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The Viscoelastic Properties of Processed Cheeses Depend on Their Thermal History and Fat Polymorphism[‡]

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ABSTRACT: Both the composition and the thermal kinetics that are applied to processed cheeses can affect their texture. This study investigated the effect of the storage conditions and thermal history on the viscoelastic properties of processed cheese and the physical properties of the fat phase. The microstructure of processed cheese has been characterized. Using a combination of physical techniques such as rheometry, differential scanning calorimetry, and X-ray diffraction, the partial crystallization of fat and the polymorphism of triacylglycerols (TG; main constituents of milk fat) were related to changes in the elastic modulus and tan δ as a function of temperature. In the small emulsion droplets (<1 μm) dispersed in processed cheeses, the solid fat phase was studied at a molecular level and showed differences as a function of the thermal history. Storage of processed cheese at 4 °C and its equilibration at 25 °C lead to partial crystallization of the fat phase, with the formation of a β' 2 L (40.9 Å) structure; on cooling at 2 °C min^{-1}, the formation of an α 3 L (65.8 Å) structure was characterized. The cooling of processed cheese from 60 to −10 °C leads to the formation of a single type of crystal: α 3 L (72 Å). Structural reorganizations of the solid fat phase characterized on heating allowed the interpretation of the elastic modulus evolution of processed cheese. This study evidenced polymorphism of TG in a complex food product such as processed cheese and allowed a better understanding of the viscoelastic properties as a function of the thermal history.

KEYWORDS: processed cheese, triacylglycerols, rheology, differential scanning calorimetry, X-ray diffraction

INTRODUCTION

Although the texture is of primary importance with regard to the quality of processed cheeses, it is not yet fully controlled by the dairy industry. The textural and sensorial properties of processed cheeses depend on their chemical composition, microstructure, and the properties of their main constituents, that is, proteins and fat. Particularly, the physical properties of the fat phase, which are highly affected by the thermal kinetics, may play a key role in the textural properties of processed cheese. However, to date, few studies have focused on these aspects.

The microstructure of spreadable processed cheeses can be characterized thanks to the use of a confocal laser scanning microscopy (CLSM) technique, which was recently optimized to characterize selectively fat and proteins in processed cheeses.[1−3] From a structural point of view, processed cheeses can be considered as oil-in-water emulsions in a gel state in which fat is dispersed in an aqueous continuous phase.

Processed cheese spread is a viscoelastic material, the rheological properties of which are affected by its composition, especially by fat[2,4] and protein contents.[5] Moreover, the textural and functional properties of processed cheeses are widely influenced by the temperature and by the thermal kinetics that are imposed on them.[6−9] Processed cheeses are subjected to various changes in temperature from their manufacture (melting process then cooling stage of the molten mass) to their transport (at room temperature or at low temperatures) and later to their storage often under isothermal condition at refrigerator temperature. Then, processed cheeses are generally consumed at room temperature, for example, 20−25 °C, or at higher temperatures in hot countries (for example, in South America, Africa, India). This corresponds to a total temperature range from melting temperature (T = 60−140 °C depending on the process) to the storage one ($T \sim$ 4−7 °C) during the shelf life of the product. Even if it is known that variation of the temperature over this wide interval and thus that the thermal history of both the protein and fat phases may influence the physical properties of processed cheeses, the mechanisms are not fully understood. Studies mainly focused on the effect of the cooling rate on the rheological properties of processed cheese.[10] The effect of temperature and/or thermal history on proteins was not directly evidenced in processed cheese, but rather in model casein systems, which may be analogous to processed cheeses.[10] Thus, an increase in the understanding of the influence of thermal history on the properties of the fat phase is expected to be beneficial to improve the functional quality of processed cheeses. This was a strong motivation in the development of the present work.

Milk fat has the capacity to be partially crystallized in a wide range of temperatures (−40 to 40 °C), and thus at the temperatures of processing, storage, and consumption of dairy products. As observed for most of the lipids, each triacylglycerol (TG) of milk fat can exhibit several crystalline forms, the

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occurrence of which strongly depends on its thermal history.[11,12] Fox et al.[13] reported that fat crystallization is an important parameter contributing to cheese structure formation upon cooling as it is for protein−protein interactions and protein−fat interactions. The properties of cheese fat are influenced by its crystallization and melting behavior through TG molecule polymorphism and morphology changes of the fat crystals formed in fat droplets, as well as by the amount of the crystallized fraction (i.e., the solid fat content as a function of temperature). Recently, Lopez et al.[14,15] studied the thermal properties of fat in Emmental cheese, quantified the solid fat content, and identified the crystalline structures formed by TG molecules at a molecular level. To the authors' knowledge, only one similar work was more recently focused on processed cheese.[16]

Few studies considered the effect of thermal history on the viscoelastic properties of oil-in-water emulsions.[17] Phase transitions and polymorphic evolutions of TG were previously characterized by Lopez et al.[11,12] on heating of milk fat precooled at different rates and by Gliguem et al.[16] on heating of processed cheese.

The combination of rheological measurements with other techniques is required to understand the changes in the physical properties of the processed cheeses induced by process conditions or occurring during temperature scans. The identification of temperature-induced transitions due to the polymorphism of TG is possible in complex dairy products thanks to the use of X-ray diffraction (XRD) and differential scanning calorimetry (DSC) techniques.[11,12] DSC was used to characterize the thermal properties of fat in cheeses such as Emmental[14,18] and processed cheese.[16] XRD allowed the characterization of the solid fat phase of food products stored at low temperature.[19] Recently, Lopez et al.[15] identified the crystalline structures formed by TG in Emmental cheeses stored at 4 °C. The coupling of XRD recorded as a function of temperature (XRDT) with DSC was used to characterize the structural and thermal behaviors of anhydrous milk fat,[12,20] milk fat dispersed in emulsions,[11] and, more recently, the dispersed fat in processed cheese.[16]

The aim of this work was to investigate the effect of the thermal history of processed cheeses on their viscoelastic properties and on the physical properties of the fat phase, that is, the organization of TG in the solid fat phase and the thermal behavior of fat.

■ MATERIALS AND METHODS

Samples. Processed cheeses were provided by a French dairy company (Fromageries Bel, Vendôme, France). All samples originated from the same batch of production to avoid any differences in their chemical composition and in their microstructure. The samples were stored at 4 °C for 7 days prior to analysis, and then two protocols were used. For the low-temperature protocol, the processed cheese samples were equilibrated at 25 °C for one night before their characterization. For the high-temperature protocol, the samples were heated to and maintained at 60 or 70 °C for a few minutes to erase their thermal history through melting of all previously existing fat crystalline nuclei.

Physicochemical Analysis. Processed cheese was analyzed for pH, dry matter, fat content, total nitrogen as protein content, and lactose and ash contents, as already reported in Gliguem et al.[16] All analyses were performed in triplicate from three independent samples.

