JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Crystallization Properties and Polymorphism of Triacylglycerols in Goat's Milk Fat Globules†

WAFA BEN AMARA-DALI,‡,§ CHRISTELLE LOPEZ,*,‡,|| PIERRE LESIEUR,⊥,# AND MICHEL OLLIVON‡

Equipe Physico-Chimie des Systèmes Polyphasés, UMR 8612, CNRS, Université Paris XI, 5 rue J. B. Clément, 92296, Châtenay-Malabry, France, Laboratoire d'Analyses Alimentaires, ENIS, BPW 3038 Sfax, Tunisia, UMR Science et Technologie du Lait et de l'Oeuf, INRA, Agrocampus, 65 rue de Saint-Brieuc, 35042 Rennes Cedex, France, Laboratoire pour l'Utilisation du Rayonnement Electromagnétique, Bâtiment 209D, Université Paris-sud, 91898 Orsay, France, and Physico-Chimie des colloïdes, Université Henri Poincaré, UMR 7565 BP 239, 54506 Vandœuvre-lès-Nancy, France

The sensorial, functional, and nutritional properties of goat dairy products result from the specific fatty acid composition of goat's milk fat. However, information on the physical and thermal properties of goat's milk fat is scarce. In this study, crystallization of triacylglycerols (TG) in goat's milk fat globules was investigated using polarized light microscopy and the coupling of time-resolved synchrotron radiation X-ray diffraction (XRD) and high-sensitivity differential scanning calorimetry (DSC). The molecular organization of the solid fat phase was characterized for cooling rates between 3 and 0.1 °C/min. Quenching of goat's milk fat globules from 50 to −8 °C and 4 °C was also examined to identify the most unstable polymorphic forms of TG. Then, the melting behavior of fat crystals was studied on subsequent heating at 1 °C/min. Triple chain length (3L: 68.6−70 Å) and double chain length (2L: 37−45.4 Å) structures were characterized and 5 polymorphic forms, α, sub-α, β'_1, β'_2, and β were identified. Polymorphic transitions were observed within goat's milk fat globules as a function of time after quenching and as a function of temperature on heating. From a technological point of view, this work will contribute to a better understanding of the rheological properties as well as on the flavor evolutions of goat's milk-based products.

KEYWORDS: Emulsion; triglycerides; X-ray diffraction; differential scanning calorimetry

INTRODUCTION

Over the past 20 years, a new and increased interest in goat's milk and dairy products (mainly cheeses) has occurred everywhere in the world. Although the world production of goat's milk is relatively minor compared with that of cow's milk (2% vs 84.2% of the total world milk production, respectively (*1*), its consumption is considerable in some Mediterranean countries, mainly in Greece and Spain. Increasing the knowledge of the sensorial and nutritional but also the physical properties of goat's milk fat is essential to improve the quality of existing goat's dairy products and to find innovative strategies allowing the development of new food products.

Fat is dispersed in milk in the form of droplets called fat globules, which are enveloped by a biological membrane rich in phospholipids and (glyco)proteins. The core of the milk fat globules consists of triacylglycerols (TG; 98% of total milk lipids), which are triesters of fatty acids (FA) and glycerol. Milk fat globules are characteristically abundant in goat's milk with sizes less than 3.5 μm (*2*). Mens (*3*) reported that 65% of fat globules have a diameter lower than 3 μm. Attaie et al. (*4*) calculated that the average diameter of particles based on a volume to surface area ratio was 2.76 μm. Studies found that the average fat globule size is smallest in goat's milk compared to cow's milk (*2, 5, 6*), which may be advantageous for digestibility and a more efficient lipid metabolism (*7*).

From a compositional point of view, goat's milk fat is rich in saturated FA with 69.5 ± 0.5% of total FA and contains 23.7 ± 5.0% monounsaturated FA (mainly $C_{18:1}$) and 3.7 ± 0.8% polyunsaturated FA (mainly $C_{18:2}$ and $C_{18:3}$) (**Table 1** (*8–12*)). Quantitatively, five FA ($C_{10:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, and $C_{18:1}$) account for more than 75% of total FA in goat's milk (*2*). To explain the nutritional characteristics (action of digestive lipolytic enzymes), the physical (crystallization behavior and melting properties) and the technological properties of goat's milk fat, its composition must be known in terms of TG

† This paper is dedicated to Michel Ollivon who passed away on June 16th, 2007.

* To whom correspondence should be addressed. Phone: +33 2 23 48 56 17. Fax:+33 2 23 48 53 50. E-mail:Christelle.Lopez@rennes.inra.fr.

‡ CNRS, Université Paris XI.

§ ENIS.

|| INRA.

⊥ Université Paris-sud.

Université Henri Poincaré.

10.1021/jf073491g CCC: $40.75
Published on Web 05/22/2008

Table 1. Fatty Acid Composition of Goat's Milk Fat

	goat's milk fat (%)[a]					
fatty acids (FA)	(a)	(b)	(c)	(d)	(e)	mean ± std
$C_{4:0}$	2.6	5.1	3.4	3.1	2.2	3.3 ± 1.0
$C_{6:0}$	2.9	4.4	2.4	2.7	2.4	3.0 ± 0.8
$C_{8:0}$	2.7	4.2	2.6	3.2	2.7	3.1 ± 0.6
$C_{10:0}$	8.4	12.9	6.8	11.7	10.0	10.0 ± 2.2
$C_{12:0}$	3.3	5.6	3.2	5.6	5.0	4.5 ± 1.1
$C_{14:0}$	10.3	9.9	8.4	13.6	9.8	10.4 ± 1.7
$C_{14:1}$		0.4			0.2	0.3 ± 0.1
$C_{16:0}$	24.6	25.4	24.0	24.0	28.2	25.2 ± 1.6
$C_{16:1}$	2.2	1.4	2.1	1.6	1.6	1.8 ± 0.3
$C_{17:0}$		1.3			0.8	1.0 ± 0.3
$C_{18:0}$	12.5	7.2	11.6	10.0	8.9	10.0 ± 1.9
$C_{18:1\ (n-9)}$	28.5	15.5	25.8	19.8	19.3	21.8 ± 4.7
$C_{18:2\ (n-6)}$	2.2	2.8	2.9	2.6	3.2	2.7 ± 0.3
$C_{18:3\ (n-3)}$		0.4	1.1	1.5	0.4	0.8 ± 0.5
$C_{18:2\ c,\ t\ conjugate}$		0.6			0.7	0.6 ± 0.1
saturated FA	63.7	74.6	62.4	73.9	69.2	69.5 ± 5.0
unsaturated FA	32.9	21.0	31.8	25.4	25.4	27.3 ± 5.0
monounsaturated FA	30.7	17.3	27.9	21.3	21.1	23.7 ± 4.9
polyunsaturated FA	2.2	3.8	3.9	4.1	4.3	3.7 ± 0.8

[a] (a) From ref *8*; (b) from ref *9*; (c) from ref *10*; (d) from ref *11*; (e) from ref *12*.

molecular species. Fontecha et al. (*9*) reported that the most important TG present in goat's milk fat in quantitative terms are constituted by medium-chain FA ($C_{8:0}$, $C_{10:0}$, $C_{12:0}$) and $C_{18:1}$ as unsaturated FA.

The understanding of the physical properties of milk fat requires the characterization of its thermal and crystallographic properties, such as the crystallization and melting behavior, the solid fat content at a fixed temperature, and the shape, size, and polymorphic forms of fat crystals. Fat crystallization in bulk proceeds quite differently from the same fat in a dispersed or emulsified form, such as in milk fat globules (*13, 14*). This is largely due to the fact that the kinetics of crystallization depends on nucleation events and crystal growth (*15*). Fat crystals may lead to the destabilization of the emulsion droplets, for example, during mechanical deformation or storage at low temperature, which is dependent on (i) the wettability of the crystals at the interface, (ii) the interfacial film rheology, (iii) the crystals microstructure (polymorphism and morphology), (iv) the localization of fat crystals in fat globules, and (v) the crystalline/liquid fat ratio (*16, 17*). The thermal properties of natural fats, in bulk and in emulsion, are often investigated using differential scanning calorimetry (DSC). However, the complex DSC recordings are difficult to interpret due to the broad distribution of FA composition and the polymorphism of TG (*18*). Thus, DSC experiments require to be completed with a technique such as X-ray diffraction (XRD) able to identify the crystalline structures formed by TG molecules. While small-angle XRD provides information on the longitudinal stacking of TG molecules in layers, generally in double or triple chain length structures (noted 2L and 3L, respectively), the wide-angle XRD yields the lateral packing of the FA chains in these layers and the subcells they adopt (*19*). Using the coupling of XRD as a function of temperature (XRDT) and DSC, it has been shown that it is possible to monitor the crystallization of TG and to follow polymorphic evolutions in anhydrous milk fat from cows (*14, 20*), dromedaries (*21*), and goats (*22, 23*). Moreover, the crystallization properties of TG have been studied within milk fat globules of various species, e.g. cow and dromedary (*13, 24–26*). However, the crystallization properties of TG in goat's milk fat globules remain to be elucidated.

The objective of this study was to investigate the thermal and structural properties of TG molecules in goat's milk fat globules. Different cooling rates, including quenching, were applied to goat's milk fat globules concentrated in creams to examine the formation of the crystalline structures and to mimic processing conditions as applied by the fat industry. Their evolutions were followed during a subsequent heating at 1 °C/min. The fat crystals formed in goat's milk fat globules were also observed at the microscopic level.

