Milk Fat Thermal Properties and Solid Fat Content in Emmental Cheese: A Differential Scanning Calorimetry Study

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ABSTRACT

The experiments reported in this study give deeper insight into the crystallization of milk fat in Emmental cheese, which is the most widely consumed hard cheese in France. Differential scanning calorimetry (DSC) was used to monitor the thermal properties of milk fat after the main stages involved during manufacture of Emmental cheese. By heating the samples to 60°C to eliminate their thermal history and cooling them at 2°C/min, the liquid → solid phase transition of fat was investigated. Confocal laser scanning microscopy was used to characterize *in situ* the supramolecular organization of milk fat dispersed in the casein matrix. The destabilization of fat globules by aggregation or coalescence and the formation of free fat during the manufacture altered the thermal properties of milk fat by increasing the initial temperature of crystallization and by the formation of 2 overlapping exotherms. The melting properties of the crystalline structures formed by fat at the temperatures used for ripening (12, 21, and 4°C) were examined. Differential scanning calorimetry was used to determine the ratio of solid to liquid fat; that is, the amount of fat that is crystallized, by dividing the partial enthalpy of melting of the fat for ripening temperature by the total enthalpy of melting of the same fat extracted from cheese. This study shows, for the first time, that milk fat is partially crystallized in Emmental cheese: about $55.7 \pm 3.5\%$ of fat is solid at 4°C at the end of ripening. Polymorphic phase transitions of milk fat are also suggested during ripening of Emmental cheese.

Key words: triacylglycerol, polymorphism, fat crystal, confocal laser scanning microscopy

INTRODUCTION

Milk fat determines the specific properties of fat-rich products, such as butter and cream. In food products, fat may be dispersed as oil in water emulsion (as

in cream, yogurt, and cheese) or may be the continuous phase (as in butter). Moreover, the fat phase may be crystallized at the temperature of storage and consumption, as is the case for dairy products. Thus, examination of the thermal properties of fat in complex food products and determination of the solid fat content are of particular importance in understanding their rheological, sensorial, and nutritional properties.

Milk fat is composed mainly of triacylglycerols (TG), esters of fatty acids and glycerol, which represent 97 to 98% of total fat. More than 400 fatty acids have been identified, varying in chain length and unsaturation (Gresti et al., 1993; Jensen and Newburg, 1995). The extreme diversity of milk fatty acids and TG, each characterized by its own melting point, induces a wide melting range, which spans from about –40 to 40°C. At intermediate temperature, milk fat is partially crystallized and corresponds to a mixture of solid fat (crystals) and liquid fat (oil).

Fat is dispersed in milk in the form of droplets, called the milk fat globules, which have a volume-weighted diameter of approximately 4 μm . A biological membrane composed mainly of phospholipids and proteins organized as a trilayer envelops the milk fat globules (Keenan and Dylewski, 1995). Due to technological processes, the structure of milk fat globules can be greatly modified and fat can be dispersed in dairy products as coalesced fat globules, aggregates of fat globules, tiny homogenized fat globules, or nonglobular fat (Lopez, 2005a).

The thermal properties of milk fat are usually studied by differential scanning calorimetry (DSC). Differential scanning calorimetry is a useful tool for determining the temperature of final melting and initial crystallization of fat, as well as for following polymorphic evolutions. Studies using DSC have given an insight into the thermodynamics of milk fat and milk fat fraction transitions in bulk (Timms, 1980; Lavigne, 1995; Marangoni and Lencki, 1998; ten Grotenhuis et al., 1999) and in emulsions (Walstra and van Beresteijn, 1975). Differential scanning calorimetry studies monitored on milk fat globules showed that the temperature of the beginning of crystallization is delayed as a function of the decrease of their size (Lopez et

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al., 2002a; Michalski et al., 2004b). Timms (1980) and Marangoni and Lencki (1998) studied anhydrous milk fat (AMF) by DSC and observed that it crystallizes and melts in several steps. Researchers agree on the existence of 3 overlapping endotherms recorded on heating corresponding to separate groups of TG that melt separately (Timms, 1980; Marangoni and Lencki, 1998). These 3 groups of milk fat TG are called the low melting point (LMP), medium melting point (MMP), and high melting point (HMP) fractions. Lavigne (1995) identified the main TG of each fraction and related them to the thermal and structural properties of the whole milk fat. The complex DSC recordings result from both the broad distribution of TG composition and the polymorphism of TG (Hagemann, 1988). Triglycerides have the ability to crystallize in different polymorphic forms, of which only one is completely stable in given conditions. This polymorphism being mainly monotropic, forms are metastable and the transitions are irreversible. The different polymorphic forms have different compositions, different crystal lattices, and different melting points, which increase with increasing stability (Small, 1986).

Recently, the use of DSC coupled to synchrotron radiation x-ray diffraction allowed identification of the crystalline structures formed by TG molecules as a function of temperature and time in AMF (Lavigne, 1995; Lopez et al., 2001a,b,c) and in milk fat globules (Lopez et al., 2000, 2001c, 2002a,b, 2005b; Michalski et al., 2004b). Lopez et al. (2001c, 2002a,b) showed that the dispersion state of milk fat; for example, in fat globules or in bulk as AMF, alters both its thermal and structural properties.

Milk fat contributes to the physical properties of dairy products, especially of those with low water content. The functional properties of milk fat are strongly related to its composition and to the amount of fat crystals of various types and sizes that are formed at the temperature of the application. Crystallization of milk fat affects many properties such as 1) its rheological properties, 2) the resistance of fat globules to disruption, and 3) the consistency and mouth feel of high-fat content products. Furthermore, milk fat crystallization may be important for technical applications. Thus, it is of interest to better understand the thermal properties of milk fat in dairy products.

Although DSC is convenient, few studies exist on the thermal properties of fat in complex dairy products, and particularly in cheese. Cheese can be considered as a heterogeneous material, mainly composed of a complex mixture of TG molecules, proteins, and water. Differential scanning calorimetry was used to distinguish natural Mozzarella cheese from imitation Mozzarella made with calcium caseinate (Tunick et

al., 1989) and to distinguish Mozzarella cheeses made from cows' milk and water buffalo milk (Tunick and Malin, 1997). Tunick (1994) examined the effects of homogenization on the melting profiles of cheese fat and free oil in Mozzarella cheese. Results from DSC experiments also showed that the melting profile of milk fat in Mozzarella cheese changes during storage (Rowney et al., 1998). Famelart et al. (2002) and Michalski et al. (2004a) used DSC to investigate the melting behavior of Emmental cheese.

The objective of this study was to investigate the thermal properties of the fat phase dispersed in cheese as a function of time and temperature during both its manufacture and ripening. Among cheeses, Emmental is of particular interest as it is successively ripened at different temperatures; that is, 12, 21, and 4°C. Differential scanning calorimetry was used to characterize in situ the crystallization and melting of TG molecules considering the supramolecular organization of fat during the manufacture of Emmental cheese. The melting properties and polymorphic evolutions of fat were characterized and the solid fat content was determined during the ripening of Emmental cheese.

MATERIALS AND METHODS

Samples

Emmental Cheese Manufacture and Ripening.

Three separate trials of Emmental cheese were manufactured, from separate milks, using a specialized pilot plant based on the research center of INRA, Rennes (Chalon Megard, La Cluse, France).