The industrially processed cheeses investigated in this study were characterized by a dry matter (DM) content of 43.30 ± 0.15% (w/w) and a fat in dry matter (FDM) content of 52.00 ± 0.21% (w/w). This is in accordance with the data found in the literature because for spreadable-type processed cheeses, the DM contents range from 42.7 to 51.8% (w/w) and the FDM contents vary from 49 to 56% (w/w).[21,22] The total protein content was 10.50 ± 0.11% (w/w), and the pH value was 5.52 ± 0.01. Lactose and ash contents were 6.80 ± 0.07% (w/w) and 3.70 ± 0.01% (w/w), respectively. Thus, the chemical composition of the processed cheeses characterized in this study corresponds to a standard type product.

Microstructural Analysis. Processed cheese samples (5 × 5 × 2 mm) were examined using a confocal laser scanning microscope CLSM 510 (Zeiss, Germany), coupled to a LSM 5 Image Brother photo camera (Zeiss, Germany). Cheese sections were cut at 4 °C and placed on a glass microscope slide. They were labeled with a mixture of Nile Red (Fluo Probes, Interchim, France) and Nile Blue (Certistain, Merck, Germany) fluorescent dyes. Nile Red was used to label fat (fat phase was arbitrarily stained in red), whereas Nile Blue was used to label proteins (the protein phase was arbitrarily stained in green). The probe mixture (50% v/v) was prepared with PEG 200 as solvent and added as a liquid (30 μL) onto the upper surface of the cheese samples. After 10 min in the dark at 4 °C, a coverslip was added and labeled cheese was imaged. Fat and protein were imaged simultaneously at 543 and 633 nm laser excitation, respectively. The CLSM observations were performed using a Plan Apochromat X63 immersion oil objective with a numerical aperture of 1.4.

Rheological Dynamic Measurements. Rheological characterization of processed cheese was undertaken as described by Gliguem et al.[16] In this study, specimens of 40 mm in diameter and 12 mm in height were cut at 4 °C by using a cylindrical tube and placed manually onto the lower plate surface of the rheometer. Independent samples of processed cheese were cooled to 3 °C after their conditioning according to the low-temperature and high-temperature protocols (see below). Before each temperature scan, samples were equilibrated at 25 or 70 °C for 2 min between the ridged plates of the rheometer (gap = 2 mm). Measurements were performed at 2 °C min^{-1} in the linear viscoelastic region (determined by preliminary experiments) by applying a constant strain of 0.1% and a constant frequency of 1 rad s^{-1}. The evolution of the storage modulus, G', and the loss modulus, G'', as well as tangent δ ($= G''/G'$), versus temperature was recorded in triplicate from three independent experiments for both cooling and heating cycles.

Thermal Properties. The thermal properties of processed cheese samples were investigated by DSC using a Perkin-Elmer DSC-7 apparatus (St Quentin en Yvelines, France). Lauric acid (99.95% purity, melting point, T_m = 43.7 °C; ΔH_m = 8.53 kcal/mol) was used as a standard to calibrate the apparatus. The samples of cheeses, in the range of 13−20 mg, were first hermetically sealed into 40 μL aluminum pans. An empty pan was used as a reference. Samples were prepared according to the two following protocols. For the low-temperature protocol, the samples were equilibrated at 25 °C for one night and then transferred to the calorimeter 25 °C. The samples were cooled from 25 to −10 °C at the rate of −2 °C min^{-1} and then heated from −10 to 60 °C at 2 C min^{-1}. The samples were also heated from 25 to 60 at 2 °C min^{-1} to evidence the presence of a solid fat phase at 25 °C. For the high-temperature protocol, the samples were heated to 60 °C to melt all existing nuclei, cooled from 60 to −10 °C at −2 °C min^{-1}, and then heated from −10 to 60 at 2 °C min^{-1}. The objective of the second protocol was to characterize the thermal properties of fat dispersed in the processed cheeses, after elimination of their thermal history. DSC measurements were performed in triplicate with independent cheese samples.

XRDT and DSC Measurements. XRD experiments were performed using the high-energy of synchrotron at Elettra (Trieste, Italy), on the small-angle X-ray scattering (SAXS) beamline (8 keV, λ = 1.54 Å). Small-angle (SAXD) and wide-angle (WAXD) XRD patterns were acquired simultaneously with DSC using the Microcalix calorimeter.[23] The cell of the calorimeter was positioned with the sample-containing

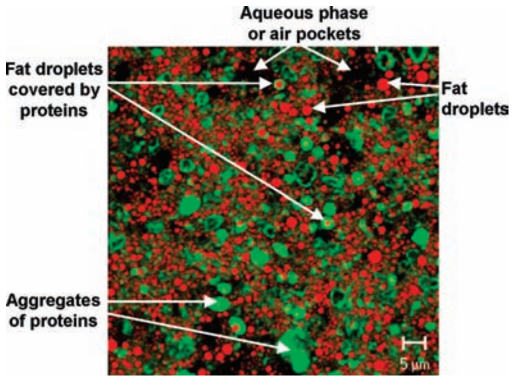

Figure 1. Microstructure of processed cheese: confocal laser scanning micrograph showing the dispersion of fat as small droplets in the protein matrix of processed cheese. The protein network is coded in green, and fat is coded in red.

capillary perpendicular to the X-ray beam in such a way that the diffraction patterns were recorded in the vertical plane including the beam. Two linear detectors were used (see Lopez et al.[22]) to record the XRD patterns by transmission using glass capillaries (diameter = 1.5 mm, wall thickness = 0.01 mm) (GLAS W. Muller, Berlin, Germany). XRD patterns were recorded as a function of time, with a duration of 60 s per XRD pattern. Each cheese sample was fitted into the capillary using a laboratory-made special syringe. Two experiments were conducted to identify the crystalline structures formed by the TG molecules dispersed in fat droplets of processed cheese. After having followed the low-temperature protocol, processed cheese was sampled in the capillaries at 25 °C and further transferred into the calorimeter cell pre-equilibrated at 25 °C. With regard to the high-temperature protocol, the capillaries were filled before heating to 60 °C. In both cases, XRD patterns were recorded during temperature ramps down to −10 °C and then up 60 °C. Analysis of the XRD patterns was performed by using IGOR PRO 4.0 software (Wavemetrics, Portland, OR). For more detailed analysis, each XRD pattern recorded as a function of time was analyzed using PEAKFIT software (Jandel Scientific, Erkrath, Germany). The diffraction peaks were fitted by the sum of Gaussian and Lorentzian functions as previously described by Lopez et al.[24]

RESULTS AND DISCUSSION

Microstructure of Processed Cheese. The microstructure of processed cheese was investigated using CLSM, with the labeling of both fat and protein phases (Figure 1). The fat phase is dispersed in processed cheese as droplets that have a spherical globular structure and diameters ranging from 0.5 to 2.5 μm, with most of the fat droplets having a diameter of <1 μm. Similar observations have been previously reported by Lopez and Briard-Bion.[3] Fat droplets are distributed uniformly in the matrix of the cheese, as individual particles or as aggregates sometimes with a pearl necklace organization. The double labeling of fat and proteins reveals that some fat droplets are covered by proteins, as indicated by arrows in Figure 1. CLSM experiments also show that fat droplets are entrapped in the protein network, which is coded in green in the CLSM micrograph. Green light spots with irregular shapes and dimensions mainly correspond to protein structures with high emission fluorescence.