MATERIALS AND METHODS

Samples. Goat's milk was obtained from a goat herd (Alpine race) belonging to the experimental farm of INRA (Jouy en Josas, France). Sodium azide (NaN_3) was added to milk at a concentration of 0.02% (w/v) to prevent the growth of bacteria. Cream was obtained by centrifugation of milk at 300*g* for 20 min (25 °C) on a Jouan GR 20 22 centrifuge (Jouan, Saint Herblain, France). Goat's cream, which corresponds to the concentration of fat globules (fat content ~500 g/kg) from milk (fat content ~38 g/kg), was maintained at room temperature (T ~18 °C) to avoid partial coalescence of fat globules enhanced by TG crystallization at lower temperature.

Polarized Light Microscopy. The microstructure of fat globules was observed in goat's milk using polarized light microscopy between crossed polarizer and with a $\lambda/4$ retarder in white light using a Nikon E600 Eclipse direct microscope (Champigny/Marne, France) equipped with a long focus objective (LWD 40× 0.55; 0−2 mm) for light field microscopy. The camera Nikon coolpik 950 was used as a picture recorder with a resolution of 1600 × 1200 pixels. A homemade sample holder was used to monitor the sample temperature, as previously detailed in ref *13*. Goat's milk was heated to 60 °C in a steam room, in order to ensure that all crystals were melted, and then it was placed between two circular lamellae located in a cavity of a peltier-cooled stage thermostatted at 40 °C. The observation of fat crystals in goat's milk fat globules was monitored after cooling of the samples at rapid rate, that is, 10 °C/min, from 40 to −8 °C.

Fat Globule Size Measurements. The size of goat's milk fat globules was determined by laser light scattering using a Mastersizer 2000 (Malvern Instruments, Malvern, U.K.), equipped with an He/Ne laser (λ = 633 nm) and an electroluminescent diode (λ = 466 nm). The refractive index of milk fat was taken to be 1.460 at 466 nm and 1.458 at 633 nm. The fat globule size distribution was measured after dispersion in distilled water to reach 10% obscuration. One milliliter of EDTA (>98%, disodium salt, and 2 H_2O, Prolabo, France) was added to disrupt casein micelles. All analyses were performed in triplicate. From the size distribution, the average diameter was calculated by the instrument software.

Coupled XRDT and DSC Measurements. X-ray diffraction (XRD) experiments were performed using synchrotron radiation on the D22 beam line (8 keV) of LURE (Laboratoire pour l'Utilization du Rayonnement Electromagnétique, Orsay, France). The recording of XRD data was carried out as a function of temperature (XRDT) simultaneously at small and wide angles with sample-to-detector distances of 177 and 30 cm, respectively. The channel to scattering vector q calibration of the detectors was made at small angles with silver behenate and at wide angles with high purity tristearin, as in Lopez et al. (*13*). Differential scanning calorimetry (DSC) experiments were performed using Microcalix (CNRS, Châtenay-Malabry, France), a Peltier temperature-controlled calorimeter developed by the group of M. Ollivon. This instrument allows simultaneous XRDT and DSC measurements, as detailed in ref *27*. This setup, which has been previously used for the study of TG crystallization in cow's milk fat globules, is presented in ref *19*.

The cream of goat's milk fat globules (about 20 μL) was loaded into thin-glass Lindeman capillaries (GLAS Muller, Berlin, Germany), especially designed for X-ray studies since they allow minimum attenuation of the beam and parasitic scattering, and since they guarantee minimum thermal losses (*14*). Goat's milk fat globules were heated to 50 °C in order to melt all existing crystal and nuclei. Then, the crystallization behavior of goat's cream was conducted on cooling at 0.1, 3, and 1 °C/min from 50 to −8 °C. Quenching of goat's milk fat globules from 50 to −8 °C and at 4 °C was also examined. The melting behavior of TG was monitored by heating of the samples at 1 °C/min.

Each XRD pattern recorded at small and wide angles as a function of temperature was analyzed using IGOR Pro 4.0 software (Wavemetrics, U.S.A.). Mathematical treatments were performed to determine the position and the maximum intensity of each XRD peak using a software procedure, called ≪peak_detection_init≫ written for IGOR Pro 4.0. In the figures, the size of the symbol is proportional to the maximum of intensity of the peak, while the circle center position indicates the period. Moreover, the DSC curve superimposed to the XRD data recorded simultaneously correlates thermal events to structural changes on the same scale of temperature. This presentation of the structural and thermal data has been already employed in refs *22 and 23*. Moreover, Peakfit software (Jandel scientific, Erkrath, Germany) was used to determine the position of overlapped XRD peaks, by fitting the XRD patterns with the Gaussian−Lorentzien (sum) equation (*25*).

RESULTS AND DISCUSSION

Fat Globules and Crystals Observed at the Microscopic Scale. Figure 1A and **C** shows the size distribution of fat globules in goat's milk as observed by light microscopy at 40 °C, that is, over the final melting point of goat's milk fat. Goat's milk fat is a discontinuous phase, which is predominantly made up of numerous and spherical droplets dispersed in the milk aqueous phase. The size distribution of fat globules in goat's milk was monomodal and spanned from 0.8 to 10 μm with a mean diameter of 3.50 ± 0.06 μm, as determined by laser light scattering (results not shown). These results are in accordance with the data reported in the literature (*2–4*). The larger fat globules, that is, with diameters <10 μm, which were observed in the micrographs, may result from the fusion of smaller globules by coalescence at high temperature. The microstructure of goat emulsion revealed the particularity of goat fat globules to be smaller and more uniform in size than those of bovine fat globules (*13*). This higher frequency of smaller fat globules implies an advantage for their digestibility compared with bovine milk fat globules and a more efficient lipid metabolism since it may lead to more efficient hydrolytic cleavage by intestinal tract enzymes (*6*).

The observation of fat crystals in goat's milk fat globules was performed by polarized light microscopy after cooling of the milk from 40 to −8 °C at 10 °C/min, directly in the temperature-controlled stage of the microscope (**Figure 1B** and **D**). This technique allows the characterization of fat crystals thanks to their property of birefringence. At 40 °C, the TG molecules in goat's milk fat globules were in their liquid state and then appeared not birefringent (**Figure**s **1A** and **C**), whereas at −8 °C, partial crystallization of TG molecules occurred (**Figure**s **1B** and **D**). Fat crystals were clearly observed in the largest fat globules (**Figure 1B**). Their morphology and location within the globules were rather close to those of the needle type (N) previously observed by authors (*13, 28*). Some fat globules showed a layer of birefringence at their periphery (**Figure 1B**). As the largest fat globules resulted from coalescence, the crystals observed in these globules may not be representative of native milk; therefore, great care in the interpretation of these observations is required. In the smallest fat globules (diameter <0.5 μm), the absence of birefringence was attributed to the very small size of the crystals, which did not allow their visualization since fat globules may be at least partially crystallized at −8 °C. Lopez et al. (*13*) showed using polarized light microscopy that the cooling rate of milk affects the size and the localization of the crystals in bovine milk fat globules. The characterization of fat crystals in goat fat globules may be important for food applications and to better understand the impact of fat crystals on the stability of the emulsion and its rheological properties.

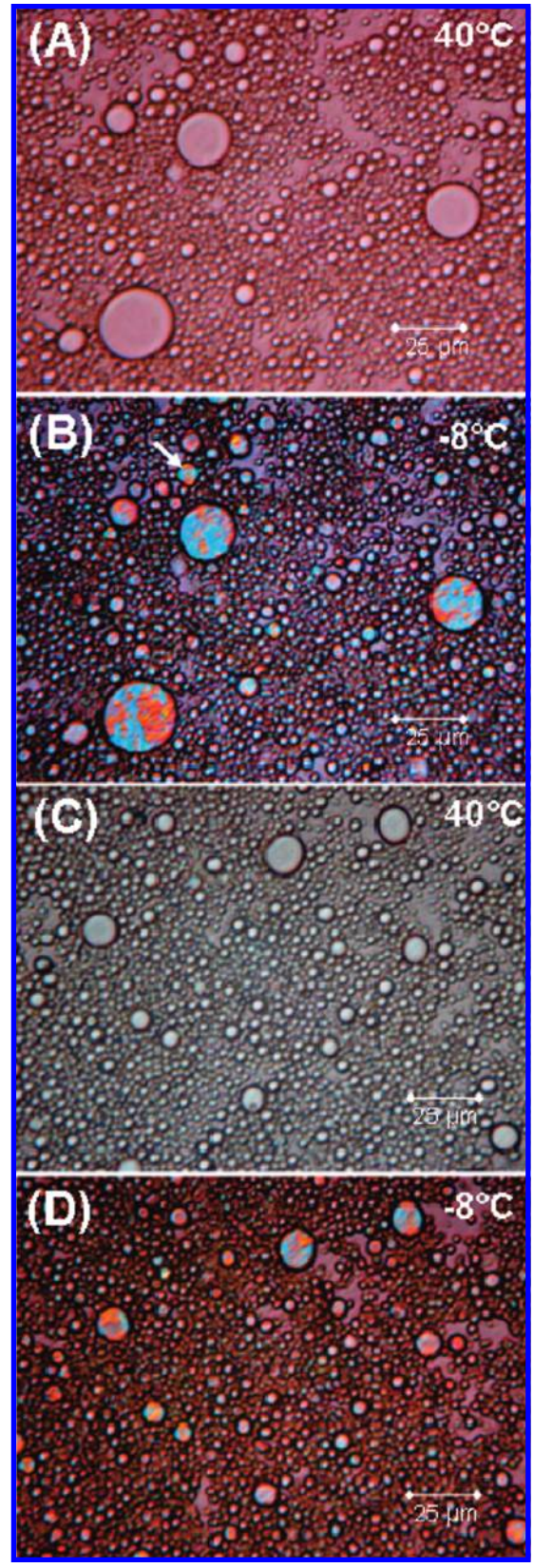

Figure 1. Polarized light microscopy photos of goat's milk taken at 40 °C (**A** and **C**) and after rapid cooling at −8 °C (**B** and **D**). The arrow shows a layer of birefringence at the periphery of a fat globule.