Day -1. Raw whole milk, purchased from a local dairy plant (Triballat, Noyal-sur-Vilaine, France), was collected the day before Emmental production (d -1). The milk was adjusted to a fat content:total N ratio of 0.86 using skimmed milk, and then stored overnight at 4°C.

Day 0. Standardized milk was pasteurized using an Actijoule pilot pasteurizer (Actini, Evian les Bains, France) at 63°C for 20 s. The cheese milk (850 L) was heated to 31°C in a temperature-controlled milk vat. A lysozyme solution (Delvosyme, Gist Brocades, Seclin, France) was added to milk at a concentration of 0.01 mL/kg. The milk was supplemented with 0.01 mL/kg of a 510 g/L CaCl₂ solution. Milk was inoculated as follows: lactococci (0.4 units/850 kg; EZAL MM100, Rhodia, Dangé-Saint-Romain, France); thermophilic lactobacilli (LH 100, Rhodia) inoculated in the milk at 0.47 mL/kg; thermophilic streptococci (PAL ITG ST 82-87, Standa, France) inoculated in the milk at 1.65 mL/kg, propionic bacteria (0.05 g/kg; PAL ITG P9, Standa). After a 30-min incubation at 31°C, milk pH

Table 1. Fat content and DM during the manufacture of Emmental cheese¹

Sample	Fat (g/kg)	DM (g/kg)
Rennet-induced curd	52.2 ± 0.6	211.5 ± 2.5
Heated curd grains	190.1 ± 0.8	322.0 ± 3.1
Emmental cheese after pressing	268.3 ± 1.4	569.8 ± 2.8
Emmental cheese at 1 d	287.5 ± 1.5	611.3 ± 0.8
Emmental cheese after brining	290.3 ± 1.4	615.6 ± 1.2
Emmental cheese at 52 d	295.0 ± 1.1	627.7 ± 5.7

¹Presented values are means (±SD) of 3 replicate experiments.

was adjusted to 6.62 using CO₂ dissolved directly in milk. Calf rennet was added at 0.25 mL/kg (145, Berthelot, ABIA S.A. Meursault, France). Curd was formed after 22 min and hardened for 4 to 5 min (20% of the clotting time). The curd was cut and curd grains were mixed and heated for 20 min to a temperature of 51°C. Curd was drained off under vacuum (30 kPa) in the racking unit and molded at 47°C in a 780-mm diameter and 27-mm height mold (Doryl, France). The curd was pressed for 4 h at 0.4 kPa (room temperature = 24°C). After pressing, the curd was turned over and acidified for 19 h in a temperature-controlled room at 24°C.

Day +1. The curd was demolded (pH 5.17 ± 0.03), weighed, and placed in a cold brine bath for 48 h (saturated NaCl solution: 350 g/L; 12°C; pH 5.2).

Day +1 to 52. Cheese was ripened in a temperate room for 16 d at 12°C, 85% relative humidity, and then in a warm room for 28 d at 21°C, 80% relative humidity, and finally in a cold room for 7 d at 4°C, for a total ripening time of 52 d. Some Emmental cheeses were kept for 7 d more at 4°C. Three Emmental cheeses of about 80 kg each were manufactured in total.

AMF Extracted from Emmental Cheese. Anhydrous milk fat was extracted from Emmental cheese 1) in the 12°C room, 2) in the 21°C room, 3) in the 4°C room, and 4) at the end of ripening, as detailed below.

Cream and AMF. Creams (fat content ~320 g/kg) were obtained by skimming fresh raw milk collected in Brittany and purchased from a local dairy plant (Triballat, Noyal-sur-Vilaine, France). The creams were collected during the same season and the same year as cheese milk used to manufacture Emmental cheese. Anhydrous milk fat was extracted from creams as detailed below.

Physicochemical Analysis

Determination of Fat Content and DM. Fat content was determined using the SBR (Schmid, Bondzynski, Ratzlaff) method (IDF/FIL, 1986). Dry matter was measured by drying 2 g of cheese mixed with sand at 102°C (±2°C) for 7 h (IDF/FIL, 1987). Table 1 shows

the fat content and DM of the samples characterized after the main stages involved during the manufacture of Emmental cheese. The DM increased due to loss of whey after cutting and heating of curd grains and as a consequence of pressing. The DM slightly increased during the ripening period indicating continuous loss of moisture. The increase in DM is responsible for the increase in fat concentration during the manufacture of Emmental cheese. At the end of ripening, the composition of the cheeses was within specified limits for Emmental (IDF/FIL, 1981).

Fatty Acid Composition During the Manufacture of Emmental Cheese

Extraction of Fat. Samples of cheeses were stored at -80°C until required for analysis and then stored at 4°C during 24h before analysis. The protocol of extraction of fat was adapted from Sehat et al. (1998).

Preparation of Methyl Esters. In a screw-capped tube, a 5-mL fat solution in hexane was prepared at a concentration of 10 mg/mL. Then, 50 µL of sodium methoxide (Sigma-Aldrich, Saint Quentin Fallavier, France), a solution of internal standard of glycerol tripelargonate (Sigma-Aldrich) stored at -20°C under nitrogen, and a trace of anhydrous sodium sulfate were added. The reagents were incubated for 15 min at room temperature, and centrifuged at 1,000 × g at 20°C for 10 min (SV-11-TH model, Firlabo, France). The upper layer was recovered and diluted 10 times in hexane before injection into the gas chromatograph (Ulberth et al., 1999). Fatty acid methyl esters were measured on a Varian gas chromatograph (model 3800, Walnut Creek, CA) equipped with a flame-ionization detector, a programmed temperature injector, and a capillary column (50 m × 0.33 mm; film thickness 0.25 µm) coated with 70% cyanopropyl polysilphenylene-siloxane (BPX-70, SGE, Ringwood, Victoria, Australia). Experimental conditions were as follows: initial temperature of on-column injection (1 µL) was 40°C for 0.2 min; the temperature injector was then programmed to increase to 200°C at a rate of 200°C/min; an isotherm at 200°C for 6 min; and a decrease in temperature to 40°C at a rate of 200°C/min. Detector temperature was 250°C; carrier gas was hydrogen at a pressure of 24.1 kPa. Oven temperature was programmed as follows: 40°C for 10 min followed by an increase to 205°C at a rate of 5°C/min; oven was maintained at 205°C for 20 min.

Microstructural Analysis

Emmental cheese microstructure was examined after the main stages involved during its manufacture

using confocal laser scanning microscopy (CLSM). Thin slices of cheese, measuring approximately $5 \times 5 \times 3$ mm thick, were prepared from the freshly cut samples, using a scalpel. The protein network was stained using acridine orange fluorescent dye (Aldrich Chemical Company, Inc., Milwaukee, WI). A lipid-soluble Nile Red fluorescent dye (Sigma-Aldrich, St Louis, MO) was used to label fat. About 0.5 mL of each staining solution was put on the coverslip. Then, the coverslip was placed on the slice of cheese to permit the diffusion of the stains for 30 min in the dark at 4°C. Microstructural analysis was performed using a confocal Leica TCS NT microscope (Leica Microsystems, Heidelberg, Germany), which used an argon/krypton laser in dual-beam fluorescent mode, with excitation wavelengths of 568 and 488 nm for fat and protein, respectively. The two-dimensional images had a resolution of $1,024 \times 1,024$ pixels, and the pixel scale values were converted into micrometers using a scaling factor. In the double-stained samples, the fat phase was colored red and the protein phase was colored green; the aqueous phase appeared as black areas in the confocal micrographs.

