (β_1, β'_1, β'_2). The absence of 3*L* crystals in the UFA-enriched TAG emulsions was due to decrease in the melting point of these TAG crystals rich in UFAs (Saturated-Unsaturated-Unsaturated TAGs vs. Saturated-Saturated-Unsaturated TAGs in control milk fat) that remain in the liquid TAG phase upon storage of the emulsion droplets at 4 °C.

As a conclusion, the enrichment of milk TAGs in UFAs affects both their crystallization and melting behaviours.

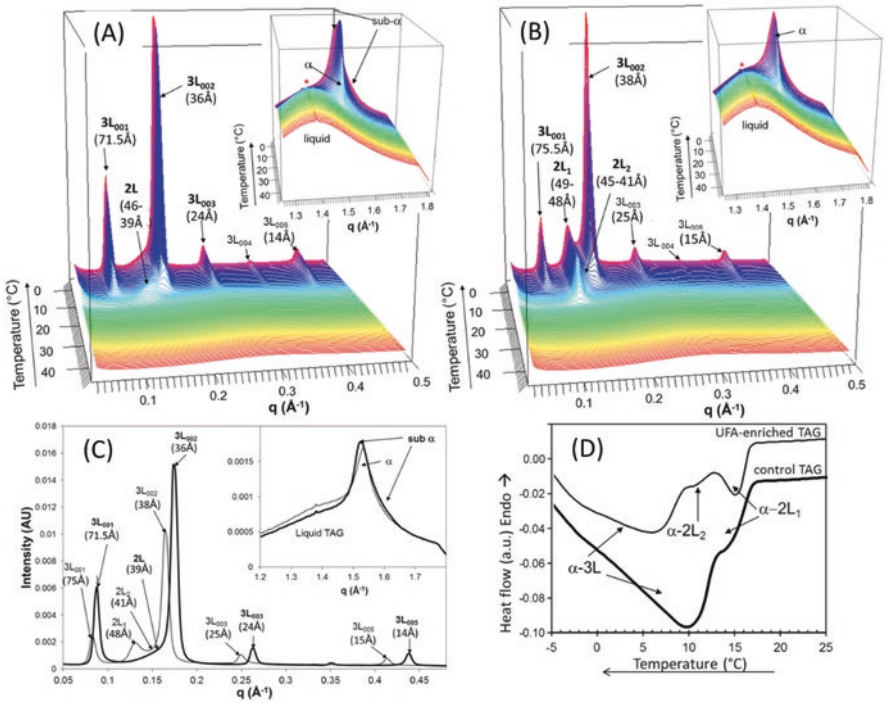

Fig. 9.18 Comparison of the crystallization behavior of unsaturated fatty acid (UFA)-enriched TAGs and control TAGs examined on cooling at 3 °C/min from 60 to −5 °C using the coupling of SR-XRD and DSC. SR-XRD patterns recorded at small and wide (insert) angles during cooling of (**A**) control TAGs and (**B**) UFA-enriched TAGs. (**C**) XRD patterns recorded at −5 °C for control (thin line) and UFA-enriched (thick line) TAGs after cooling. (**D**) DSC curves recorded simultaneously to XRD experiments. Adapted from Bugeat et al. (2015)

6.3 Milk Fat from Various Mammal Species

Most of the studies about milk fat crystallization have been performed with bovine milk fat since bovine milk represents about 84% of the global worldwide milk production (information from the International Dairy Federation). However, the FA and TAG compositions of milk depend on mammal species and changes in milk fat composition can affect the crystallization and melting properties between milk fats from various origins (i.e. goat, sheep, water buffalo, donkey, horse, camel; Smiddy, Huppertz, & van Ruth, 2012). The crystallization properties of milk TAGs have been characterized by the coupling of DSC and synchrotron radiation XRD in AMF and fat globules from goat milk (Ben Amara-Dali et al., 2007; Ben Amara-Dali, Lopez, Lesieur, & Ollivon, 2008), dromedary milk (Karray, Lopez, Lesieur, & Ollivon, 2005; Lopez, Karray, Lesieur, & Ollivon, 2005) and human milk (Lopez, Briard-Bion, Bourgaux, & Perez, 2013).