To complement the microstructural analysis, which was performed with goat's milk using polarized light microscopy,

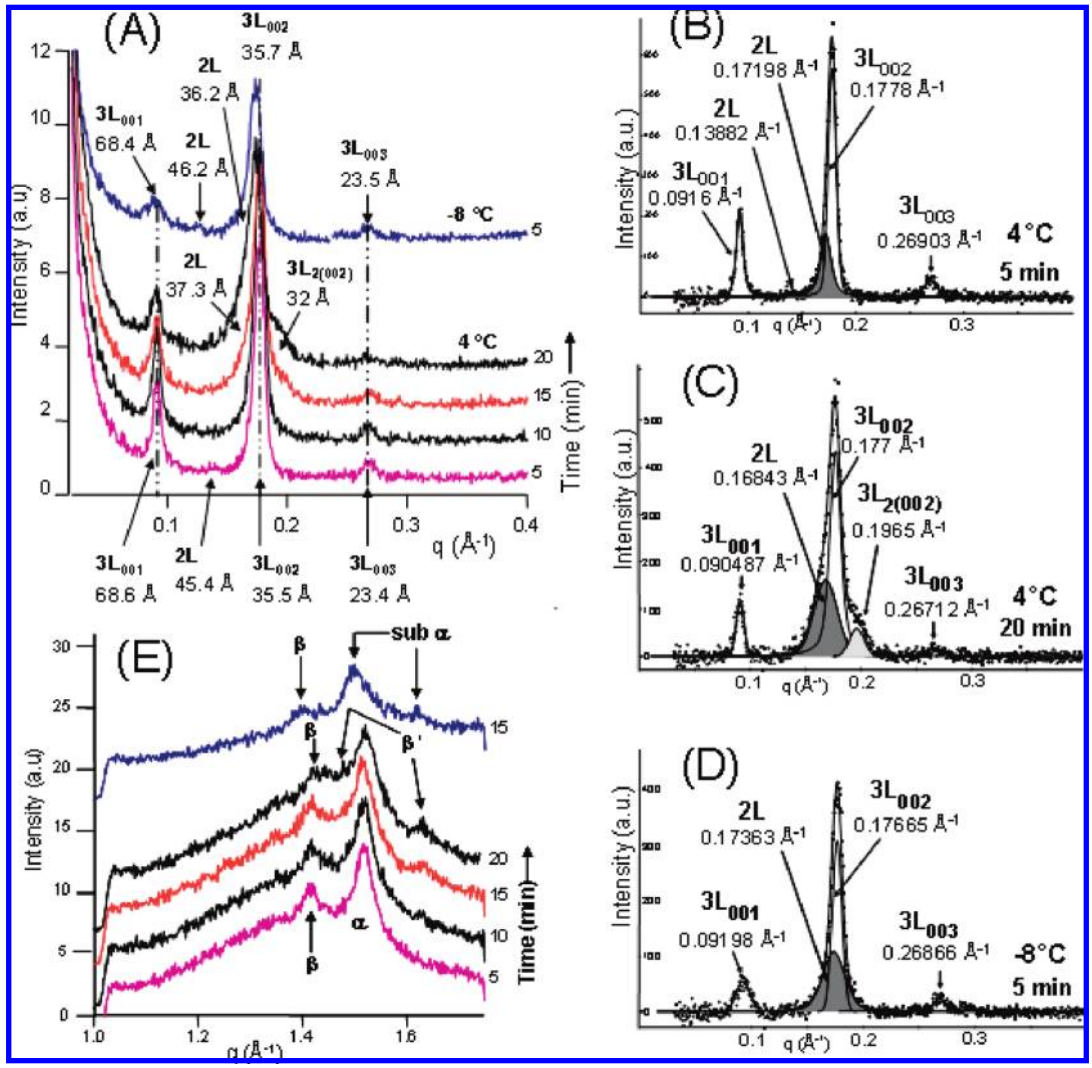

Figure 2. Characterization of the crystalline structures formed by triacylglycerols after quenching of goat's milk fat globules. X-ray diffraction (XRD) patterns recorded at small (**A**) and wide (**E**) angles after quenching at 4 and −8 °C. (**B**, **C**, and **D**) Analysis of the XRD patterns recorded at small angles, as indicated in the figure.

the organization of TG molecules was investigated at a molecular level using the coupling of X-ray diffraction (XRD) and differential scanning calorimetry (DSC).

Crystallization Properties of Goat's Cream after Quenching: Unstable Species of Triacylglycerols. Goat's milk fat globules concentrated in a cream were quenched from 50 °C to low temperature, 4, and −8 °C, in order to study the most unstable crystalline structures formed by TG molecules in fat globules and their evolution in isothermal conditions.

Quenching at 4 °C and Isothermal Conditioning. The crystalline structures formed by TG molecules after quenching of goat's milk fat globules from 50 at 4 °C were identified by XRD (**Figure 2**). At small angles, three sharp diffraction peaks were recorded at 68.6 Å, 35.5 Å, and 23.4 Å, immediately after quenching (**Figure 2A**). These peaks were related to the formation of a 3L stacking of TG molecules ($3L_{001}$ = 68.6 Å with its superior order of diffraction noted $3L_{002}$ and $3L_{003}$). However, the careful analysis of the small-angle XRD pattern performed using peakfit software evidenced the presence of a XRD peak below the signal related to the $3L_{002}$ structure (**Figure 2B**). This peak corresponds to a double-chain length structure: 2L (36.5 Å). Moreover, a small XRD peak was recorded at 45.4 Å and related to the crystallization of a bilayered structure 2L (**Figure 2A**). The wide-angle XRD patterns (**Figure 1E**) show two XRD peaks after quenching, attributed to the formation of a α polymorphic form (q = 1.51 Å^{-1}; d = 4.16 Å) coexisting with a β form, (q = 1.4 Å^{-1}; d = 4.49 Å). The XRD peaks were located on a XRD bump, which corresponds to the liquid phase of TG molecules in goat's milk fat globules. Thus, the liquid → solid phase transition of TG molecules that occurred within goat's milk fat globules after quenching at 4 °C was the following: liquid → 3L (68.6 Å) + traces of 2L (45.4 Å) + 2L (36.5 Å), with α and β polymorphic forms.

Following quenching from 50 to 4 °C, the polymorphic evolution of TG molecules dispersed in goat's milk fat globules have been monitored in isothermal conditions at 4 °C as a function of time for 20 min (**Figure 2**). The vanishing of the XRD peak related to the 2L (45.3 Å) structure, after 5 min storage of goat's milk fat globules at 4 °C was attributed to the metastable character of these crystals. The stacking of the 3L structure increased as a function of time until $3L_{001}$ = 69.4 Å, whereas the intensity of the XRD peaks related to this structure decreased. From 10 min, the asymmetry of the peak roughly related to the second order of the 3L structure that was clearly visible. A more detailed analysis of the small-angle XRD pattern recorded after 20 min storage at 4 °C after quenching shows that the main XRD peak results in fact from the presence of three overlapped peaks (**Figure 2C**). While the peak centered

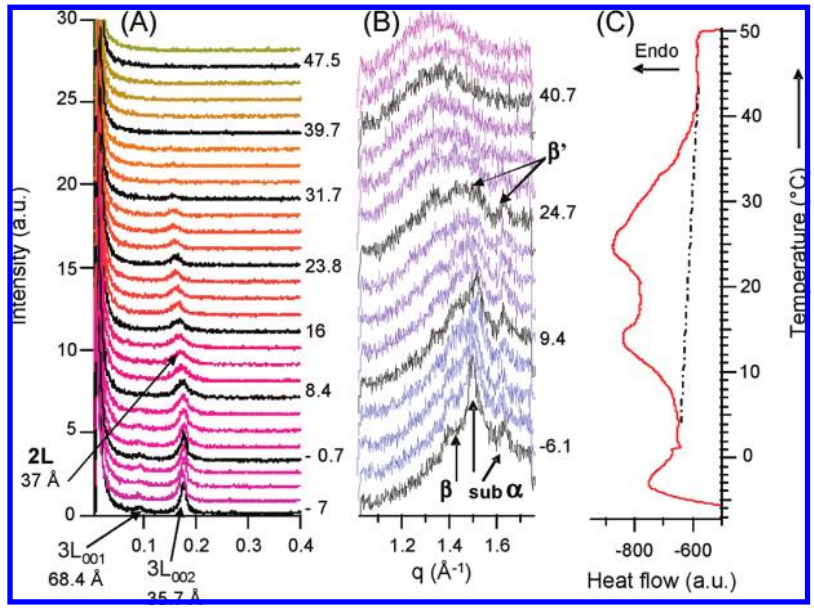

Figure 3. Three-dimensional plots of small (**A**)- and wide (**B**)-angle X-ray diffraction patterns recorded as a function of temperature, during the heating of goat's milk fat globules at 1 °C/min. (**C**) Differential scanning calorimetry curve recorded simultaneously. The crystalline structures are identified in the figure.