Particle Size Measurements

The fat globule size distributions in Emmental samples and cream were measured by laser light scattering using a Mastersizer 2000 (Malvern Instruments, Malvern, UK), equipped with a He/Ne laser (wavelength = 633 nm) and an electroluminescent diode (wavelength = 466 nm).

To determine the fat globule size in the curd and curd grains, 1 g of the sample was dissociated with 5 mL of dissociation buffer [6 mol/L urea, 100 mmol/L EDTA, 20 mmol/L imidazole buffer, pH 6.6 ($\leq 99\%$, Prolabo, Fontenay sous Bois, France)], and stirred for 30 min at room temperature before measurement (Dalglish, 1984). The fat globule size distribution in cream was measured after dispersion in distilled water.

The refractive index of milk fat was taken to be 1.460 at 466 nm and 1.458 at 633 nm as previously determined in our laboratory (Michalski et al., 2001). All analyses were performed in triplicate. From the size distribution, the average volume-weighted diameter, $d_{43} = \sum n_i d_i^4 / \sum n_i d_i^3$ (where n_i is the number of fat globules in a size class of diameter d_i), was calculated by the instrument software.

Thermal Properties

The thermal properties of the samples were monitored by DSC using a TA Q-1000 calorimeter (TA Instruments, New Castle, DE). Calibration was made

with an indium standard, melting point = 156.66°C, ΔH melting = 28.41 J/g.

Emmental Cheese. About 50 to 70 mg of cheese was accurately weighed in a hermetic stainless steel pan of 100 μ L. An empty pan was used as a reference, and both pans were hermetically sealed. Two protocols were used to study the thermal properties of Emmental cheese. In the first protocol, the structures formed after elimination of the thermal history were characterized. The samples were heated to 60°C to melt all existing nuclei, cooled from 60 to -5°C at $2^\circ\text{C}/\text{min}$, and heated from -5 to 60°C at $2^\circ\text{C}/\text{min}$. In the second protocol, the structures formed at the temperature of ripening were characterized. The samples of cheese were conditioned at the temperature of ripening, $T_{\text{ripening}} = 12, 21, \text{ and } 4^\circ\text{C}$, and then transported in an isothermal box to the calorimeter precooled to T_{ripening} . The samples of cheese were tempered for 5 min at the chosen temperature. Then, the cooling and heating kinetics were monitored as follows: 1) From T_{ripening} (12, 21, 4°C) to 60°C at $2^\circ\text{C}/\text{min}$; 2) From T_{ripening} (12, 21, 4°C) to -5°C at $2^\circ\text{C}/\text{min}$, then from -5 to 60°C at $2^\circ\text{C}/\text{min}$. Measurements were performed in triplicate with independent cheese samples.

Cream. About 5 to 10 mg of cream was weighed in a hermetic aluminium pan of 50 μ L. An empty hermetic aluminium pan was used as reference, and the pans were hermetically sealed. The creams were 1) heated to 60°C , 2) cooled from 60 to -5°C at $2^\circ\text{C}/\text{min}$; and 3) heated from 5 to 60°C at $2^\circ\text{C}/\text{min}$. Measurements were performed in triplicate with independent creams.

AMF. About 5 to 10 mg of AMF extracted from Emmental cheese samples and cream were weighed in a hermetic aluminium pan of 50 μ L. An empty hermetic aluminium pan was used as reference. The pans were hermetically sealed. Two protocols were used as follows. In the first protocol, AMF samples extracted from cream were heated to 60°C to melt all existing nuclei, cooled from 60 to -5°C at $2^\circ\text{C}/\text{min}$, and heated from -5 to 60°C at $2^\circ\text{C}/\text{min}$. In the second protocol, AMF samples extracted from Emmental cheeses were heated to 60°C to melt all nuclei, cooled from 60 to -40°C at $2^\circ\text{C}/\text{min}$, and heated from -40 to 60°C at $2^\circ\text{C}/\text{min}$. Measurements were performed in triplicate with independent AMF samples.

The temperature of the beginning of crystallization (T_{onset} , in $^\circ\text{C}$), of final melting (T_{offset} , in $^\circ\text{C}$), and the enthalpy of melting (ΔH expressed in J/g of sample) were calculated by the software (Advantage Software version 2, TA Instrument). Because the enthalpy of melting of TG is proportional to the amount of fat in the samples, the results were divided by the concentration of fat determined for each sample.

Table 2. Fatty acid composition (% wt/wt) of the samples characterized during the manufacture of Emmental cheese¹

Fatty acids	Curd	Heated curd grains	Cheese after pressing	Cheese at 1 d	Cheese after brining	ANOVA ²
C4:0	3.06 ± 0.02	3.06 ± 0.02	2.98 ± 0.08	3.02 ± 0.09	3.00 ± 0.04	NS
C6:0	1.93 ± 0.05	1.94 ± 0.01	1.98 ± 0.06	1.94 ± 0.02	1.98 ± 0.05	NS
C8:0	1.35 ± 0.01	1.35 ± 0.01	1.35 ± 0.01	1.33 ± 0.04	1.35 ± 0.01	NS
C10:0	3.18 ± 0.01	3.18 ± 0.01	3.19 ± 0.01	3.17 ± 0.05	3.18 ± 0.01	NS
C12:0	3.95 ± 0.09	3.92 ± 0.16	3.95 ± 0.17	3.83 ± 0.10	4.03 ± 0.10	NS
C14:0	11.71 ± 0.08	11.70 ± 0.07	11.69 ± 0.01	11.71 ± 0.08	11.72 ± 0.01	NS
C16:0	31.76 ± 0.02	31.78 ± 0.08	31.78 ± 0.04	31.75 ± 0.22	31.77 ± 0.05	NS
C16:1	1.60 ± 0.01	1.59 ± 0.00	1.59 ± 0.02	1.61 ± 0.02	1.61 ± 0.01	NS
C18:0	11.05 ± 0.12	10.84 ± 0.13	10.83 ± 0.06	10.93 ± 0.20	11.03 ± 0.01	NS
C18:1 <i>cis</i> -9	26.52 ± 0.13	26.48 ± 0.12	26.42 ± 0.07	26.51 ± 0.15	26.47 ± 0.12	NS
C18:3 <i>cis</i> -9,12,15	0.75 ± 0.01	0.74 ± 0.00	0.74 ± 0.01	0.74 ± 0.03	0.74 ± 0.01	NS
C20:0	0.67 ± 0.01	0.68 ± 0.01	0.67 ± 0.01	0.68 ± 0.10	0.68 ± 0.01	NS

¹Presented values are means (± SD) of 3 replicate experiments.

²NS = not significant at $P > 0.05$.

Statistical Analyses

Analyses of variance were performed using the GLM procedure of Statgraphics Plus version 5 (Statistical Graphic Corp., Englewood Cliffs, NJ) to determine 1) the effect of the stage of manufacture of Emmental cheese on the concentration of fatty acids and on the thermal parameters (T_{onset} , T_{offset}), and, 2) the effect of the stage of ripening on the thermal parameters (ΔH , T_{offset}) and on the calculated solid fat content. Differences between the treatment means were compared at the 5% level of significance using the Fisher's least significance difference (LSD) test.