6.3.1 Crystallization Properties of Goat Milk Fat

Goat milk fat globules have a mean diameter of about 3–3.5 μm. They are rich in saturated FAs, about 70% of total FAs, and the five FAs C10:0, C14:0, C16:0, C18:0 and C18:1c9 account for more than 75% of total goat milk FAs (Ben Amara-Dali et al., 2008). The most important TAGs present in goat's milk fat contain medium-chain length saturated FAs (C8:0, C10:0, C12:0) and C18:1c9 as unsaturated FA. The molecular organization of the solid TAG phase formed within goat milk fat globules was investigated on cooling at the rates of 0.1 °C/min (slow cooling) and 1000 °C/min (quenching) and on subsequent heating at 1 °C/min. The lamellar structures 3*L* (69–70 Å) and 2*L* (37–45 Å) were characterised and the five polymorphic forms α, sub-α, β'_1, β'_2 and β were identified. The two main types of crystals correspond to a segregation of goat TAG molecules in the solid state as a result of different compositions, as observed for bovine TAGs. Polymorphic transitions were observed within goat's milk fat globules as a function of time after quenching from the melt and as a function of temperature on heating. Increasing the knowledge about the physical properties of goat's milk fat is essential to improve the quality of existing dairy products and to increase the technolocal application of goat's milk fat crystallization to contribute in the development of new food products.

6.3.2 Crystallization Properties of Dromedary Milk Fat

In camel milk, fat represents about 3.6% of the composition. Differences exist in the FA composition of camel milk compared to bovine milk. Short-chain FAs (C4:0–C12:0) are present in very small amounts and the amount of long-chain saturated FAs (C14:0–C22:0) is higher for camel milk fat than those for bovine milk fat. These differences lead to the formation of specific TAG crystals.

On cooling of anhydrous dromedary milk fat from the melt, the crystalline structures formed by TAG molecules correspond to 2*L* type crystals. The absence of short-chain FAs in dromedary milk fat prevents the formation of 3*L*-type crystals. On cooling at 1 °C/min, two successive 2*L* crystals are formed, α 2*L* (47 Å) from 24 °C and β′ 2*L* (42.2 Å) from 21 °C. These crystals successively melt on subsequent heating. On fast cooling at 5 °C/min a four-chain length longitudinal organization 4*L* (84.5 Å) was characterized (Karray et al., 2005). On slow cooling at 0.1 °C/min, the crystals formed from about 29.5 °C correspond to a lamellar structure with a double-chain length longitudinal organization of the TAG molecules (2*L* = 42.3 Å) associated with a β′ lateral packing of the chains. These crystals progressively melt on subsequent heating and disappear above 42 °C. Investigations of TAG crystallization within dromedary milk fat globules revealed the successive formation of two double-chain length (2*L*) lamellar structures: α $2L_1$ (46.7 Å) from 22 °C and β′ $2L_2$ (41.7 Å) from 9 °C, which coexist until the end of the cooling process. The same lamellar structures, α $2L_1$ and then β′ $2L_2$, are formed on cooling in the dispersed and bulk states. However, crystallization in the unstable α form is favoured in fat globules (Lopez, Karray, et al., 2005). Increasing the knowledge

about dromedary milk fat crystallization contributes in developing the technological applications and textural properties of creams and dromedary milk fat-rich products, for example butter, that strongly depend on the thermal and structural properties of dromedary milk TAGs.

6.3.3 Crystallization Properties of Human Milk Fat

Human milk contains about 3–5% fat dispersed in fat globules having a mean diameter of about 5 μm. Human milk TAGs that contain 48–57% saturated FAs with about 28% of C16:0 contribute some 40–55% of the total energy intake for the breast-fed infants. The efficient digestion of TAGs is therefore of primary importance for the optimal growth of newborns. However, storage of breast milk in the fridge at 4 °C leads to the partial crystallization of TAGs within milk fat globules (Lopez et al., 2013). Microscopic observations of breast milk stored at 4 °C revealed the non-spherical distorted shape of fat globules due to the presence of TAG crystals (Fig. 9.19A). Synchrotron-radiation XRD experiments allowed the identification of