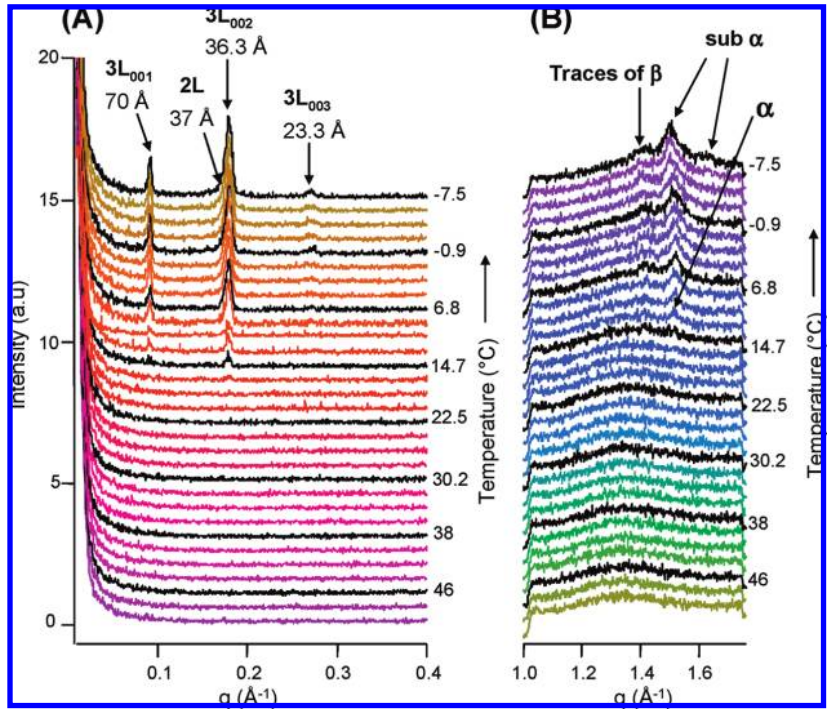

Figure 4. Three-dimensional plots of the X-ray diffraction patterns recorded at small (**A**) and wide (**B**) angles during the cooling of goat's milk fat globules at 1 °C/min from 50 to −8 °C. The crystalline structures are identified in the figure.

at 35.5 Å corresponds to the second order of the 3L (69.4 Å) structure, the peak located at 37.3 Å corresponds to the 2L structure identified after quenching, and the peak at 32 Å was interpreted as the second order of a trilayered structure (3L ∼64 Å) since this thickness is too low to be assimilated to a 2L structure. However, the poor signal/noise ratio prevented us from detecting the first order of the $3L_2$ (∼64 Å) structure. In isothermal condition at 4 °C, the β form (peak at 4.5 Å) coexisted with the α form (peak at 4.16 Å). Moreover, the formation of a XRD peak at $q = 1.63$ Å^{-1} (3.85 Å) showed the formation of a β' form with a $\alpha \rightarrow \beta'$ polymorphic transition, which occurred in isothermal conditions after quenching (**Figure 2E**). The changes in the structural organization of TG molecules in goat's milk fat globules characterized at 4 °C as a function of time showed the dynamic character of the system. Similar changes were previously reported by Lopez et al. (*26*), who studied the unstable species formed by TG molecules after the quenching of cow's milk fat globules at 4 °C and their polymorphic evolution as a function of time. At small angles, the decrease in intensity of the peaks related to the 3L (68.6 Å) structure was concomitant with the growth of the $3L_2$ (64 Å) structure and the 2L (37.3 Å) structure (**Figure 2A**). At wide angles, the α form progressively transformed in the β' form as a function of time (**Figure 2E**). Thus, the following polymorphic transition was characterized in goat's milk fat globules: α 3L

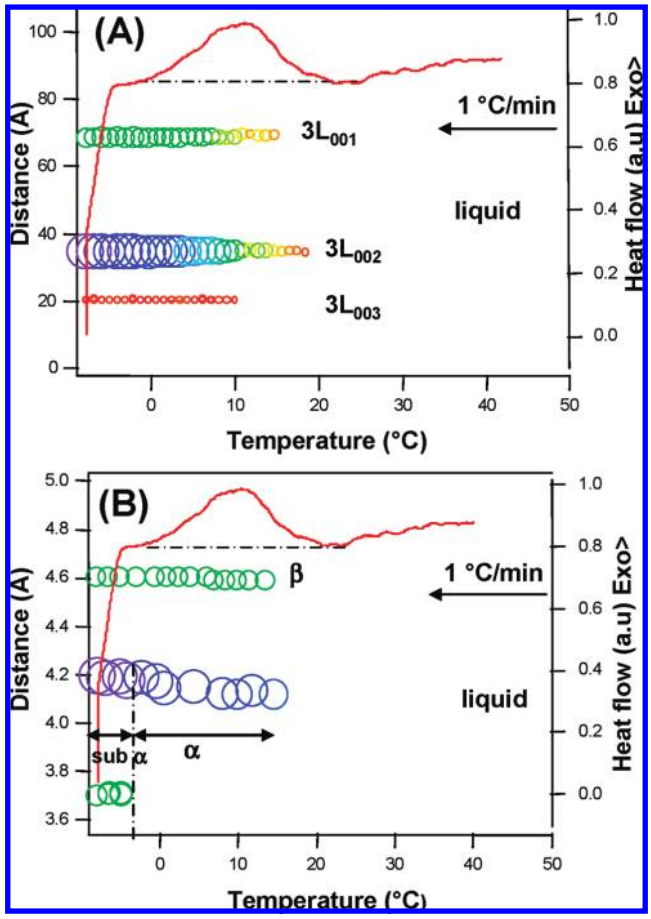

Figure 5. Structural and thermal properties of triacylglycerols in goat's milk fat globules during cooling at 1 °C/min. Evolutions, as a function of temperature, of the long (**A**) and short (**B**) spacings recorded at small and wide angles, respectively (**Figure 4**). Symbol sizes, centered on distance and temperature, are proportional to the maximal intensities of the lines. The structural data are superimposed with the crystallization curve recorded simultaneously by differential scanning calorimetry.

(68.6 Å) + 2L (45.4 Å) + β 2L (36.5 Å) → α 3L (69.4 Å) + β 2L (37.3 Å) + β' $3L_2$ (64 Å).

The organization of the FA chains in the α polymorphic form is not surprising after such a rapid cooling rate since it is the subcell with the lowest density (hexagonal packing). The characterization of the β polymorphic form after quenching, which is the most stable lateral organization of FA chains, was more questionable. As this β form has not been reported after the quenching of cow's milk fat globules at 4 °C (*26*), we deduced that its formation is specific to the FA composition of goat's milk fat.

As for many foods, dairy products require storage at 4 °C to prevent bacterial growth. Thus, the quenching of goat's milk fat globules and the study of the structural behavior of TG contributed to a better understanding of what physically happens in the fat phase of dairy products as a function of time when they are placed and kept at the conservation temperature of 4 °C.

Quenching at −8 °C and Melting Behavior. Goat's milk fat globules were quenched from 50 to −8 °C by direct introduction of the capillary into the calorimeter precooled to this temperature. The quenching temperature, −8 °C, was chosen low enough to crystallize the TG of the sample in the less stable polymorphic varieties, without the formation of ice crystals. The crystalline structures formed by TG dispersed in goat's milk fat globules have been identified by recording isothermal XRD patterns for 5 min at both small and wide angles (**Figure 2A** and **E**). The small-angle XRD pattern showed four peaks at 68.4, 46.2, 35.7, and 23.5 Å (**Figure 2A**). The peak at 68.4 Å was related to the formation of a 3L structure, with its second order located at 35.7 Å ($3L_{002}$) and its third order at 23.5 Å ($3L_{003}$). The peak recorded at 46.2 Å corresponded to the formation of a 2L organization. A deeper analysis of the XRD pattern evidenced the asymmetry of the peak with the highest intensity and showed the presence of two overlapped peaks corresponding to a bilayered structure 2L (36.2 Å) and to the $3L_{002}$ (**Figure 2D**). The simultaneous wide-angle XRD pattern recorded at −8 °C **Figure 2E**) shows the occurrence of two diffraction lines centered at 4.22 Å and 3.8 Å, which were attributed to the formation of a sub-α form. Moreover, the third diffraction line recorded at 4.5 Å was attributed to the presence of the β form. Thus, after quenching of goat's milk fat globules at −8 °C, we characterized the formation of the following structures: 3L (68.4 Å) + 2L (46.2 Å) + 2L (36.2 Å) with sub-α and β polymorphic forms.

Similar crystalline structures were formed within goat's milk fat globules, whatever the temperature of quenching, for example, 4 °C or −8 °C. However, the peaks associated with the $3L_{001}$ structure (68.6 Å and 68.4 Å) were sharper after quenching at 4 °C compared with −8 °C. This may be due to (i) the size of the crystals, which may be smaller at −8 °C (higher peak width), (ii) the level of organization of TG molecules in the crystals (less organized at −8 °C), and (iii) the composition of TG in the crystals formed after the quenching since more TG may have been crystallized at a lower temperature (−8 °C) compared with 4 °C.