RESULTS

Fatty Acid Composition

The fatty acid composition of the samples analyzed during the manufacture of Emmental cheese is given in Table 2. The results showed that the fatty acid composition did not evolve significantly ($P > 0.05$) during the manufacture of Emmental cheese; that is, from the formation of the curd until after brining.

Thermal Properties and Supramolecular Organization of Fat

Thermal Properties of Emmental Cheese. Figure 1 shows the DSC curves recorded on cooling at 2°C/min of the samples characterized after the main stages involved during the manufacture of Emmental cheese. The samples were first heated to 60°C (above the final melting point of milk fat) to melt all existing nuclei and eliminate their thermal history. The thermal parameters calculated from the DSC curves are presented in Table 3.

The DSC curve recorded during cooling of the rennet-induced curd showed a single broad exothermic peak. Considering heated curd grains, the DSC curve showed the formation of a small exothermic event (a shoulder) before the main exothermic peak. The DSC curve recorded for Emmental cheese after pressing showed 2 exothermic peaks, with an increased enthalpy of crystallization for the first peak, compared with the Emmental cheese before pressing. After 1 d, the DSC curve was similar to the one recorded after pressing. After brining, at least 2 minor exothermic peaks were recorded before the major exothermic peak. The crystallization behavior of Emmental cheese at 52

Figure 1. Differential scanning calorimetry curves recorded during cooling at $|dT/dt| = 2^\circ\text{C}/\text{min}$, from 60 to -5°C , of rennet-induced curd (A), heated curd grains before pressing (B), and Emmental cheese after pressing (C), at 1 d (D), after brining (E), and at the end of ripening (52 d; F). Heat flow is given in arbitrary units; Endo > indicates the direction of the endothermic reaction.

Table 3. Thermal characteristics of the differential scanning calorimetry curves recorded on cooling and subsequent heating of Emmental cheese at 2°C/min after the main stages involved during its manufacture¹

Sample	Cooling ²			Heating ²	
	T _{onset} (°C)	Temp. of second exo (°C)	No. of exo	T _{offset} (°C)	Events
Rennet-induced curd	15.34 ± 0.31 ^a	—	1	39.38 ± 0.45 ^{ab}	3 endo
Heated curd grains	16.17 ± 0.17 ^b	15.35 ± 0.21 ^a	2	39.49 ± 0.35 ^a	3 endo
Cheese after pressing	16.45 ± 0.21 ^c	14.48 ± 0.21 ^b	2	39.16 ± 0.33 ^{ab}	3 endo
Cheese at 1 d	16.48 ± 0.25 ^c	14.22 ± 0.22 ^b	2	39.36 ± 0.35 ^{ab}	3 endo
Cheese after brining	16.45 ± 0.14 ^c	14.37 ± 0.31 ^b	2 to 3	38.80 ± 0.51 ^b	3 endo + 1 exo
Cheese at the end of ripening (52 d)	15.48 ± 0.11 ^a	14.05 ± 0.32 ^b	2	38.13 ± 0.11 ^c	3 endo + 1 exo

^{a-c}Values with the same subscript were not significantly different according to the LSD test ($\alpha < 0.05$). Probability of *F*-test: $P < 0.001$ for temperatures of crystallization and $0.001 < P < 0.01$ for T_{offset}.

¹Presented values are means (±SD) of 3 replicate experiments.

²T_{onset} = initial temperature of crystallization; T_{offset} = final temperature of melting; Exo = exothermic peak; Endo = endothermic peak.

d showed 2 exothermic peaks overlapping each other. Caseins do not exhibit DSC thermal transitions in the temperature region investigated (Tunick, 1994). Furthermore, cheese samples were not cooled at $< -5^{\circ}\text{C}$ to avoid water crystallization. Thus, the exothermic peaks recorded on cooling were only due to the crystallization of fat in the samples of cheeses and corresponded to the liquid $\rightarrow$ solid phase transition of TG molecules. The initial temperature of crystallization recorded for the curd, T_{onset} = 15.34°C, was significantly lower than the T_{onset} recorded for the other samples during manufacture (Table 3). The T_{onset} significantly increased during the manufacture of Emmental cheese until after the pressing, from 15.34 ± 0.31°C to 16.45 ± 0.21°C. These results were interpreted considering the supramolecular organization of fat (see below). Because the supramolecular organization of fat did not change during ripening (Lopez et al., 2006a), the decrease of T_{onset} to 15.48 ± 0.11°C at the end of ripening may be related to the hydrolysis of TG by lipolytic enzymes, as long-chain saturated fatty acids have higher melting points. The extent of lipolysis in Emmental cheese is about 1 to 2% of fat (Steffen et al., 1993). Furthermore, the decrease in T_{onset} can result from the presence of lipolysis products; for example, monoacylglycerols, diacylglycerol, and free fatty acids. Recent studies showed that nucleation and crystal growth of AMF are affected by the presence of monoacylglycerols and diacylglycerols (Wright et al., 2000; Foubert et al., 2004). The effect of lipolysis products on milk fat crystallization was investigated during ripening of Emmental cheese (Lopez et al., 2006b).

The thermal properties of fat recorded on cooling changed during the manufacture of Emmental cheese. Thus, the DSC crystallization curves were delimited in 3 temperature ranges or domains as indicated in

Figure 1. The first domain delimited on cooling corresponded to TG molecules in their liquid state. The second domain corresponded to the liquid $\rightarrow$ solid phase transition with the formation of the first exothermic peak. The third domain corresponded to the formation of the second exothermic peak. The 2 exotherms recorded on cooling were related to crystallization of 2 independent groups of TG molecules (Lopez et al., 2002a).

Figure 2 shows the DSC curves recorded during heating from -5 to 60°C at 2°C/min of the samples previously cooled in the calorimeter (Figure 1). All the DSC melting curves showed 1) an endothermic peak from the beginning of the experiment to about 15°C, 2) a second endothermic event from about 15 to 20°C, and 3) an endothermic event with at least 2 overlapped peaks, from about 20°C to the final melting temperature of the samples, where the DSC curve reached the baseline. For the DSC melting curves corresponding to Emmental cheese after brining (Figure 2E) and at 52 d (Figure 2F), an exotherm was clearly recorded, between 13 and 16°C, between the first and the second endothermic peaks recorded on heating. The overlapped peaks, endothermic and exothermic, showed that several crystalline reorganizations occurred during heating.

Thermal Properties of Cream and AMF. The crystallization and melting properties of cream and AMF (which is the continuous fat phase without the physical barrier of membranes extracted from the cream) were studied on cooling and subsequent heating at 2°C/min. The influence of fat dispersion was investigated using DSC in the same experimental conditions as those used to characterize the samples of Emmental cheese. The milk fat globule size distribution of the cream (fat content ~320 g/kg) was monomo-

Figure 2. Differential scanning calorimetry curves recorded during heating at 2°C/min, from -5 to 60°C, of rennet-induced curd (A), heated curd grains before pressing (B), and Emmental cheese after pressing (C), at 1 d (D), after brining (E), and at the end of ripening (52 d; F), after cooling at 2°C/min from 60 to -5°C (Figure 1). Heat flow is given in arbitrary units; Endo > indicates the direction of the endothermic reaction.

dal and spanned from 1.3 to 10 μm with a volume-weighted diameter of $4.33 \pm 0.02 \mu\text{m}$ (data not shown). The DSC cooling curve of the cream showed a single broad exotherm with $T_{\text{onset}} = 17.61 \pm 0.16^\circ\text{C}$ (Figure 3). The DSC cooling curve of AMF extracted from the cream showed 2 well-separated exothermic events (Figure 3). The first exotherm corresponded to a sharp peak with $T_{\text{onset}} = 18.03 \pm 0.05^\circ\text{C}$.