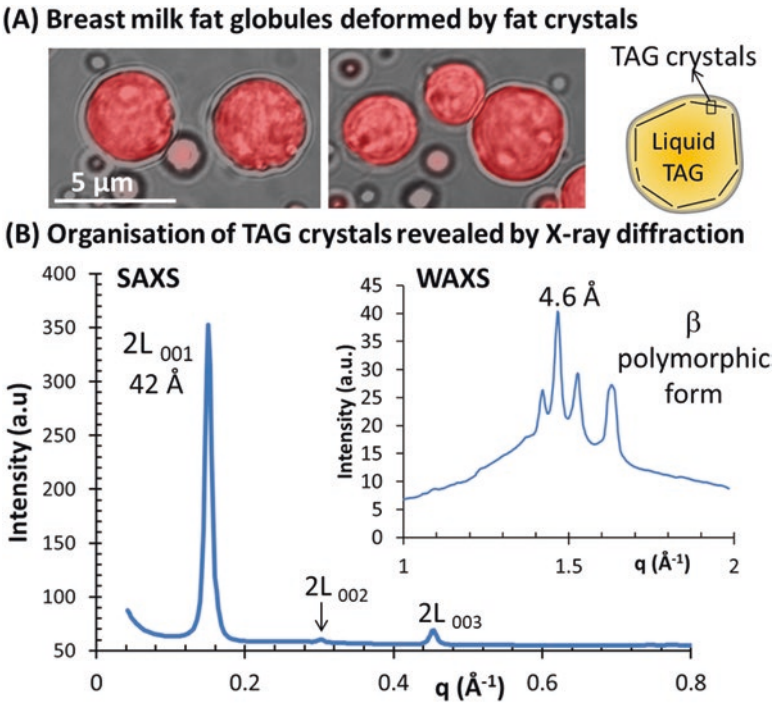

Fig. 9.19 TAG crystals formed within human milk fat globules upon storage at 4 °C. (**A**) Polarized light microscopy images showing the deformation of milk fat globules by TAG crystals, (**B**) Identification of the organization of TAG molecules performed using SR-XRD at both small and wide (insert) angles. Adapted from Lopez et al. (2013)

the TAG crystals that are formed within breast milk fat globules upon storage at 4 °C, i.e. β-2*L* (41.7 Å) lamellar structures (Fig. 9.19B). The β crystals correspond to the most thermodynamically stable polymorphic form of TAGs with a compact organization of the FA chains. The crystals formed in human milk fat globules upon storage at 4 °C are different from those characterized in bovine milk, which confirms that the chemical composition of milk TAGs govern their crystallization properties. The final melting point of the β-2*L* (41.7 Å) human TAG crystals was 41.1 ± 1.6 °C, which is above the in-body temperature of milk digestion by newborns.

The presence of solid TAGs in the core of breast milk fat globules after storage at 4 °C raises the question of the action of the digestive lipolytic enzymes on a solid substrate, on their solubilization and then on the absorption and metabolism of milk lipids. The influence of the physical state of TAGs and particularly the proportion of solid TAGs and type of crystals on lipid digestion and absorption remains poorly documented. Lopez et al. (2013) hypothesized that crystallization of milk TAGs could decrease the amount of utilizable fat for the recipient infant in the case breast milk is consumed at a temperature below the final melting temperature of TAG crystals. Warming breast milk at about 45–50 °C, i.e. above the final melting point of human β-2L TAG crystals, is then important to ensure optimal breast milk TAG digestibility.