Physical Properties of Processed Cheese on Cooling. *Solid Fat Phase in Processed Cheese.* The organization of the solid fat phase was investigated in situ in processed cheeses using X-ray diffraction as a function of temperature. The crystalline structures formed by TG molecules were characterized at a molecular level on cooling at −2 °C min^{-1} for the processed cheeses (i) equilibrated at 25 °C after storage at 4 °C (low-temperature protocol) (Figure 2) and (ii) heated at 60 °C to melt the dispersed fat phase (high-temperature protocol) (Figure 3). The crystallization properties of fat have been compared to determine the influence of the processed cheeses thermal history.

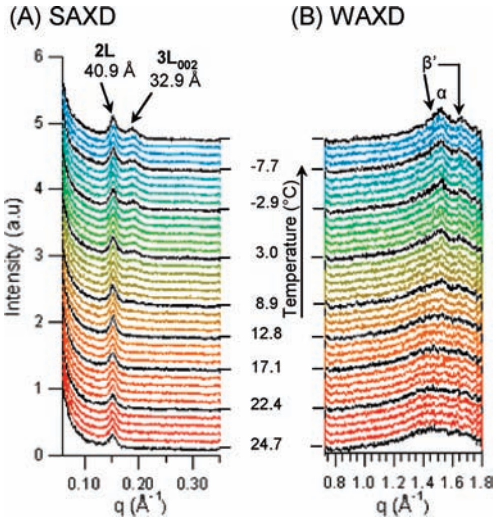

Figure 2. X-ray diffraction patterns recorded as a function of temperature (A) at small angles and (B) at wide angles, on cooling of processed cheese at 2 °C min^{-1} from 25 to −10 °C. The cheeses have been previously stored at 4 °C and equilibrated for one night at 25 °C. The identification of the crystalline structures is indicated in the figure. 2L, double-chain length organization; 3L, triple-chain length organization.

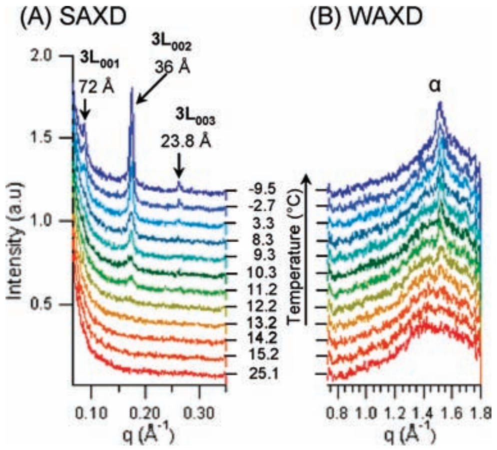

Figure 3. X-ray diffraction patterns recorded as a function of temperature (A) at small angles and (B) at wide angles, on cooling of processed cheese at 2 °C min^{-1} from 60 to −10 °C. The samples have been heated from 4 °C (storage temperature) to 60 °C to melt all existing fat crystals. Only the XRD patterns recorded from 25 °C are presented in the figure for comparison with Figure 2. The identification of the crystalline structures is indicated in the figure. 3L, triple-chain length organization.

Figure 2 shows the XRD patterns recorded simultaneously at small and wide angles on cooling of processed cheese from 25 to −10 °C (Figure 2, panels A and B, respectively). At 25 °C, the diffraction line recorded at q = 0.152 Å^{-1} (repeat distance = 40.9 Å; Figure 2A) was attributed to the crystallization of TG molecules in a double-chain length structure (2L). At wide angles, the presence of two diffraction peaks at q = 1.643 Å^{-1} (3.83 Å) and q = 1.475 Å^{-1} (4.26 Å) corresponds to the β' packing of the fatty acid chains (Figure 2B). The XRD experiments revealed (i) that the storage of processed cheese at 4 °C and then its equilibration at 25 °C lead to the partial crystallization of milk fat and (ii) that the crystalline variety which corresponds to a β' 2L (40.9 Å) structure may be composed of high-melting point TG molecules with long-chain saturated fatty acids. Lopez et al.[15] previously revealed the presence of a solid fat phase in Emmental cheese stored at 4 °C and identified the coexistence of two lamellar structures (2L and 3L) with different polymorphic forms (α, β', and β). On cooling of processed cheese, a broad peak of diffraction centered at q = 0.188 Å^{-1} (32.9 Å) was recorded from about 11.8 °C (Figure 2A). According to the TG composition of milk fat, a thickness of 32.9 Å is too low to correspond to crystallization of milk TG in a 2L structure. Then, this peak is more reasonably related to the second order of a triple-chain length structure ($3L_{002}$ line). The first-order peak of the 3L structure may correspond to 65.8 Å, but the low signal/noise ratio prevented its detection (Figure 2A). At wide angles, the formation of a diffraction line at q = 1.505 Å^{-1} (4.16 Å) was recorded for $T \leq 11.8$ °C. It was attributed to the formation of a hexagonal packing of aliphatic chains (α polymorphic form). Formation of the α 3L (65.8 Å) structure on cooling of processed cheese corresponds to an additional crystallization of TG molecules containing fatty acids with various chain lengths and saturation. The two types of crystals, for example, β' 2L (40.9 Å) and α 3L (65.8 Å), coexist until the end of the cooling process at −10 °C. From the characteristics of the polymorphs, we can observe that the crystals initially formed in the processed cheeses after storage at low temperature correspond to metastable structures (β' form), whereas the crystals formed on subsequent cooling correspond to unstable structures (α form) that may evolve as a function of time.

Figure 3 shows some XRD patterns recorded on cooling of processed cheeses from 60 to −10 °C. On cooling from 60 °C (data are not reported on the figure), no diffraction line was observed at small angles for $T \geq 25$ °C. An X-ray bump centered at q = 1.40 Å^{-1} (4.5 Å) associated with the lateral packing of the fatty acid chains in their liquid state[25] was characterized at wide angles. The XRD experiments show (i) that the fat phase is liquid at 25 °C on cooling of processed cheese from 60 °C and (ii) that the thermal history of processed cheese affects the physical properties of the fat phase because the presence of a β' 2L (40.9 Å) structure has been revealed at 25 °C after storage at low temperature. On cooling of processed cheese from 60 °C, the formation of a solid fat phase was observed for $T < 13$ °C. This solid fat phase corresponded to a single α 3L (72 Å) structure, characterized by 3 orders of diffraction at small angles ($3L_{001}$, 72 Å; $3L_{002}$, 36 Å; and $3L_{003}$, 23.8 Å, where the indices stand for the h,k,l values; Figure 3A) and a diffraction peak at wide angles with q = 1.514 Å^{-1} (4.15 Å) (Figure 3B). These results are in accordance with those previously reported in Gliguem et al.[16] Crystallization of TG molecules was observed through the liquid → α 3L (72 Å) phase transition characterized within the submicronic size of fat droplets dispersed in processed cheese (Figure 1). Such a result is in accordance with the work of Lopez et al.[11] Indeed, Lopez et al.[11] reported that crystallization in milk fat emulsions with fat droplets ranging from 0.38 to 1.25 μm occurred only in the 3L α (72 Å) structure on cooling at −1 °C min^{-1} from 60 to −7 °C. Lopez et al.[11] also reported that the crystallization of TG in natural milk fat globules (diameter ~ 4 μm) upon cooling at $1 < |dT/dt| < 3$ °C min^{-1} starts at about 18 °C with the formation of a 2L α (47 Å) and a 2L α (42 Å) structure, whereas a 3L α (71.2 Å) structure is formed at about 15 °C. Our experiments show that crystallization of milk fat in processed cheeses is similar to crystallization in small-sized fat droplet emulsions.