The melting behavior of cream and AMF were monitored on heating at 2°C/min (Figure 4). The DSC melting curve recorded for cream showed 3 endothermic events that were overlapped: 1) from the beginning of the heating to 14°C, 2) from 15 to 21°C, and 3) from 22°C to the final melting temperature of TG dispersed in fat globules. The DSC melting curve of AMF was composed by well-separated thermal events: 1) a first endothermic peak from the beginning of the heating process to about 13°C, 2) an exothermic peak from 13°C to 17°C, 3) an endothermic peak from 17°C to 21°C, and 4) an endothermic event with overlapped

Figure 3. Differential scanning calorimetry curves recorded during cooling of cream and anhydrous milk fat extracted from the cream, from 60 to -5°C at 2°C/min. Heat flow is given in arbitrary units; Endo > indicates the direction of the endothermic reaction.

peaks from 22°C to the final melting temperature of TG. The exothermic peak recorded in the 13 to 17°C range was enhanced for AMF compared with cream. Thus, the supramolecular organization of milk fat; that is, dispersed in fat globules vs. in bulk, affects its thermal properties.

Supramolecular Organization of Fat. Figure 5 shows the CLSM micrographs taken after the main stages involved during the manufacture of Emmental cheese. Fat appears in red and the protein phase ap-

Figure 4. Differential scanning calorimetry curves recorded during heating of cream and anhydrous milk fat extracted from the cream, from -5 to 60°C at 2°C/min, after cooling from 60 to -5°C at 2°C/min. Heat flow is given in arbitrary units; Endo > indicates the direction of the endothermic reaction.

Figure 5. Confocal laser scanning micrographs of Emmental cheese taken after the main stages involved during manufacture: A) rennet-induced curd, B) heated curd grain before pressing, C) Emmental cheese after pressing, D) Emmental cheese after brining. Fat is colored red; proteins are colored green; the serum phase is black.

pears in green. In the rennet-induced curd, the fat globules, which were entrapped in the serum pores of the casein network, had a spherical globular structure (Figure 5A). The size of fat globules were in agreement with laser light scattering measurements, which gave a mean diameter of $d_{43} = 4.47 \pm 0.06 \mu\text{m}$ (Figure 6). In the heated curd grains before pressing, fat was present as individual fat globules, aggregates of fat globules, and coalesced fat globules that resulted from the fusion of several fat globules of smaller size (Figure 5B). The increase of mean fat globule size, which was observed in the CLSM micrograph, was related to the increase of size measured by laser light scattering: from 4.47 to 5.18 μm (Figure 6). After pressing, Emmental cheese microstructure consisted of cavities con-

taining fat and serum phase surrounded by protein strands. Milk fat globule disruption was clearly observed (Figure 5C). Fat was present in different forms: milk fat globules that had the same size as that detected following milk coagulation (~ 4 to $5 \mu\text{m}$), aggregates of fat globules, coalesced fat globules, and free fat, which was defined as the fat not protected by the milk fat globule membrane and susceptible to oiling off or removal. Following pressing, the particle size distribution determined by laser light scattering revealed the presence of large fat globules, ranging from 1 to 40 μm with a mean diameter of $8.79 \pm 0.18 \mu\text{m}$ (Figure 6). Some free fat was also observed during sample preparation. After brining, the pools of fat were surrounded by pockets of serum and the protein net-

Figure 6. Fat globule size distribution measured after dissociation of the casein matrix of (◆) rennet-induced curd, (○) heated curd grains, and (△) Emmental cheese after pressing.

work seemed to be more continuous and compact than after pressing (Figure 5D).

Crystallization of Milk Fat During Ripening of Emmental Cheese

Thermal Properties. During ripening, Emmental cheeses were successively stored in a temperate room (12°C, 16 d), a warm room (21°C, 28 d), and a cold room (4°C, 7 d). The thermal properties of fat dispersed in Emmental cheese during ripening were investigated in these different temperature-controlled rooms. Figure 7 shows the DSC melting curves recorded during heating of Emmental cheeses at 2°C/min from $T_{\text{ripening}} = 12, 21$, or 4°C, to 60°C. The DSC signal recorded on heating corresponded to the melting of the crystalline structures formed by fat in Emmental cheese. The final melting temperatures of fat are presented Table 4.

The DSC melting curve that corresponded to Emmental cheese ripened for 16 d at 12°C showed several overlapped endotherms, that may be divided into 1) a first endotherm from 12 to 21°C, 2) an endothermic peak from 21 to about 28°C, with a maximum at 24.2°C, and 3) an endothermic massif from about 28°C to the final melting temperature of TG, $T_{\text{offset}} = 40.03 \pm 0.15^\circ\text{C}$ (Figure 7A). The DSC melting curve recorded for Emmental cheese after 28 d at 21°C showed a minor endothermic event from 21 to 27°C, and an endothermic massif from 27°C to the final melting temperature of TG, $T_{\text{offset}} = 40.84 \pm 0.08^\circ\text{C}$ (Figure 7B). The DSC melting curve recorded for Emmental cheese after 7 d in the cold room at 4°C showed 1) an endothermic peak from 4 to 10°C, 2) a second endothermic peak from 15 to 27°C, and 3) an endothermic massif from 27°C to

Figure 7. Differential scanning calorimetry melting curves recorded upon heating of Emmental cheese from the temperature of ripening to 60°C at 2°C/min: A) after 16 d at $T_{\text{ripening}} = 12^\circ\text{C}$; B) after 28 d at $T_{\text{ripening}} = 21^\circ\text{C}$; C) after 7 d at $T_{\text{ripening}} = 4^\circ\text{C}$; and D) Emmental cheese at the end of ripening, stored at 4°C. Heat flow is given in arbitrary units; Endo > indicates the direction of the endothermic reaction.

the final melting temperature, $T_{\text{offset}} = 40.45 \pm 0.67^\circ\text{C}$ (Figure 7C). Below 10°C, the DSC signal was considered to correspond to the equilibration of the calorimeter. The DSC melting curve recorded for ripened Emmental cheese stored 14 d at 4°C was similar to that recorded after 7 d in the 4°C room (Figure 7D).

The melting properties of milk fat globules concentrated in a cream (fat content ~320 g/kg) were investigated for comparison with the melting properties of cheese fat during ripening of Emmental. The DSC melting curve of the cream stored 7 d at 4°C showed an endothermic event composed of overlapping peaks with a maximum at 21.8°C and a final melting temperature of $40.9 \pm 0.1^\circ\text{C}$ (Figure 8A). Figure 8B shows the DSC melting curve of the cream first heated to 60°C to eliminate its thermal history and kept at room temperature (i.e., 19 to 21°C) during 8 h. The endothermic peak recorded on heating, from 26 to 41°C with a maximum at 32.2°C, showed that some fat crystals were formed in fat globules at room temperature.