After rapid cooling and identification of the crystalline varieties formed at −8 °C, the goat's cream sample was heated to 50 at 1 °C/min. This heating rate has been chosen as intermediate between high heating rate values ($dT/dt \geq 2$ °C/min) capable of blocking the sample evolution during heating and values low enough ($dT/dt \leq 1$ °C/min) to allow good time-resolved X-ray recording, especially with dispersed systems. The plots of the XRD patterns recorded at small and wide angles, as a function of time, are presented in **Figure 3A** and **B**, respectively. At small angles (**Figure 3A**), we observed until about 9 °C the vanishing of the diffraction lines related to the 3L (68.4 Å) structure formed after quenching and the formation of a single peak corresponding to a bilayered structure, 2L (37 Å). The 2L (37 Å) peak was broader than the peaks that were associated to the 3L (68.4 Å) structure. Moreover, the thickness of the 2L structure progressively increased from 37 to 40.4 Å as a function of temperature until its final melting over 38 °C. At wide angles (**Figure 3B**), the main diffraction lines identified at −8 °C were attributed to the formation of sub-α (lines at 3.75 and 4.21 Å) and β (line at 4.55 Å) polymorphic forms after the quenching of goat's milk fat globules. On heating, the sub-α polymorphic form transformed into a β' form, which is characterized by a less intense and broader peak at ∼4.2 Å. No diffraction lines were recorded for $T > 38$ °C, meaning that all of the TG of goat's milk fat globules were in their liquid state. Information provided by the coupling of the small- and wide-angle XRD experiments allowed the characterization of the following polymorphic evolution on heating: 3L (68.4 Å) sub-α + 2L (36.2 Å) with traces of β → 2L (37 Å - 40.4 Å) β' → liquid.

The complex melting curve recorded by DSC during the heating of goat's milk fat globules at 1 °C/min is shown in **Figure 3C**. The DSC melting curve shows two overlapped

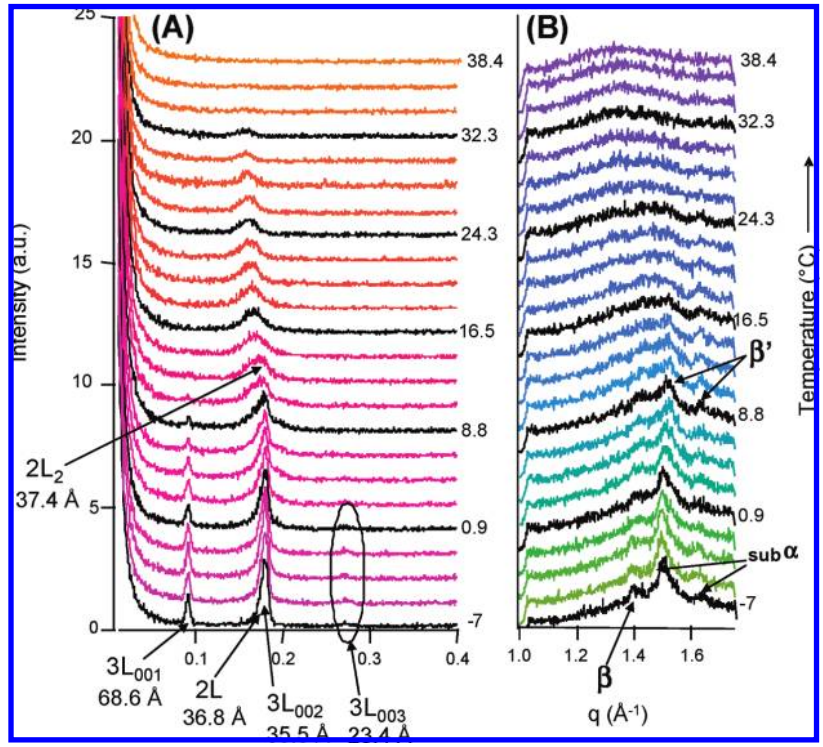

Figure 6. Three-dimensional plots of the X-ray diffraction patterns recorded at small (**A**) and wide (**B**) angles as a function of temperature during the heating of goat's milk fat globules at 1 °C/min, after cooling at 1 °C/min. The crystalline structures identified are noted in the figure.

endotherms until the final melting temperature at 39 ± 0.5 °C. The DSC signal recorded for $T < 2$ °C corresponds to the transition from static to dynamic equilibrium of the calorimeter. The first endotherm, which spans until about 19 °C with a maximum at 13.8 °C, has been assigned to the melting of the sub-α 3L (68.4 Å) form. The second endotherm, which is observed in the range 20 °C < T < 39 °C with a maximum at 24.8 °C, coincides with the melting of the β' 2L (37 Å) structure characterized by XRD experiments (**Figure 3A** and **B**).

Crystallization Behavior of Triacylglycerols on Cooling at 1 °C/min and Subsequent Heating. The crystalline structures formed in goat's milk fat globules were studied at intermediate cooling rates with $|dT/dt| = 1$ and 3 °C/min. As similar results were obtained for these two cooling rates, only the experiments performed on cooling at 1 °C/min are detailed below.

Cooling of Goat'S Milk Fat Globules at 1 °C/min. On cooling of goat's milk fat globules at 1 °C/min from 50 to −8 °C, 62 XRD patterns of 60 s each were recorded as a function of time. **Figure 4** shows the plots of the XRD patterns recorded simultaneously at small and wide angles, which were summed by two to increase the signal/noise ratio. At small angles (**Figure 4A**), the simultaneous recording of three sharp XRD peaks, centered at 70 Å, 36.3 Å, and 23.3 Å, occurred from about 18 °C. These peaks were related to the crystallization of a triple-chain length organization, 3L (70 Å), with its superior orders of diffraction. Below $T \sim 0$ °C, the broadening of the $3L_{002}$(36.3 Å) peak may correspond to the formation of a new crystalline organization, with a double-chain length organization, 2L (37 Å). The formation of a new 3L structure with $3L_{002} = 37$ Å has been excluded since the $3L_{001}$ (70 Å) peak did not broaden on cooling. The wide-angle XRD patterns (**Figure 4B**) show, from about 15 °C, the occurrence of a diffraction line at $q = 1.51$ Å^{-1} (4.15 Å), which is characteristic of TG crystallization with a hexagonal packing of the acylglycerols chains (α form). In the XRD pattern recorded at −8 °C, the peak centered at $q = 1.49$ Å^{-1} (4.2 Å) and the occurrence of a second XRD peak at 1.68 Å^{-1} (3.73 Å) were related to the formation of a sub-α subcell. The XRD peak at $q = 1.36$ Å^{-1} (4.6 Å) corresponded to TG crystallization in the β form and was related to the 2L (37 Å) structure.

The DSC curve recorded simultaneously with XRDT experiments and the evolutions of maximum intensity of the diffraction peaks recorded at small and wide angles on cooling of goat's milk fat globules at 1 °C/min are plotted in **Figure 5**. The results are expressed as a function of temperature in order to relate the thermal (DSC experiments) to the structural (XRD experiments) properties of milk fat. Moreover, the longitudinal organization of the TG molecules recorded at small angles (**Figure 4A**) were related to their lateral packing recorded at wide angles (**Figure 4B**), with the symbol sizes being proportional to the maximum line intensities (**Figures 5A** and **B**). The DSC recording shows a single broad exotherm with an initial temperature of crystallization at about 18 °C. This exotherm was related to crystallization of the α 3L (70 Å). At small angles, the intensity of the XRD peaks related to the $3L_{001}$ (70 Å) structure increased from their formation at about 18 to 10 °C showing the growth of this crystalline structure in goat's milk fat globules. For $T < 10$ °C, the intensity of the XRD did not evolve significantly (**Figure 5A**). The low proportion of the β 2L (37 Å) variety prevented the detection of its formation using DSC. Thus, the XRDT data are much sensitive and informative compared to DSC.

Cooling of cream at 1 °C/min lead to the following crystallization behavior of TG molecules in goat's milk fat globules: liquid → α 3L (70 Å) → sub-α 3L (70 Å) + β 2L (37 Å).

Subsequent Heating of Goat'S Milk Fat Globules at 1 °C/min. After cooling of goat's milk fat globules at 1 °C/min, they were heated from −8 to 50 °C at 1 °C/min, with the recording of XRD patterns, each 60 s (62 frames were recorded). The plots of the XRD patterns recorded at small and wide angles as a function of time are presented in **Figure 6A** and **B**, respectively (the XRD patterns recorded were summed by 2).

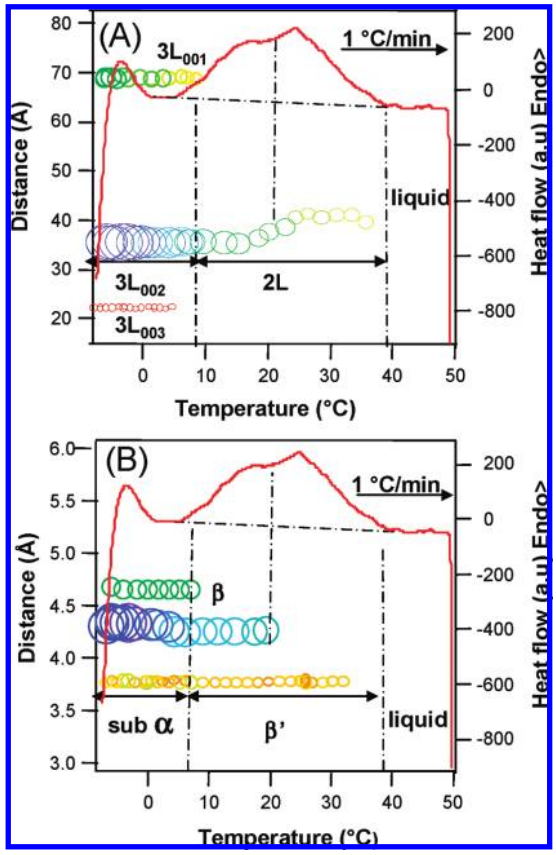

Figure 7. Structural and thermal properties of triacylglycerols in goat's milk fat globules. The evolutions, as a function of temperature, of the long (**A**) and short (**B**) spacings deduced from the diffraction peaks of **Figure 6A** and **B**, respectively. The structural data are superimposed with the differential scanning calorimetry curve recorded simultaneously during heating at 1 °C/min.