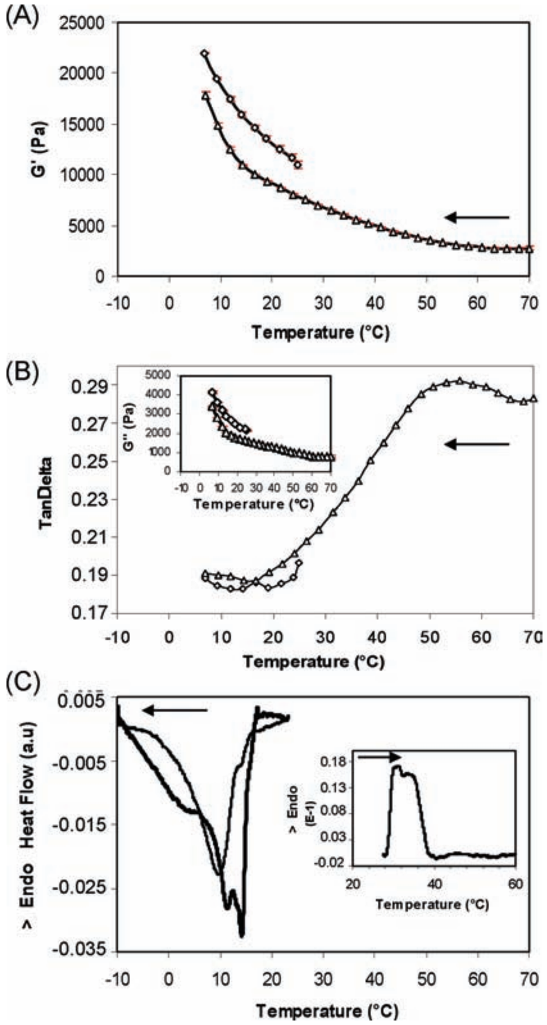

Figure 4. Viscoelastic and thermal properties recorded on cooling at 2 °C min^{-1} of processed cheese samples stored either at low temperature or heated at 70 °C. (A) G' and (B) tan δ and G'' (inset) evolution versus temperature was recorded during cooling of processed cheeses from 25 to 3 °C (diamonds) and from 70 to 3 °C (triangles) as indicated in the figure. Standard deviation is indicated for each point. (C) Differential scanning calorimetry curves were recorded on cooling of processed cheese at 2 °C min^{-1}, from 60 to −10 °C (thick line) (data are not shown from 60 to 25 °C) and from 25 to −10 °C (thin line). (Inset) Heating of processed cheeses from 25 to 60 °C.

From a structural point of view, it is interesting to note that the 3L structures identified in the processed cheeses had different characteristics as a function of the thermal treatment applied to processed cheeses. After equilibration at 25 °C, the thickness of the 3L structure was 65.8 Å, and it was characterized by broad XRD peaks. On cooling from 60 °C, the 3L structure had a thickness of 72 Å and was characterized by sharp diffraction lines. As this structure corresponds to a hexagonal packing, the fatty acid chains are expected to be perpendicular to the plane formed by the hydrophilic glycerols (tilt angle = 0°). The 6.2 Å difference in the thicknesses of the two 3L structures was interpreted as being due to differences in the TG composition of the crystalline structures as a function of the thermal treatment applied to processed cheeses.

Viscoelastic Properties of Processed Cheese. The role played by the thermal history of processed cheeses on their rheological properties was determined by the investigation of the viscoelastic properties. After storage at 4 °C, the processed cheeses were (i) equilibrated at 25 °C for one night (low-temperature protocol) or (ii) heated to 70 °C (high-temperature protocol). The viscoelastic properties of the cheeses were afterward determined as a function of temperature on cooling at 2 °C min^{-1}. Panels A and B of Figure 4 show, respectively, the evolutions of the storage modulus (G') and the tan δ including loss modulus (G'') on cooling (i) from 25 to 3 °C and (ii) from 70 to 3 °C. Whatever the initial temperature of cooling, G' and G'' moduli increased with decreasing temperature with a predominantly elastic behavior (e.g., $G' > G''$) over the whole range of temperatures (Figure 4A,B(inset)). For T = 25 °C, the G' value is higher for the cooling process starting from 25 °C (G' = 10970 ± 300 Pa, low-temperature protocol) compared to the G' value recorded for the cooling process starting from 70 °C (G' = 7799 ± 9 Pa, high-temperature protocol). The differences in G' values that have been recorded at 25 °C show that they depend on the thermal history of processed cheese (Figure 4A). This difference in the magnitude of the elastic modulus of about 25% was maintained from 25 °C to the end of the cooling process at 3 °C (Figure 4A).

Thermal Properties of Fat in Processed Cheese. The thermal properties of fat were investigated by DSC on cooling of processed cheeses at 2 °C min^{-1} (i) from 25 to −10 °C (low-temperature protocol) or (ii) from 60 to −10 °C (high-temperature protocol) (Figure 4C). On cooling from 60 °C, processed cheese presents an initial temperature of crystallization of TG of 15.5 ± 0.3 °C (Figure 4C, thick line curve). The DSC signal recorded from 60 to 25 °C was not reported because no thermal event was recorded, meaning that the TG remain in the liquid state for T > 25 °C. Three exothermic events were successively recorded on cooling of processed cheese: two overlapped sharp peaks (with maximum heat flow near 13 and 10 °C) and then a broad event with a maximum centered toward 4 or 5 °C. On cooling from 25 °C, two exothermic events have been recorded (Figure 4C, thin line curve). A small event (shoulder) is first recorded with an initial temperature of crystallization of 16.1 ± 0.3 °C. Then follows the major exothermic event with an onset at 13.8 ± 0.2 °C. The exothermic peaks recorded on cooling (Figure 4C) were related only to the crystallization of TG molecules in fat droplets because the other constituents of processed cheeses such as proteins do not exhibit thermal transitions in the range of temperatures investigated.[26] The thermal properties of the processed cheese equilibrated at low temperature (4 then 25 °C) were investigated on heating from 25 to 60 at 2 °C min^{-1} (Figure 4C(inset)). The two overlapped endotherms recorded on heating correspond to the melting of fat in processed cheese. The first one spans from about 25 to 32 °C and the second endotherm from about 32 °C to the final melting temperature of TG in processed cheese recorded presently at 38.6 ± 0.1 °C. The endothermic events recorded upon heating of processed cheese confirm the presence of the solid fat phase in the processed cheese stored at low temperature, which has been revealed by the XRD experiments (Figure 2). These results are in accordance with the work previously performed by Lopez et al.,[15,18] who reported that fat is partially crystallized at the usual temperature of storage (4 °C).