Solid Fat Content During Ripening of Emmental Cheese. It is generally accepted that the partial

Table 4. Solid fat content determined using differential scanning calorimetry (DSC) after the main stages involved during the ripening of Emmental cheese¹

Samples of Emmental cheese	Ripening temperature (°C)	Thermal parameters ²				Calculated solid fat content (%)
		T _{offset} (°C)	Cheese fat $\Delta H_{\text{partial}}$ (J/g of cheese)	Cheese fat $\Delta H_{\text{partial}}$ (J/g of fat)	AMF ΔH_{total} (J/g of fat)	
After 16 d in the temperate room	12	40.03 $\pm$ 0.15 ^a	9.94 $\pm$ 1.11	33.71 $\pm$ 3.75	76.47 $\pm$ 1.44	44.08 $\pm$ 5.73 ^a
After 28 d in the warm room	21	40.84 $\pm$ 0.08 ^b	4.61 $\pm$ 0.46	15.37 $\pm$ 1.53	81.92 $\pm$ 0.72	18.76 $\pm$ 2.03 ^b
After 7 d in the cold room	4	40.45 $\pm$ 0.67 ^{ab}	12.63 $\pm$ 1.55	42.09 $\pm$ 5.17	78.90 $\pm$ 1.44	53.35 $\pm$ 4.52 ^c
Cheese at the end of ripening	4	40.66 $\pm$ 0.05 ^b	14.14 $\pm$ 0.84	47.14 $\pm$ 2.80	84.60 $\pm$ 0.25	55.72 $\pm$ 3.48 ^c

^{a-c}Values with the same subscript were not significantly different according to the LSD test ($\alpha < 0.05$). Probability of *F*-test: $P < 0.05$ for T_{offset} and $P < 0.001$ for calculated solid fat content.

¹Presented values are means ($\pm$ SD) of 3 replicate experiments; DSC melting curves were recorded on heating at 2°C/min from the ripening temperature to 60°C.

²T = Temperature; T_{offset} = final temperature of melting; ΔH = enthalpy of melting; AMF = anhydrous milk fat.

area under the melting peak (endothermic event) is equivalent to the percentage of solid fat remaining at the selected temperature (Ali and Dimick, 1994). The ratio of solid to liquid fat; that is, the amount of fat that is crystallized, was calculated using the DSC technique in the 12°C room, the 21°C room and the 4°C room during ripening of Emmental cheese (Table 4). The ratio of the partial to the total enthalpy of melting was determined over the melting range of the fat. First, the area between the DSC melting curve and the baseline was calculated to obtain the partial enthalpy of melting of fat dispersed in Emmental cheese at T_{ripening} ($\Delta H_{\text{partial}}$ cheese fat, expressed in J/g of fat). Then, milk fat was extracted from each Emmental cheese characterized during the successive stages of ripening to obtain the respective AMF: AMF_{12°C}; AMF_{21°C}; AMF_{4°C}. This procedure ensured that Emmental cheese and AMF had the same composition of

fat and avoided differences that may be due to lipolysis; that is, hydrolysis of TG during ripening. The melting behavior of the AMF samples was characterized upon heating from -40 to 60°C at 2°C/min. The total enthalpy of melting of AMF was calculated for each AMF sample (ΔH_{total} AMF, expressed in J/g of fat), as was previously done for the corresponding Emmental cheese samples. The following ratio was calculated to determine the amount of fat that was crystallized at the different stages during ripening of Emmental cheese (Table 4):

$$\text{Solid fat content (\%)} = [\Delta H_{\text{partial}} \text{ cheese fat} / \Delta H_{\text{total}} \text{ AMF}] \times 100.$$

The amount of fat that was crystallized after 16 d in the 12°C room (44.08 $\pm$ 5.73%), 28 d in the 21°C room (18.76 $\pm$ 2.03%), and 7 d in the 4°C room (53.35 $\pm$ 4.52%) were significantly different ($P < 0.001$). A longer storage of Emmental cheese at 4°C after the end of ripening did not significantly ($P > 0.05$) increase the amount of solid fat (Table 4).

The solid fat content within Emmental cheese stored at 4°C after its ripening process was calculated as a function of temperature by constructing an integral curve from the DSC melting thermogram (Figure 9). This curve shows that 38% fat is crystallized in Emmental cheese at 20°C, 22% is crystallized at 25°C, and that 3% is crystallized at the mouth temperature (37°C).

Influence of Thermal History on the Thermal Properties of Milk Fat in Emmental Cheese. Figure 10 shows the influence of the thermal history of Emmental cheese on the thermal properties of milk fat. Two protocols were used to compare the melting behavior of fat: protocol 1 permitted the characterization of the crystalline structures naturally formed in Emmental cheese during the ripening process,

Figure 8. Differential scanning calorimetry melting curves recorded on heating at 2°C/min of milk fat globules concentrated in a cream stored for A) 7 d at 4°C, and B) 8 h at 20°C. Heat flow is given in arbitrary units; Endo > indicates the direction of the endothermic reaction.

whereas protocol 2 permitted the characterization of the melting behavior of Emmental cheese after elimination of its thermal history (see Materials and Methods section).

The DSC curves recorded from -5 to 60°C and corresponding to the melting of fat dispersed in the Emmental cheeses at $T_{\text{ripening}} = 12, 21$, and 4°C showed different shapes and number of endothermic peaks (Figure 10). Furthermore, no exothermic events were recorded on heating. The DSC melting curve recorded after 16 d in the 12°C room showed an endothermic event, constituted by the overlapping of at least 5 endothermic events: 2 overlapped endotherms and a broad event that may be formed by the overlapping of 3 endotherms (Figure 10A). Considering the Emmental cheese stored in the 21°C room, the DSC curve recorded during cooling of the sample from $T_{\text{ripening}} = 21$ to -5°C at $2^{\circ}\text{C}/\text{min}$ showed an exothermic peak, corresponding to crystallization of TG molecules from about 12.2°C (Figure 10B insert). The DSC melting curve showed 2 well-separated endothermic peaks; the first peak spanned from about 0 to 28°C with a maximum at 17°C and the second peak spanned from 28°C to the final melting temperature of fat in Emmental cheese (Figure 10B). The DSC melting curves recorded for Emmental cheese during ripening in the 4°C room and at the end of ripening were similar and showed a first endotherm from the beginning of the experiments to about 18°C , a second endotherm from about 19°C to about 28°C with a maximum at 24.3°C , and a third endotherm that lasted until the final melting temperature of fat in Emmental cheese (Figure 10C and 10D).

Figure 9. Evolution of the solid fat content as a function of temperature determined in Emmental cheese at the end of ripening (52 d), using differential scanning calorimetry.

The DSC melting curves recorded after elimination of the thermal history of Emmental cheese showed similar shapes and thermal events (Figure 10). They were constituted by overlapped peaks: a first endotherm between -5 and 13 to 14°C , an exotherm between 14 and 16°C , a second endotherm between 16 and 19 to 20°C , and a broad endothermic event from about 20°C to the final melting temperature of fat in Emmental cheese.

Comparing the DSC melting curves recorded in the same range of temperature; that is, from -5 to 60°C , and at the same heating rate, e.g., $dT/dt = 2^{\circ}\text{C}/\text{min}$, differences due to the thermal history of Emmental cheeses were observed (Figure 10; Table 5). The melting peaks and suggested polymorphic transitions were different (Figure 10). Furthermore, the crystalline structures formed by fat during ripening of Emmental cheese had a significantly ($P < 0.05$) higher final melting temperature (T_{offset}) than the same fat after elimination of its thermal history (Table 5). The differences in final temperature of melting between unstable and more stable crystalline species were in the range 1.72 to 2.82°C (Table 5).