The evolution of the lines recorded at small angles showed successive phase transitions of the crystalline structures (**Figure 6A**). In the interval of temperature -7 °C $< T <$ 9 °C, the intensity of the diffraction lines related to the 3L crystalline variety ($3L_{001}$ = 68.6 Å; $3L_{002}$ = 35.5 Å; $3L_{003}$ = 23.4 Å) decreased. For $T >$ 9 °C, the absence of the $3L_{001}$ (68.6 Å) line was interpreted as the melting of the 3L structure formed on cooling at 1 °C/min. Then, the single peak of XRD characterized at small angles was related to the formation of a bilayered structure, $2L_2$ (37.4 Å). The intensity of the peak related to the $2L_2$ structure increased until 24 °C and then decreased until its final melting for $T >$ 36 °C. The period of the $2L_2$ structure increased from 37.4 Å (16.5 °C) to 40.4 Å (32.3 °C) (**Figure 6A**). The XRDT data recorded at wide angles show the coexistence of the sub-α and β polymorphic forms from -8 to about 9 °C (**Figure 6B**). Above this temperature, the β' form was characterized until the final melting point of goat's milk fat globules. For $T >$ 36 °C, the absence of diffraction peaks at small angles and the recording of the broad peak of scattering observed at wide angles means that all TG of the goat's milk fat globules are in their liquid state. The crystalline organization formed on heating of goat's milk fat globules, $2L_2$ (37.4 Å), may be constituted in part of the TG molecules integrated in the 2L (36.8 Å) structure formed on cooling and which melted over 9 °C, and/or part of the TG molecules that were incorporated in the 3L (68.6 Å) structure. It is also possible that all of the TG molecules incorporated in the β 2L (36.8 Å) structure melted at $T >$ 9 °C.

The evolution of both the position and the maximum intensity of diffraction peaks recorded at small and wide angles during heating of goat's milk fat globules were superimposed to the DSC recorded simultaneously as a function of temperature (**Figure 7**). The DSC melting curve revealed the overlapping of at least two endotherms until the final melting temperature at about 38 °C. Above this temperature, all of the TG dispersed in goat's milk fat globules were in their liquid state. For $T <$ 0 °C, the signal of DSC corresponds to the equilibration of the calorimeter. For $0 \leq T \leq 8$ °C, the DSC signal may correspond to the superimposition of an endotherm corresponding to the melting of both the sub-α 3L (68.6 Å) structure with an exotherm associated with the formation of the β' $2L_2$ (37.4 Å) structure with the sub-α $\rightarrow$ β' polymorphic transition. The melting of the β 2L (36.8 Å) structure was not clearly detected. The broad endotherm was interpreted as the progressive melting of the β' $2L_2$ (37.4 $\rightarrow$ 40.4 Å) structure. The overlapping of at least 2 endotherms may correspond to structural changes of the $2L_2$ structure, on heating. The first endotherm corresponds to the increase in the period of the $2L_2$ structure, which may be due to its enrichment in long-chain saturated fatty acids, while the second endotherm may be related to the melting of this structure without any changes in the period (**Figure 7**A).

In summary, the following polymorphic transitions were observed on heating: 3L (68.6 Å) sub-α + 2L (36.8 Å) $\beta \rightarrow$ $2L_2$ (37.4 $-$ 40.4 Å) $\beta' \rightarrow$ liquid.

Crystallization of Triacylglycerols in Goat's Milk Fat Globules on Slow Cooling at 0.1 °C/min and Subsequent Heating. The crystallization properties of TG molecules in goat's milk fat globules were studied on slow cooling from 50 to -8 °C at $|dT/dt| = 0.1$ °C/min, by the recording of XRD patterns with a duration of acquisition of 600 s per frame. These XRD experiments required the use of synchrotron radiation for ~10 h. **Figure 8** shows the XRD patterns that were recorded simultaneously at small and wide angles as a function of time. Since the XRD patterns recorded on slow cooling were summed by 2, each XRD pattern presented in **Figure 8** corresponds to the information that has been recorded in a temperature range of 2 °C. In the plot of the XRD patterns recorded at small angles (**Figure 8A**) the detection of the first peaks of diffraction from about 17 °C corresponds to the crystallization of TG molecules in goat's milk fat globules. Crystallization was initiated by the simultaneous formation of a 2L (40.2 Å) structure and a 3L (68.7 Å) structure characterized by 2 peaks ($3L_{001}$ = 68.7 Å and $3L_{002}$ = 35.2 Å). The evolutions of maximum intensity of the diffraction peaks recorded at small angles during slow cooling of goat's milk fat globules are presented in **Figure 8C**. The intensity of the 2 peaks related to the 3L (68.7 Å) structure increased until about 5 °C and then decreased. As a function of the decrease in temperature, the 2L structure developed and its thickness decreased from 40.2 Å (17 °C) to 37.2 Å (-6.7 °C). From about 7 °C, the recording of an additional peak at 30.7 Å was interpreted as the second order of a second 3L structure, $3L_2$ (~ 61.4 Å), the first order of which was not detected because of its low intensity compared to the signal/noise ratio of the experiments.

At wide angles, the recording of a bump centered at about 1.34 $Å^{-1}$ from 50 °C to about 18 °C corresponded to the scattering signal of TG molecules in their liquid state (**Figure 8B**). The first crystalline structures formed in goat's milk fat globules corresponded to the α polymorphic form (peak at 4.16 Å) and the β'_1 form (peaks at 4.35 Å and 3.73 Å). Then, as a function of the decrease in temperature, we characterized the formation of the β'_2 (peaks at 4.21 Å and 3.82 Å) and the β polymorphic forms (peak at 4.68 Å) (**Figure 8B**).

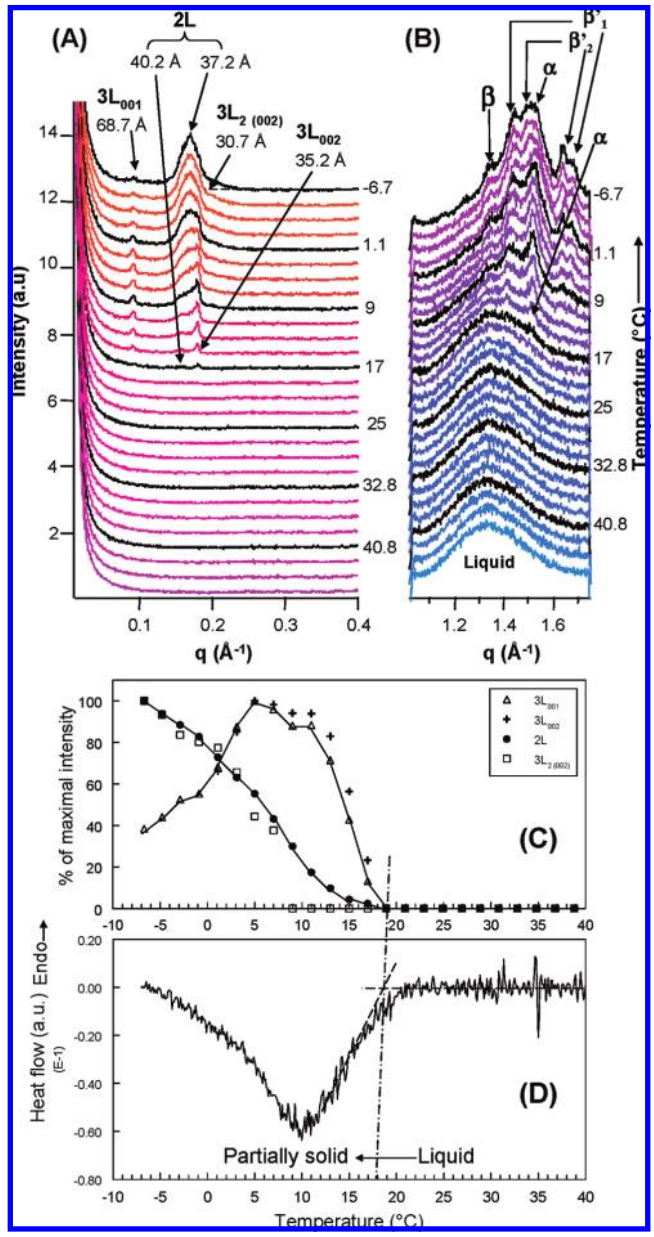

Figure 8. Structural and thermal analysis of goat's milk fat globules during cooling at 0.1 °C/min. Three-dimensional plots of small (**A**) and wide (**B**) angle X-ray diffraction patterns recorded as a function of temperature. (**C**) Evolution of the maximal intensity of the peaks recorded at small angles and (**D**) the differential scanning calorimetry curve recorded simultaneously on cooling from 50 to −8 °C. The crystalline structures are identified in the figure.