7 Liquid TAG Phase

For temperatures above the melting point of milk TAGs, the liquid TAG phase exhibits an organization. Within milk fat globules, the synchrotron radiation X-ray patterns of milk TAGs in their liquid state correspond to scattering peaks at both small (SAXS) and wide (WAXS) angles, respectively centred at 22.4 nm and 4.5 Å (Lopez, Lavigne, et al., 2001a). In anhydrous milk fat and the primary fractions, stearin and olein, the synchrotron radiation X-ray scattering from the liquid-crystalline organization of milk TAGs in their liquid state recorded at 60 °C was centered at 23.3 Å (SAXS) and about 4.5 Å (WAXS) (Lopez & Ollivon, 2009). Differences were characterized as a function of the FA composition of TAGs, with a higher thickness *d* for UFA-enriched TAGs compared to control TAGs (d = 2.26 ± 0.01 vs. 2.21 ± 0.01 nm; Bugeat et al., 2015). The thickness value d of 22.1–23.3 Å supports the existence of liquid-crystalline like lamellae and corresponds to the stacking of TAGs in a single layer of the acyl chains along the long-chain axis integrating FAs with different chain length (from 4 to 18 atoms of carbon) and unsaturation (Fig. 9.20). The scattering peak recorded at about 4.5 Å corresponds to a disordered mesophase with short-range order of the FA chains. These synchrotron radiation XRD data support evidence that complex TAG blends such as milk TAGs display anisotropy with a lamellar ordering in the liquid state.

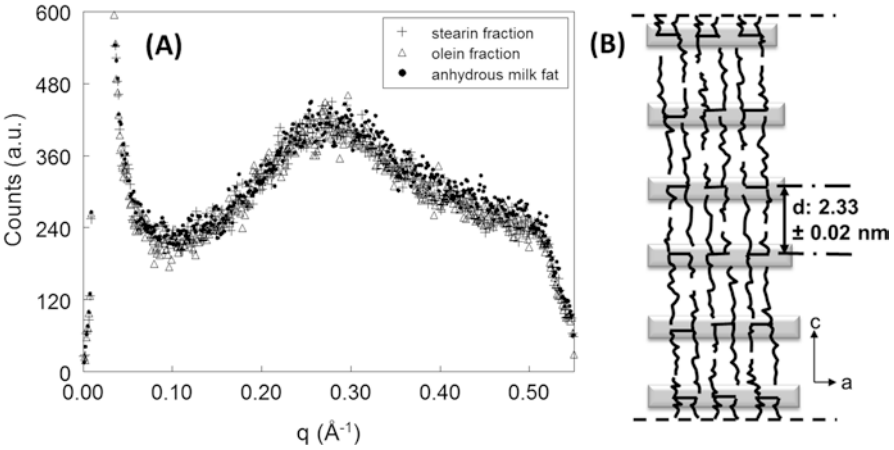

Fig. 9.20 Structural information on the liquid phase of milk TAG molecules. (**A**) SR-XRD patterns recorded at small angles with milk fat, olein fraction and stearin fractions at 60 °C. (**B**) Proposed structure for the molecular packing of TAG molecules in their liquid state, as seen in the ca projection. Short, medium and long-chain fatty acids, saturated and unsaturated fatty acids are stacked in monolayers, between glycerol groups. Adapted from Lopez and Ollivon (2009)

8 Conclusions

Crystallization of TAGs is a complex phenomenon, especially for milk fat due to its extremely wide FA composition leading to many TAG molecular species. As reviewed in this chapter, extensive research has provided considerable insight into the crystallization properties of milk fat in the anhydrous state, in emulsion (natural milk fat globules, processed lipid droplets, recombined cream) and in complex dairy products (butter, cheeses). This book chapter highlights the recent research demonstrating from scientific points of views that the crystallization properties of milk fat are affected by (1) its FA and TAG compositions, (2) cooling rates and tempering, (3) shear, (4) presence of minor lipid compounds (FFAs, MAGs, DAGs, phospholipids), (5) its dispersion state, i.e. anhydrous bulk *versus* emulsified in numerous droplets. Polymorphic evolutions have been characterized as a function of temperature on heating and in isothermal conditions, e.g. after rapid cooling from the melt. Understanding the functional properties of milk TAG crystals networks requires investigations at several scale levels (microscopic level, nanoscale, molecular scale) performed as a function of temperature or as a function of time in isothermal conditions and then the combination of complementary techniques (rheology, polarised light microscopy, electron microscopy, NMR, DSC, XRD including USAXS, SAXS and WAXS). Undoubtedly, the pursuit of fundamental knowledge in the area of TAG crystallization can yield fascinating new insights that will increase further the value of milk fat in food applications.

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