DISCUSSION

The increased knowledge of the thermal properties and crystallization of fat in food products might be a certain value in understanding their functional properties, to develop superior cooling strategies for storage and for technical applications. More particularly, the existence of milk fat crystals can have important consequences on the textural and rheological properties of cheese (Prentice, 1987). Furthermore, the amount, size and type of crystals may have primordial importance for final product consistency, acceptability, and functional properties (Walstra and Jenness, 1984). The crystallized part of the fat determines to a large extent the firmness of fat-rich products and the physical stability of products in which fat is present as the dispersed phase, because the stability of the fat globules is dependent on the proportions of crystalline and liquid fat (Hinrichs and Kessler, 1997).

Quantification of Milk Fat Crystals in Emmental Cheese Using DSC

In this study, the amount of fat crystals was determined by calculating the ratio of solid to liquid fat (solid fat content) using DSC for the 3 temperatures used in the ripening process of Emmental cheese (Table 4). Solid fat content values were derived from DSC melting curves by sequential integration. The calculation of the solid fat content involved the implicit as-

Figure 10. Differential scanning calorimetry melting curves recorded during heating of Emmental cheese from -5 to 60°C at $2^{\circ}\text{C}/\text{min}$, following different kinetics of cooling; 1) after cooling from the temperature of ripening (T_{ripening}) to -5°C at $2^{\circ}\text{C}/\text{min}$ (solid line), and 2) after heating cheese to 60°C to eliminate the thermal history and cooling from 60 to -5°C at $2^{\circ}\text{C}/\text{min}$ (dashed line). The melting properties of Emmental cheese were determined after the main stages involved during its ripening: A) $T_{\text{ripening}} = 12^{\circ}\text{C}$, B) $T_{\text{ripening}} = 21^{\circ}\text{C}$; the insert shows the crystallization curve recorded on cooling from 21 to -5°C ; C) $T_{\text{ripening}} = 4^{\circ}\text{C}$; and D) Emmental cheese at the end of ripening, stored at 4°C . Heat flow is given in arbitrary units; Endo > indicates the direction of the endothermic reaction.

sumption that the heat of melting of TG was constant. In fact, the heat of melting had a positive correlation with melting point and, as a result, the true proportion of solids was overestimated (by approximately 7 to 10%) at high solid fat contents (Norris and Taylor, 1977). From a methodological point of view, the determination of $\Delta H_{(\text{total cheese fat})}$ is difficult. The better procedure would be to determine $\Delta H_{(\text{total cheese fat})}$ directly in Emmental cheese. However, this is not possible because the water present in cheese (DM = 627 g/kg) crystallizes below -5°C . Thus, upon heating in the -40 to 60°C range, the ΔH melting of water is superimposed on the ΔH melting of fat in cheese, hindering the determination of $\Delta H_{(\text{total cheese fat})}$. Thus, to approximate $\Delta H_{(\text{total cheese fat})}$, cheese fat was extracted from cheese

and analyzed in bulk (AMF). The authors consider that both the dispersion state of fat and the metastability of crystalline species may influence the estimation of ΔH_{total} AMF: 1) ΔH_{total} is higher in bulk than in the dispersed state (5 to 10%); that is, in Emmental cheese. Considering the dispersion state of fat in Emmental cheese, ΔH_{total} AMF is overestimated; 2) ΔH_{total} AMF recorded on heating is lower after cooling at $2^{\circ}\text{C}/\text{min}$ (formation of unstable species) than after storage for a long period at different temperatures during ripening of cheese (stable species). Considering the metastability of the crystalline species formed, ΔH_{total} is underestimated. The difference in ΔH between stable and unstable polymorphic forms is in the range from 10 to 20%. Thus, the authors consider that the ΔH_{total}

Table 5. Thermal characteristics of the DSC curves recorded on heating of Emmental cheese at 2°C/min from -5 to 60°C, after cooling from their temperature of ripening to -5°C¹

Samples of Emmental cheese	Ripening temperature (°C)	Crystalline structures formed in Emmental cheese (stable species)		Crystalline structures formed after elimination of the thermal history (unstable species)		<i>F</i> -test	ΔT_{offset} (°C)
		T_{offset} (°C)	Events	T_{offset} (°C)	Events		
After 16 d in the temperate room	12	39.92 ± 0.52	At least 5 overlapped endotherms	38.20 ± 0.03	3 main endotherms; 1 exotherm	*	1.72 ± 0.02 ^a
After 28 d in the warm room	21	40.91 ± 0.17	2 well-separated endotherms (including HMP)	38.19 ± 0.21	3 main endotherms; 1 exotherm	***	2.72 ± 0.03 ^b
After 7 d in the cold room	4	40.51 ± 0.12	3 main endotherms (LMP, MMP, HMP)	37.69 ± 0.11	3 main endotherms; 2 exotherms	***	2.82 ± 0.02 ^c
Cheese at 52 d	4	40.76 ± 0.25	3 main endotherms (LMP, MMP, HMP)	38.13 ± 0.08	3 endotherms; 1 exotherm	***	2.63 ± 0.02 ^d

^{a-d}Values with the same subscript were not significantly different according to the LSD test ($\alpha < 0.05$).

¹Presented values are means (± standard deviations) of three replicate experiments.

²T = Temperature; T_{offset} = final temperature of melting; LMP = low melting point; MMP = middle-melting point; HMP = high-melting point.

*** $P < 0.001$; * $P < 0.05$.

AMF used to calculate the solid fat content in Emmental cheese is underestimated to about 10 to 15%.

Differential scanning calorimetry allowed us to quantify the amount of fat that was crystallized as a function of temperature until its final melting, and to characterize the thermal properties and probably polymorphism of fat upon cooling and heating in a complex and high fat content food product. The method developed, which had never been used before to quantify the solid fat content in food products, is innovative. For the first time, to the author's knowledge, the solid fat content was determined in a cheese and during the ripening process of Emmental cheese.

Nuclear magnetic resonance (NMR) spectroscopy is usually used to quantify the solid fat content of fats and oils. Most NMR studies of dairy products have focused on AMF or blends of AMF and vegetable oils (Lambelet, 1983b; Marangoni et al., 2000; Singh et al., 2002). In contrast, only few NMR relaxation measurements on fatty products with intermediate water content have been carried out (Samuelsson and Vikelsoe, 1971; Chaland et al., 2000; Wiking et al., 2005). Nuclear magnetic resonance spectroscopy is not yet adapted for determining the solid fat content in complex products in which the fat is dispersed. Moreover, the polymorphism of milk fat increases the difficulty of interpreting the NMR data. Recently, Bertram et al., (2005) determined the crystallization temperatures of milk fat globules using ¹H NMR transverse (T_2) relaxation measurements during continuous cooling of

creams. The latter authors confirmed that both the chemical composition and the supramolecular structure of fat (i.e., size of fat globules) may contribute to the T_2 relaxation pattern and showed, for the first time, that phase transitions (i.e., fat crystallization) in cream can be determined using NMR transverse relaxation measurements.