The thermal properties of goat's milk fat globules were studied by DSC simultaneously with the structural analysis performed by XRD, during their cooling at 0.1 °C/min (**Figure 8D**). The DSC recording shows that crystallization of the 2L (40.2 Å) and the 3L (68.7 Å) structures occurred in a broad exothermic event. The initial temperature of crystallization was about 20 °C.

After their cooling at $|dT/dt| = 0.1$ °C/min, the goat's milk fat globules were heated from −8 to 50 °C at 1 °C/min to follow the structural and thermal properties of TG molecules (**Figure 9**). The XRD patterns recorded at small angles (**Figure 9A**) show the progressive melting of the crystalline structures formed on cooling, with an increase of the thickness of the 2L structure as a function of the increase in temperature until the final melting of TG molecules in goat's milk fat globules. The wide-angle XRD patterns recorded on heating are presented in **Figure 9B**. The changes in the structural organization of TG molecules on heating showed that the crystalline structures formed on slow cooling were metastable and transformed into more stable varieties until their final melting. The thermal analysis, which was conducted simultaneously to the structural investigations thanks to the coupling of XRDT and DSC experiments shows a broad endothermic event until the final melting of TG molecules in goat's milk fat globules (**Figure 9C**).

Influence of Cooling Rate on the Crystallization Properties of Triacylglycerols in Goat's Milk Fat Globules. Figure 10 shows the XRD patterns recorded at small (**Figure 10A**) and wide (**Figure 10B**) angles after the cooling of goat's milk fat globules at different cooling rates dT/dt, with $0.1 < dT/dt < 1000$ °C/min (quenching). **Table 2** summarizes the main structural and thermal characteristics of the crystalline structures formed by TG in goat's milk fat globules. These experiments show that the cooling rate affects the crystallization properties of TG molecules in goat's milk fat globules. Thus, the variation of the cooling rate is one of the major tools allowing the exploration of the thermal and structural behaviors of fats, at

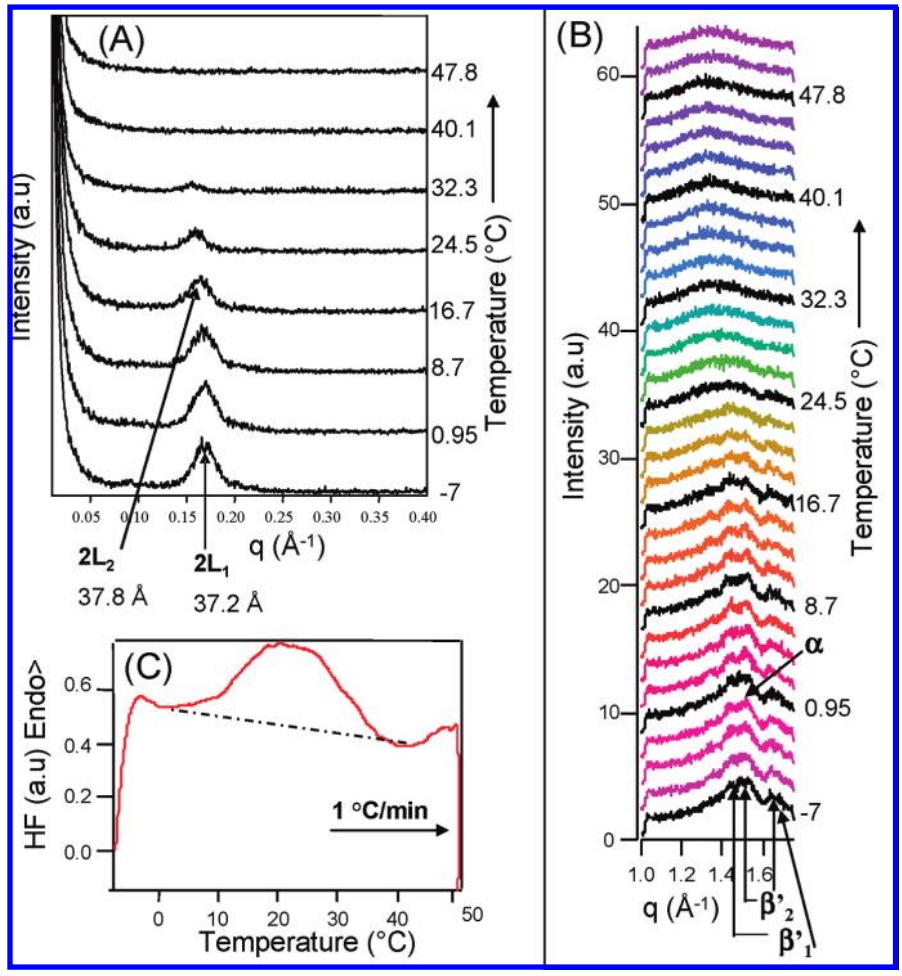

Figure 9. Structural and thermal properties of triacylglycerols in goat's milk fat globules. Three-dimensional plots of the X-ray diffraction patterns recorded at small (**A**) and wide (**B**) angles during heating at 1 °C/min. (**C**) Differential scanning calorimetry curve recorded simultaneously.

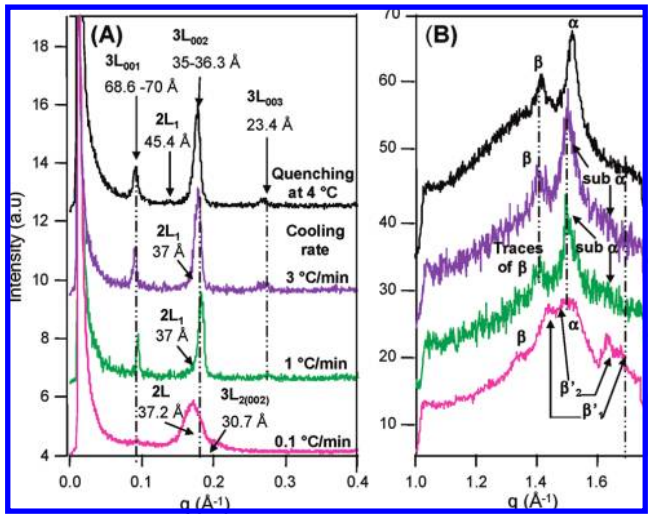

Figure 10. Comparison of the crystalline structures formed by triacylglycerols in goat's milk fat globules. Small (**A**) and wide (**B**) angle X-ray diffraction patterns recorded at −8 °C after different cooling rates indicated on the figure and at 4 °C after quenching from 50 °C. The crystalline structures are identified in the figure.

the molecular scale. XRD experiments performed at small angles allowed the identification of 3L (68.6 − 70 Å) and 2L (37 − 45.4 Å) structures in goat's milk fat globules. These two main types of crystals showed a segregation of TG molecules as a function of their properties. As previously reported, the 3L structures may correspond to the organization of TG molecules with unsaturated FA or FA with different chain lengths (with differences in the number of atoms of carbon >4) (*29*). The 2L structures may be formed by TG molecules with similar chain length and saturation. The 2L (45.4 Å) characterized after quenching of goat's milk fat globules at low temperature (−8 and 4 °C) was metastable and disappeared after 5 min of storage in isothermal conditions, whereas the 2L (∼37 Å) formed on cooling was more stable. The different behavior of these 2L crystalline structures may correspond to different compositions in TG. Similar longitudinal organizations of TG molecules were characterized in cow's milk fat globules (*13, 24–26*). Furthermore, we showed that the faster the cooling the sharper the small-angle XRD line width, which may be related to the size of fat crystals and to their degree of organization.

At wide angles, 5 polymorphic forms were characterized in goat's milk fat globules: α, sub-α, β'_1, β'_2, and β. The α polymorphic form was mainly related to the 3L structures, while the more stable polymorphic forms (β' and β) characterized on cooling were attributed to the 2L structures. The formation of the β 2L (36.2 − 37.3 Å) characterized after the quenching of goat's milk fat globules may be formed by TG molecules with similar chain length and saturation, which are able to self-assemble in the most dense and stable polymorphic form. Considering the specific FA composition of goat's milk fat (*8–12*), this structure may be formed by TG molecules with $C_{8:0}$, $C_{10:0}$, and $C_{12:0}$ FA.

Table 2. Summary of the Structural and Thermal Behaviour of Goat's Milk Fat Globules Characterized by X-ray Diffraction As a Function of Temperature (XRDT) and Differential Scanning Calorimetry (DSC), Respectively, after Cooling at the Different Rates Indicated and during Subsequent Heating at 1 °C/min

cooling rate: d*T*/d*t*	0.1 °C/min	1 °C/min	quenching (−8 °C)
- number of crystallization peaks	1	1	
- initial temperature of crystallization (°C)	17	18	
- varieties observed on cooling:			
chain packing	α, β'_1, β'_2, β	α, sub-α, traces of β	sub-α + β
molecule stacking[a]	$3L_{001}$(68.7 Å)	$3L_{001}$(70 Å)	$3L_{001}$(68.4 Å)
	$3L_{002}$(35.2 Å)	$3L_{002}$(36.3 Å)	$3L_{002}$(35.7 Å)
	$3L_{2\ (002)}$(30.7 Å)	$3L_{003}$(23.3 Å)	$3L_{003}$(23.5 Å)
	2L (40.2 Å)	2L (37 Å)	2L (46.2 Å)
	2L (37.2 Å)		2L (36.2 Å)
- thermal behavior on heating	α melts first, then β'_1, β'_2	sub-α melts first, then β and β'	sub-α melts first, then traces of β and β'
- final temperature of melting (°C)	39 ± 0.5	38 ± 0.5	39 ± 0.5

[a] 3L, triple chain length organization; 2L, double chain length organization.