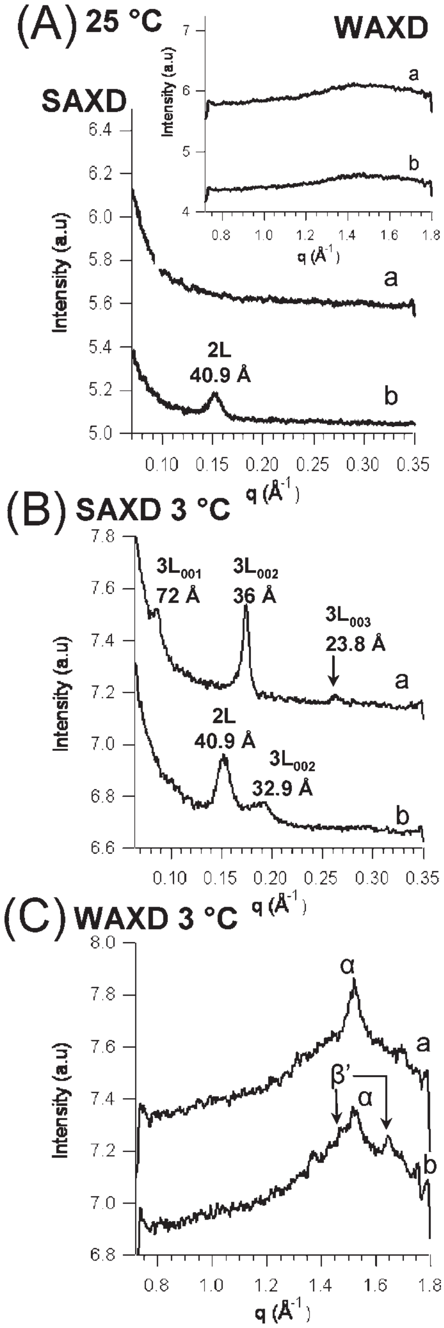

Figure 5. Crystalline structures formed by triacylglycerols in fat droplets dispersed in processed cheese on cooling at −2 °C min^{-1}. X-ray diffraction patterns (A) were recorded at 25 °C at small and wide (inset) angles during heating of processed cheese at 60 °C (a) or storage at low temperature (b). Small-angle XRD (B) and wide-angle XRD (C) patterns were recorded at 3 °C after cooling of processed cheese at −2 °C min^{-1} (a) from 60 to −10 °C and (b) from 25 to −10 °C. The identification of the crystalline structures is indicated in the figure.

Relation between the Crystallization Properties of Fat and the Viscoelastic Properties of Processed Cheese. Figure 5 shows the differences in the molecular organization of TG molecules characterized in processed cheese at 25 and at 3 °C as a function of its thermal history (XRD patterns extracted from Figures 2 and 3).

Such data contribute to a better understanding of the information obtained with viscoelastic experiments (Figure 4). The larger G' and G'' values recorded at 25 °C for the processed cheese maintained at low temperature (Figure 4) can be attributed (i) to the presence of a solid fat phase organized in the 2L β' (40.9 Å) form (Figure 5A) in comparison with the processed cheese cooled from 70 °C in which the fat phase is liquid at 25 °C (Figures 3 and 4C) and (ii) to the rigidity of the protein network as a result of the thermal treatments applied to processed cheese. It is possible that the number of bonds or their strength might be higher within the protein network in the cheese sample stored and equilibrated at low temperature compared to the one cooled from 70 °C. Storage at low temperature leads to the formation of stronger hydrogen bonding that may be favorable for the formation of a firmer product (larger dynamic moduli values). In terms of the temperature effect on the viscoelasticity of emulsion gels containing proteins, the results in the literature are focused on acid casein gels. For instance, it has been reported that acid casein gels prepared at low temperatures (20−25 °C) have much higher moduli compared with those prepared at high temperature (40−45 °C).[17,27]

On cooling from 25 °C, the increase in G' modulus recorded for the cheese stored at low temperature can be related not only to the formation of the β' 3L (65.8 Å) structure, and its coexistence with the β' 2L (40.9 Å) structure (Figure 2), but also to the protein network rigidity. In the processed cheese heated to 70 °C, the partial crystallization of the fat phase, which has been recorded from about 15.5 °C (Figure 4C) and which corresponds to the formation of the α 3L (72 Å) structure identified by XRD (Figure 3), may be responsible, at least partially, for the G' modulus increase that was characterized by the rheological experiments from about 14 °C (Figure 4A,B). The slight difference ($\Delta T \sim 2$ °C) observed between rheological measurements and thermal analysis may be due to the fact that a sufficiently large number of fat crystals must be present to modify the rheological properties of the processed cheese. One should also take into account that the rheological measurements were performed under shear, whereas static conditions were used for the DSC recordings. Because the fat phase remains in the liquid state, the variations of G' and G'' moduli observed between 25 and 14 °C can be attributed to the protein fraction and the gradual reinforcement of its network. At 3 °C, the differences in the G' and G'' values may result from differences (i) in the solid fat content, (ii) in the crystalline structures formed (β' 2L (40.9 Å) + β' 3L (65.8 Å) vs α 3L (72 Å), or (iii) the characteristics of the protein network.

The increase in the G' modulus, which was characterized for both cooling scans, is related to a more solid-like behavior, referring to a strengthening of the physical interactions especially at low temperatures, from about 20 °C, as evidenced by the lowest values of tangent δ (Figure 4B). However, below this temperature, there is no change in the microstructure organization of the cheese network because the evolution of tangent δ is not temperature dependent in the 20−3 °C domain. Besides, from 25 to 3 °C, the structuring scale of both processed cheese samples seems to present similarity and to be independent of the thermal history of the cheeses.