Whatever the cooling rate, we showed that cooling of goat's milk fat globules leads to the formation of metastable structures that evolve as a function of time (after quenching) or as a function of temperature after a subsequent heating. The formation of a β' 2L (37−40 Å) structure was characterized on heating with a $\alpha \rightarrow \beta'$ polymorphic transition. Thus, the most stable longitudinal organization of TG molecules in goat's milk fat globules corresponds to a 2L organization. Furthermore, the increase of its thickness until its final melting point corresponded to its enrichment in long chain saturated FA as a function of the increase in temperature. These changes in the organization of TG molecules show that the polymorphism of TG molecules exists in dispersed systems such as milk fat globules. Similar results were previously reported for cow's milk fat globules (*13*).

Whereas the XRD experiments performed as a function of temperature showed the coexistence of several crystalline structures (of 3L and 2L type), the DSC recordings showed broad exothermic (on cooling) and endothermic (on heating) events. Thus, the coupling of DSC with XRDT using synchrotron radiation allowed the identification of the crystalline structures that crystallize and melt in goat's milk fat globules as a function of temperature. The study of the crystallization behavior of TG is more difficult and challenging in emulsion than in bulk. Indeed, the presence of many compounds such as water and proteins decrease the intensity of the X-ray signal by a simple dilution effect. Furthermore, the crystallization properties may also be influenced by the size of droplets and the presence of an interface with water.

Lipids are the most important components of milk, in terms of cost, nutrition, and physical and sensory characteristics that they impart to dairy products. The experiments reported in this study give deeper insight into the study of the crystallization behavior of goat's milk fat globules. The use of synchrotron radiation and the coupling the XRDT and DSC experiments allowed, for the first time, to our knowledge, the identification of the polymorphism and phase transition displayed by TG molecules in goat's milk fat globules at a molecular scale. This study is important as fundamental knowledge on lipid crystallization and polymorphism, and for the food industry to better control the physical properties of milk fat, and to increase the technical application of goat's milk fat crystallization in order to contribute to the development of new products.

ACKNOWLEDGMENT

We thank Professor H. Attia and Dr. N. Karray (ENIS, Sfax, Tunisia) for valuable discussions on goat's milk properties. J. Tessier (Experimental farm, INRA, Grignon, France) is acknowledged for providing goat's milk.

LITERATURE CITED

(1) CNIEL. *L'Economie Laitière en Chiffres*, 2007 ed.; CNIEL Ed.; 2007; pp 201.
(2) Park, W. Y.; Juarez, M.; Ramos, M.; Haenlein, G. F. W. Physico-chemical characteristics of goat and sheep milk. *Small ruminant Res.* **2007**, *68*, 88–113.
(3) Mens, P. L. Propriétés Physico-Chimiques Nutritionnelles et Chimiques (Physico-Chemical Nutritional and Chemical Properties. In *Laits et Produits Laitiers. Vache Brebis Chèvre (Milk and Milk Products from Cows, Sheep and Goats)*; Luquet, F. M., Ed.; Apria, Paris, France, 1985; Vol. l, pp 349−367.
(4) Attaie, R.; Richtert, R. L. Size distribution of fat globules in goat milk. *J. Dairy Sci.* **2000**, *83*, 940–944.
(5) Mehaia, M. A. The fat globule size distribution in camel, goat, ewe and cow milk. *Milchwissenschaft* **1995**, *50*, 260–263.
(6) Lopez, C. Focus on the supramolecular structure of milk fat in dairy products. *Reprod. Nutr. Dev.* **2005**, *45*, 497–511.
(7) Park, Y. W. Hypo-allergenic and therapeutic significance of goat milk. *Small Ruminant Res.* **1994**, *14*, 151–161.
(8) Jandal, J. M. Comparative aspects of goat and sheep milk. *Small Ruminant Res.* **1996**, *22*, 177–185.
(9) Fontecha, J.; Rios, J. L.; Lozada, L.; Fraga, M. J.; Juarez, M. Composition of goat's milk fat triglycerides analysed by silver ion adsorption-TLC and GC-MS. *Int. Dairy J.* **2000**, *10*, 119–128.
(10) Haenlein, G. F. W. Goat milk in human nutrition. *Small Ruminant Res.* **2004**, *51*, 155–163.
(11) Cardak, A. D.; Yestismeyen, A.; Bruckner, H. Quantitative comparison of free fatty acids in camel, goat and cow milk. *Milchwissenschaft* **2003**, *58*, 127–130.
(12) Alonso, L.; Fontecha, J.; Lozada, L.; Fraga, M. J.; Juarez, M. Fatty acid composition of caprin milk: major, branched chain and *trans* fatty acids. *J. Dairy Sci.* **1999**, *82*, 878–884.
(13) Lopez, C.; Bourgaux, C.; Lesieur, P.; Bernadou, S.; Keller, G.; Ollivon, M. Thermal and structural behavior of milk fat. 3. Influence of cooling rate and droplet size on cream crystallization. *J. Colloid Interface Sci.* **2002**, *254*, 64–78.
(14) Lopez, C.; Lesieur, P.; Ollivon, M. Thermal and structural behavior of anhydrous milk fat. 3. Influence of cooling rate. *J. Dairy Sci.* **2005**, *88*, 511–526.
(15) Boistelle, R. Fundamentals of Nucleation and Crystal Growth, Inc. *Crystallization and Polymorphism of Fats and Fatty Acids*, Garti, N. and Sato, K., Eds.; Marcel Dekker Inc., New York, 1988; pp 189−22.
(16) Dickinson, E. and McClements, D. J. Advances in Food Colloids. *Fat Crystallization in Oil-in-Water Emulsions*; Chapman and Hall, London, 1995; pp 211−246.
(17) Rousseau, D. Fat crystals and emulsion stability: a review. *Food Res. Int.* **2000**, *33*, 3–14.
(18) Timms, R. E. The phase behaviour and polymorphism of milk fat, milk fat fractions and fully hardened milk fat. *Aust. J. Dairy Technol.* **1980**, *35*, 47–52.

(19) Lopez, C.; Bourgaux, C.; Lesieur, P.; Ollivon, M. Coupling of time-resolved synchrotron X-ray diffraction and DSC to elucidate the crystallization properties and polymorphism of triglycerides in milk fat globules. *Le Lait* **2007**, *87*, 459–480.

(20) Lopez, C.; Bourgaux, C.; Lesieur, P.; Riaublanc, A.; Ollivon, M. Milk fat and primary fractions obtained by dry fractionation. 1. Chemical composition and crystallization properties. *Chem. Phys. Lipids* **2006**, 17–33.

(21) Karray, N.; Lopez, C.; Lesieur, P.; Ollivon, M. Dromedary milk fat: Thermal and structural properties 2- Influence of cooling rate. *Le Lait* **2005**, *85*, 433–451.

(22) Ben Amara-Dali, W.; Karray, N.; Lesieur, P.; Ollivon, M. Anhydrous goat's milk fat: Thermal and structural behaviors. 1. Crystalline forms obtained by slow cooling. *J. Agric. Food Chem.* **2005**, *53*, 10018–10025.

(23) Ben Amara-Dali, W.; Lesieur, P.; Artzner, F.; Karray, N.; Attia, H.; Ollivon, M. Anhydrous goat's milk fat: Thermal and structural behaviors studied by coupled DSC and X-ray diffraction. 2. Influence of cooling rate. *J. Agric. Food Chem.* **2007**, *55*, 4741–4751.

(24) Lopez, C.; Lesieur, P.; Keller, G.; Ollivon, M. Thermal and structural behavior of milk fat. 1. Unstable species of Cream. *J. Colloid Interface Sci.* **2000**, *229*, 62–71.

(25) Lopez, C.; Lesieur, P.; Bourgaux, C.; Keller, G.; Ollivon, M. Thermal and structural behavior of milk fat. 2. Crystalline forms obtained by slow cooling of cream. *J. Colloid Interface Sci.* **2001**, *254*, 64–78.

(26) Lopez, C.; Bourgaux, C.; Lesieur, P.; Ollivon, M. Crystalline structures formed in cream and anhydrous milk fat at 4 °C. *Lait* **2002**, *82*, 317–335.

(27) Ollivon, M.; Keller, G.; Bourgaux, C.; Kalnin, D.; Villeneuve, P.; Lesieur, P. DSC and high resolution X-ray diffraction coupling. *J. Therm. Anal. Calorimetry* **2006**, *85*, 219–224.

(28) Walstra, P. On the crystallization habit in fat globules. *Neth. Milk Dairy J.* **1967**, *21*, 166–191.

(29) Sato, K. Polymorphism of Pure Triacylglycerols and Natural Fats. In *Advances in Applied Lipid Research*, JAI Press Inc., Amsterdam, The Netherlands, 1996; Vol. 2, pp 213−268.

Received for review November 29, 2007. Revised manuscript received March 26, 2008. Accepted April 1, 2008. We are grateful to the French Embassy in Tunisia for supporting the work of W. Ben Amara-Dali.

JF073491G