Physical Properties of Processed Cheese upon Heating. *Melting Properties and Polymorphism of Fat in Processed Cheeses as a Function of Their Thermal History.* The melting properties of fat in processed cheese were investigated on heating from −10 to 60 at 2 °C min^{-1} using the coupling of XRD as a function of temperature and DSC. Figure 6 shows the melting properties of fat in the processed cheese stored at low temperature. The two diffraction lines recorded at small angles, which correspond to

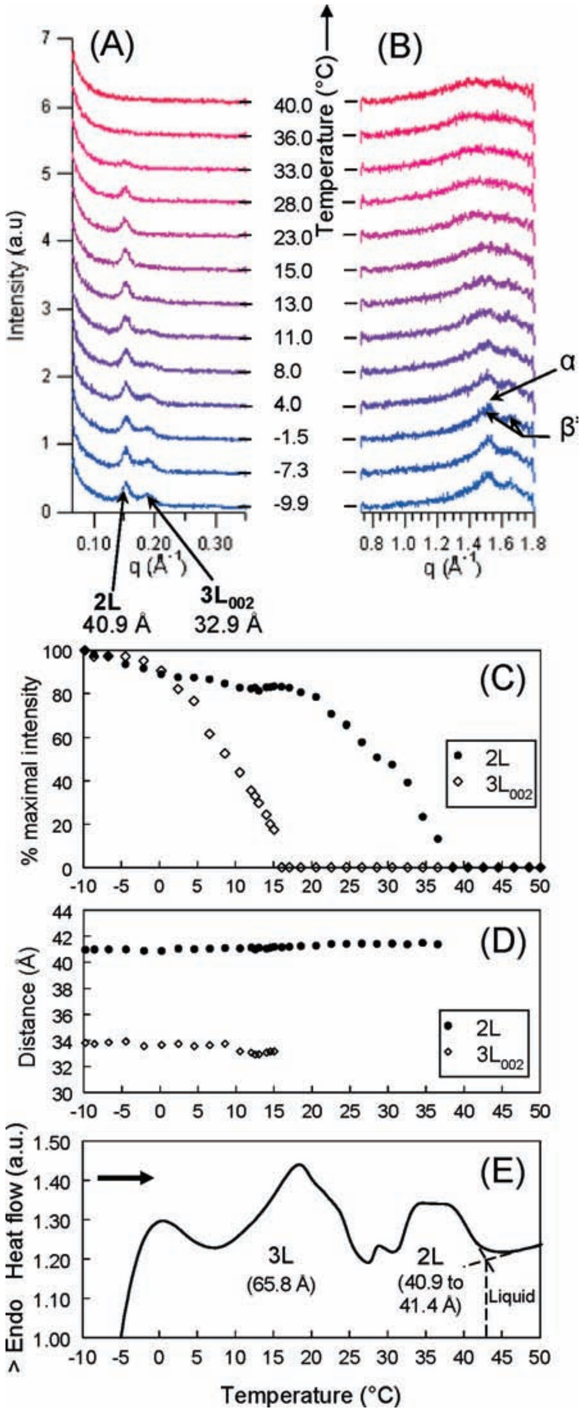

Figure 6. Physical properties of processed cheese on heating. Plots of (A) small- and (B) wide-angle X-ray diffraction (XRD) patterns were recorded during the heating at 2 °C min^{-1} from −10 to 60 °C of processed cheese stored at low temperature and cooled from 25 to −10 °C. (C) Evolution of maximal intensity of the XRD peaks was recorded at small angles as a function of temperature. (D) Evolution of the thicknesses of the lamellar structures is characterized by small-angle XRD (panel A). (E) DSC curve was recorded simultaneously with XRD experiments. Dashed lines present the construction used to determine the final melting temperature of TG.

the β' 2L (40.9 Å) structure and the second-order diffraction line of the α 3L (65.8 Å) structure, coexist in the $-10 \leq T < 15.5$ °C domain (Figure 6A). For $T \geq 15$ °C, only the 2L (40.9 Å) structure is observed at small angles and only peaks of the β' polymorphic form (3.83 and 4.26 Å) are present at wide angles (Figure 6B). For $T > 38$ °C, the absence of a diffraction line means that the fat is in its liquid state above this temperature. The evolution of the maximal intensity of the XRD lines recorded at small angles has been expressed as a fraction of the maximal intensity of the same diffraction line (Figure 6C). The decrease in intensity of the peaks corresponds to the melting of the α 3L (65.8 Å) structure, followed by that of the β' 2L (40.9 Å) one. The successive melting of these crystals is related to the two main endothermic events recorded simultaneously by DSC (Figure 6E). For $T < 5$ °C, the DSC signal was attributed to the equilibration of the calorimeter (Figure 6E). Figure 6D shows that the thickness of the α 3L (65.8 Å) structure did not change as a function of temperature, whereas the thickness of the β' 2L (40.9 Å) structure slightly increased to 41.4 Å on heating of processed cheese. Due to polydispersity in the TG molecules, β' 2L crystals may include TG composed by fatty acids with different chain lengths and unsaturations. Those crystals including the shortest TG molecules are expected to melt before others. This would lead to an increase of the average repeat distance upon heating as observed. The absence of polymorphic transformation upon heating of processed cheese at 2 °C min^{-1} shows that the β' 2L (40.9−41.4 Å) crystalline structure, which has been formed during storage of the cheese at low temperature, is a metastable polymorphic variety and that the cooling rate (−2 °C min^{-1}) is adequate to obtain a stable species even with the temporary simultaneous presence of unstable ones (i.e., the α form).

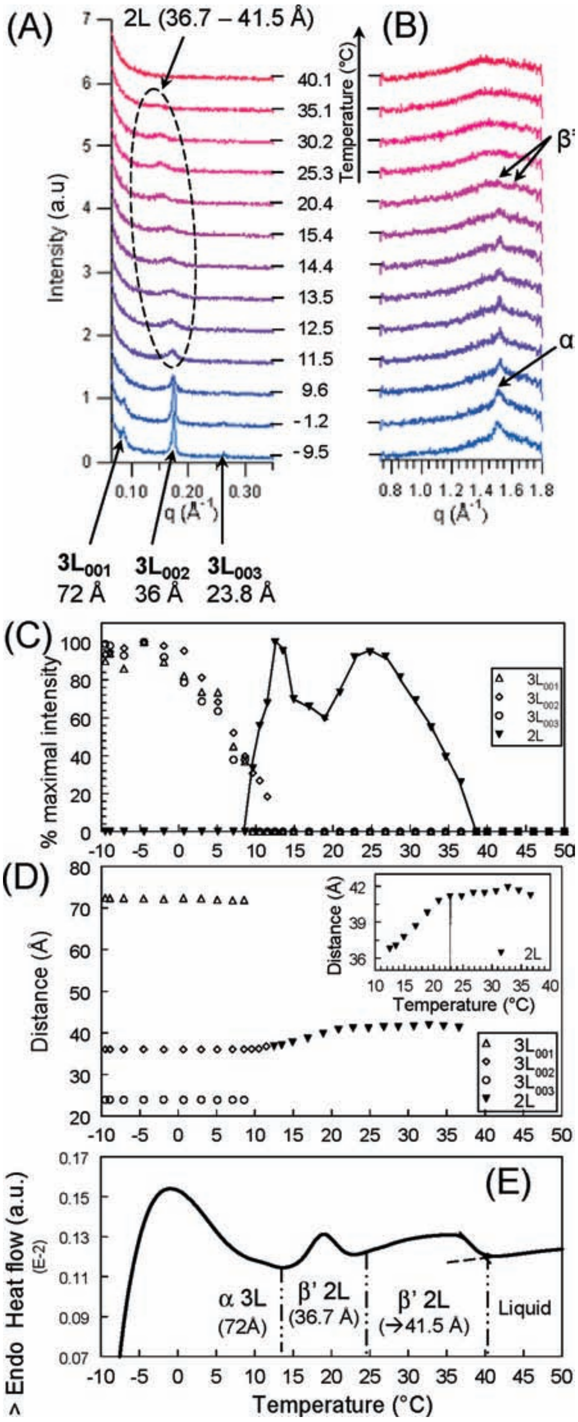

Figure 7. Physical properties of processed cheese on heating. Plots of (A) small- and (B) wide-angle X-ray diffraction (XRD) patterns were recorded during the heating at 2 °C min^{-1} from −10 to 60 °C of processed cheese previously heated to 60 °C and cooled to −10 °C. (C) Evolution of maximal intensity of the XRD peaks was recorded at small angles as a function of temperature. (D) Evolution of the thicknesses of the lamellar structures is characterized by small-angle XRD (panel A). (E) DSC curves recorded simultaneously with XRD experiments.

Figure 7 shows the melting properties of fat in the processed cheese that has been previously heated to 60 °C (high-temperature protocol; Figure 3). The plots of the XRDT patterns recorded at small angles (Figure 7A) could be divided into four temperature-delimited domains. In the first one, from −10 to 8 °C, the three diffraction peaks associated with the 3L (72 Å) structure previously formed on cooling (Figure 3A) simultaneously decrease in intensity (Figure 7C). At wide angles, the line characteristic of the α form vanishes (Figure 7B). These structural changes were related to the melting of the α 3L (72 Å) structure on heating of processed cheese. Then, in the $8 \leq T \leq 4$ °C domain, a complex phase transition is observed (Figure 7A). The broadening of the peak at 36 Å is attributed to the formation of a new double-chain length structure: 2L (36.7 Å). It is related to the recording at wide angles of two diffraction peaks at about 3.9 and 4.2 Å characteristic of a β' crystalline form (Figure 7B). The melting of this 3L (72 Å) structure occurs to the benefit of a more stable polymorphic form, with an $\alpha \rightarrow \beta'$ transition of monotropic type. Then, structural changes in the β' 2L structure are observed at small angles up to 22 °C (Figure 7A). A continuous increase of the repeat distance was observed from 36.7 Å ($T = 14$ °C) to 40.6 Å ($T = 22$ °C) (Figure 7D). Above this temperature and until the final melting of TG performed at an offset temperature of about 38 °C (Figure 7E), the period of the 2L structure remained nearly constant (with small variations from 40.6 to 41.5 Å). At wide angles, beyond 22 °C, the characteristic lines of the β' form are not clearly observed and seem to have disappeared (Figure 7B). Both the low amount of solid fat phase on processed cheeses and the low signal/noise ratio prevented the observation of diffraction lines at wide angles for $T > 20$ °C. The changes in the intensity of the β' 2L structure, which have been characterized as a function of temperature, are presented Figure 7C. From the offset temperature of 38 °C and up to the end of the heating ramp, no diffraction lines are observed as all TG remain in the liquid state. The following

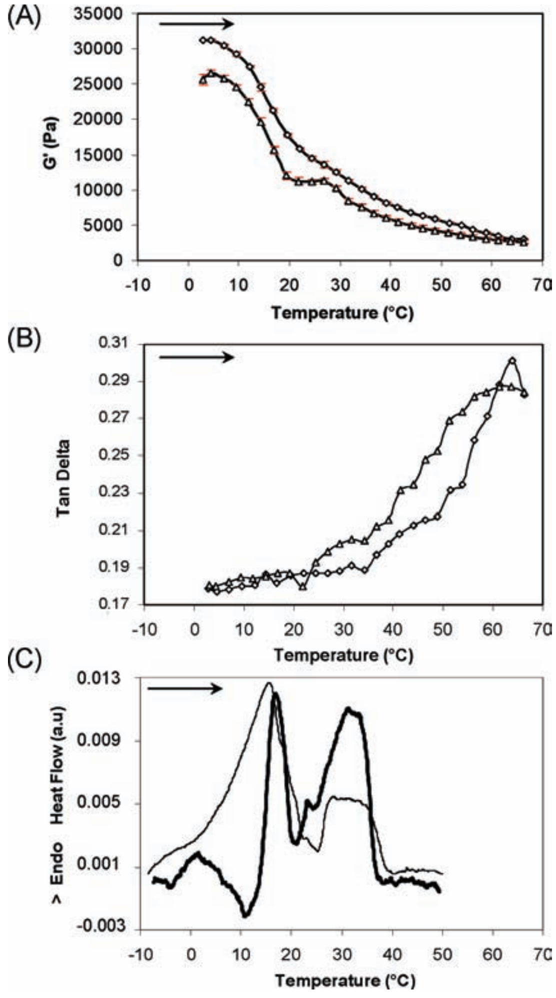

Figure 8. Changes in the viscoelastic and thermal properties of processed cheese on heating at 2 °C min^{-1}: (A) G' and (B) tan δ versus temperature from 3 to 70 °C for samples stored at low temperature and cooled from 25 to 3 °C (LT; diamonds) and for samples heated to 70 °C and cooled at 3 °C (HT; triangles); (C) DSC curves recorded during heating, from −10 to 60 °C (data not shown from 60 to 50 °C) of the samples (HT, thick line; LT, thin line) following ramps down to −10 °C.

transitions were observed on heating of processed cheese: α 3L (72 Å) → β′ 2L (36.7 − 41.5 Å) → liquid. The structural information obtained using XRDT has been related to the thermal data recorded simultaneously using DSC (Figure 7E).

Viscoelastic Properties of Processed Cheese on Heating. Panels A and B of Figure 8 show the changes in the rheological parameters recorded during the heating of processed cheese samples from 3 to 70 at 2 °C min^{-1}. For both types of samples, the values of G' measured at 3 °C (Figure 8A) were higher than those recorded at the end of the cooling process (Figure 4). This was interpreted as the formation of additive links that may have been formed in the cheeses (the cheese samples stayed about 2 min at 3 °C between the cooling and the heating scans). Figure 8A shows that G' moduli exhibit an evolution in several steps versus temperature and decreased differently according to the thermal treatment applied to processed cheese. Similar trends were observed for G'' evolution (results not shown). Considering the processed cheese stored at low temperature and further cooled from 25 to 3 °C, the G' evolution upon heating includes four temperature-delimited domains: (i) a pseudoplateau of G' from 3 to about 10 °C, (ii) a large decrease between 12 and 21 °C, (iii) a shoulder from 21 to about 38 °C, and then (iv) a much slower, continuous, and progressive decrease in the domain $38 \leq T < 70$ °C. Focusing on the G' evolution of processed cheese heated to 70 °C and cooled from 70 to 3 °C, five major domains can be defined. The evolution of G' in the two first domains of temperature (i.e., 3−12 and 12−21 °C) was similar to that described for the other type of sample. The major difference is the lower G' values measured at 3 °C: $G' = 31180 \pm 12$ Pa for the sample precooled from 25 to 3 °C and $G' = 25680 \pm 727$ Pa for the sample precooled from 70 to 3 °C. This relative difference in the magnitude of the G' moduli at 3 °C corresponds to about 20% (interestingly very close to the difference of 25% between G' moduli upon cooling in the temperature domain 25 → 3 °C). This difference was maintained between the two heating curves from 3 to 21 °C (Figure 8A). Then, the third domain in the evolution of G' spans from 21 to 25 °C, where G' evolution can be assimilated to a plateau. The fourth domain, from 25 to 38 °C, shows a fast decrease in the G' modulus. In the fifth domain, from 38 to 70 °C, a much weaker, continuous, and progressive decrease of G' was observed as was the case upon cooling.

Figure 8B shows the temperature dependence of tangent δ upon heating. As observed upon cooling (Figure 4B), tangent δ evolution is limited to the interval 0.18−0.30 and possesses a sigmoid shape from a low value toward larger ones. This is indicative of the evolution from a stiff structure to a softer one. Tangent δ is not temperature-dependent at low temperatures for both processed cheese samples. However, above 20 °C, differences in the temperature-dependent behavior of the G''/G' ratio are noted. These variations can be due (i) to the TG phase transitions, which occur until about 38 °C, and (ii) to the proteins. Comparison of the curves obtained upon cooling or heating shows a small hysteresis of <10 °C. It is consistent with a reinforcement of the network after a journey of the cheese at low temperatures, favorable to the establishment of intra- and intermolecular bonds that give stiffness to the structure.

Thermal Properties of Fat on Heating. The DSC experiments performed under similar experimental conditions as for rheological measurements and XRD experiments are reported Figure 8C. The DSC melting curves recorded during heating of processed cheese samples at 2 °C min^{-1} from −10 to 50 °C show different shapes. The melting curve of the processed cheese stored at low temperature and cooled from 25 to −10 °C shows two well-separated endothermic peaks (Figure 8C, thin line). The first one spans from −10 to about 23 °C with a maximum at 16 °C, and the second peak spans from about 25.4 ± 0.3 °C to the final melting temperature of fat in processed cheese at 38.8 ± 0.2 °C. The DSC melting curve of the processed cheese heated to 60 °C and cooled from 60 to −10 °C shows at least four endotherms and an exotherm. The first endotherm spans from −4.1 ± 0.1 to 6.1 ± 0.2 °C. The exothermic event occurs between 6.1 and 12.4 ± 0.4 °C. The second sharp endothermic peak is observed between 12.4 and 21 ± 0.1 °C. The third, small, one spans between 21 and 25 ± 0.2 °C. The fourth, broad, endotherm spans from 25 °C until the final melting of TG at 38.1 ± 0.4 °C. This latter temperature is slightly higher in the processed cheese stored at low temperature (about 0.7 °C), which corresponds to the formation of more stable polymorphic forms. The comparison performed between the DSC melting curves recorded in the same range of temperature (from −10 to

60 °C) and at the same heating rate (2 °C min^{-1}) revealed differences attributed to the thermal history of the cheeses.

Relationship between the Melting Behavior of Fat and the Viscoelastic Properties of Processed Cheese. The combination of the rheological data, thermal information, and analysis of the melting properties of the solid fat phase permitted increased understanding of the physical properties of processed cheeses, as a function of the thermal treatment applied. The differences, which have been characterized in magnitude of G' moduli between the two processed cheese samples at 3 °C (Figure 8A), were at least partially attributed to the fat fraction. Differences in the types of fat crystals were characterized using XRD (Figure 5). Unfortunately, the amount of fat that was crystallized at 3 °C within fat droplets dispersed in the processed cheeses investigated in this study was not quantified. Considering the heating curve of the processed cheese stored at low temperature and cooled from 25 to 3 °C, the decrease of G' modulus (Figure 8A) observed between 3 and 10−12 °C coincides with the first endotherm recorded by DSC (Figures 6E and 8C), that is, to the melting of the α 3L (65.8 Å) structure (Figure 6C). The subsequent decrease of G' between 13 and 21−25 °C is related to the second endotherm associated with the melting of the β' 2L (40.9−41.4 Å) structure (Figures 8C and 6C,E). Heating of the processed cheese heated to 70 °C and cooled from 70 to 3 °C leads to variations of G' modulus (Figure 8A) for $3 < T < 20$ °C related to the melting of the α 3L (72 Å) crystals and to the formation of the 2L structure with an α to β' polymorphic transition occurring within the fat droplets dispersed in the processed cheese. Above 21 °C and up to 25 °C, the plateau of G' evolution is related to the growth of the 2L structure (Figure 7C), the repeat distance of which increases as a function of temperature (Figure 7D). For $25 < T < 38$ °C, the decrease in G' value is at least partly attributed to the melting of the 2L structure. The rheological properties of processed cheeses characterized as a function of temperature also depend on the protein phase known to have an effect on the rheological properties, even in a restricted temperature domain (as previously discussed; Figure 8B). The global decrease of G' modulus upon heating indicates a very strong weakening of the cheese structure. Indeed, the change in G' under the investigated temperature range is about 90% (considering the mean values of G' for both heating curves, from 3 to 70 °C). The thermal history may affect the nature of the protein network (i.e., the nature of the interactions) and alters the strength of the physical interactions between the structural components in processed cheese.

Correlations between the behavior of the rheological parameters and the structural ones of TG evidence the influence of fat structure onto processed cheese properties, although it is present as droplets dispersed in the aqueous protein matrix.

According to our results, the viscoelasticity of processed cheeses is strongly dependent on the temperature and thermal history of the product. On the basis of our results and our preceding study[16] and those reported by Chen and Dickinson,[17] the origin of temperature irreversibility of the viscoelasticity in the thermal cycle 70 → 3 → 70 °C is presumably not due to the protein fraction, but mainly due to the fat faction and maybe to protein−fat globule surface interactions according to Chen and Dickinson,[17] in relation to the thermal history of the cheese. This temperature dependence of the emulsion rheology is mirrored at least partially by the changes at a molecular and supramolecular level in the organization of TG due to their polymorphism evidenced in fat droplets dispersed in processed cheese.

In conclusion, we showed that the thermal history applied to processed cheeses affects their rheological properties and both the structural and thermal properties of the fat phase. Moreover, using the combination of adequate techniques, we related the crystallization properties and polymorphism of the fat fraction, which is dispersed in small droplets, to changes in the viscoelasctic properties of processed cheese.

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DEDICATION

[‡]This work is dedicated to the memory of Michel Ollivon, who passed away June 16, 2007.

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