Differential scanning calorimetry and NMR were compared for determination of the solid fat content in anhydrous fats in stable polymorphic forms (Lambelet, 1983ab). Solid fat content values derived from DSC curves were higher than values obtained by NMR spectroscopy. The latter author attributed these results to differences in the chemical composition of the fats and concluded that results obtained by the NMR method were too low. Furthermore, the differences between DSC and NMR solid fat contents are not constant, but show variations as a function of temperature (Norris and Taylor, 1977). The values of solid fat content determined using NMR found in the literature are lower than the solid fat content determined in the current study using DSC (Table 4). Wiking et al. (2005) determined that after 50 min at 4°C, 27.8 to 44.2% of fat was solid in cream, depending on the fatty acid composition. Samuelsson and Vikelsoe (1971) calculated that after 72 h storage at 12°C, about 38% of fat was crystallized in cream.

The presence of fat crystals in Emmental cheese (solid fat content ~55 to 56% at 4°C) was observed in this study, but precise localization of fat crystals

within the inclusions of fat dispersed in Emmental cheese was not investigated. The determination of the size and the localization of such fat crystals, which may be dispersed in the interior of the fat inclusions or may be at the interface with the protein network, could provide some understanding of the flavor development and flavor release in complex matrices such as cheeses. Indeed, some aroma compounds are solubilized in the liquid phase of fat and their release depends on the ratio of solid to liquid fat (Relkin et al., 2004). Furthermore, the melting of fat crystals may contribute to the release of these aroma compounds and to mouthfeel during consumption of Emmental cheese.

We showed in this study that the final melting point of fat in Emmental cheese was $40.76 \pm 0.25^\circ\text{C}$ (Table 5), which means that the fat was totally liquid only over this temperature. Thus, fat crystals do not play a role in the functional properties—flowability, meltability, and stretchability—of melted Emmental cheese. Furthermore, the amount of fat that was solid above 37°C was calculated to be 3% (wt/wt; Figure 9). This fraction of solid fat can be mainly constituted by high-melting point TG; that is, TG with saturated long-chain fatty acids such as palmitic acid (C16:0). Regarding the digestive and nutritional properties of milk fat, studies found in the literature did not consider that milk fat is partially solid for temperatures $>37^\circ\text{C}$. The mechanisms involved in the hydrolysis of such solid TG molecules by the digestive lipolytic enzymes have not been investigated and thus are not well known.

Importance of the Supramolecular Organization of Fat on Thermal Properties

The crystallization and melting properties of milk fat changed during the manufacture of Emmental cheese (Figures 1 and 2; Table 3). The experiments, which were performed using DSC at the same cooling rate ($2^\circ\text{C}/\text{min}$) showed the formation of a second exothermic peak at higher temperature during the manufacture. The crystallization properties of fat may depend on 1) the composition of fat because the fatty acid composition of milk fat determines the temperature at which it begins to crystallize (Shi et al., 2001); 2) the dispersion of fat (Lopez et al., 2002a); that is, its supramolecular organization, or 3) accumulation of lipolysis products during ripening (Foubert et al., 2004). The fatty acid analysis did not show any significant ($P > 0.05$) changes in the composition of fat as a function of time and mechanical treatments during the manufacture of Emmental cheese (Table 2). Regarding the microstructural analysis, the CLSM micrographs showed

changes in situ in the supramolecular organization of fat during the manufacture of Emmental cheese (Figure 5). Furthermore, the DSC experiments performed using DSC on cream and AMF indicated the importance of the supramolecular organization of fat, fat globules vs. bulk, on its thermal properties (Figures 3 and 4). The size distribution of fat globules in cream was similar to the size distribution of fat globules entrapped in the curd ($\sim 4.5 \mu\text{m}$). Thus, the delay in the temperature of the beginning of crystallization observed in the curd, $\Delta T_{\text{onset}} = 17.61 - 15.34 = 2.27^\circ\text{C}$, may be due to the lower content of fat (lower signal to noise ratio) or explained by the environment of fat; that is, caseins and water. Lopez et al. (2002a) showed the formation of 2 overlapped exotherms during cooling of cream at 1 and $3^\circ\text{C}/\text{min}$. The differences observed may be related to the size of fat globules in the creams; the latter authors used a cream with larger fat globules (up to $30 \mu\text{m}$). Smaller fat globules show a single exotherm on cooling (Lopez et al., 2002a). Regarding our results obtained on cooling of AMF, we agree with the existence of 2 exothermic processes that correspond to crystallization of 2 groups of TG (Lavigne, 1995; ten Grotenhuis et al., 1999).

Comparing cream and AMF, initial crystallization was initiated at different temperatures and in a different way, as previously observed (Walstra and van Beresteyn, 1975; Söderberg et al., 1989; Lopez et al., 2001c, 2002ab). Initial temperature of crystallization recorded for cream ($T_{\text{onset}} = 17.61^\circ\text{C}$) was slightly lower than that of AMF ($T_{\text{onset}} = 18.03^\circ\text{C}$). The initial crystallization of fat was related to supercooling relaxation and then to the nucleation rate in the dispersed state (Walstra and van Beresteyn, 1975). The slopes of the initial peak of crystallization were 0.016 arbitrary units (a.u.)/ $^\circ\text{C}$ for cream and 0.407 a.u./ $^\circ\text{C}$ for AMF. The sharp peak was related to a higher crystal growth rate for AMF compared with individual milk fat globules of cream. Although crystal growth and material supply for growing, which occurred by molecular diffusion, were necessarily limited in fat globules, it was not so in a far greater volume of bulk fat. Considering the influence of the size of milk fat globules on their crystallization properties, Lopez et al. (2002a) showed that reducing the size of milk fat emulsion droplets by homogenization induces a higher supercooling; that is, the initial crystallization temperature was displaced toward the lower temperature. The same results were obtained with native milk fat globules of different sizes (Michalski et al., 2004b). The differences in supercooling were generally explained by the theory of nucleation for crystallization (Walstra and van Beresteyn, 1975). When the liquid fat is dispersed in emulsion droplets, at least one nucleus must be

formed in every droplet to achieve full crystallization. This implies that a lower temperature was needed for a finer dispersion because more catalytic impurities were needed. Thus, the time needed to obtain the first nuclei was inversely proportional to fat globule volume (Walstra and van Beresteyn, 1975).

The DSC recordings of Emmental cheese corresponded to a sum of the phenomenon that occurred during cooling and heating of fat dispersed in a continuous protein network. The increase of fat globule size by coalescence and the formation of free fat after pressing of Emmental cheese could explain the higher temperature of crystallization due to lower supercooling, and the faster crystal growth rate related to the first exothermic peak recorded on cooling (Figure 1).

The DSC melting curves corresponding to Emmental cheese after elimination of their thermal history were similar to those recorded for AMF (Figures 2 and 4). The DSC melting curves recorded on heating of cream and AMF were similar to the results obtained by Lopez et al. (2002a, 2005c), using coupled DSC and x-ray diffraction techniques. It has been shown that polymorphic transitions are facilitated in AMF by a comparison with cream (Lopez et al., 2002b). Thus, the exothermic processes recorded with Emmental cheese after brining and at the end of ripening could be related to monotropic transitions on heating favored by the presence of nonglobular fat in the protein matrix.

Phase Transition of Milk Fat Fractions in Emmental Cheese During Ripening

The experiments performed in this study showed that $44.08 \pm 5.73\%$ of the fat was crystallized after 16 d of ripening in the 12°C room (Table 4). Then, the increase in temperature from 12 to 21°C in the warm room ($\Delta T_{\text{ripening}} = +9^\circ\text{C}$) induced the melting of some TG crystallized at 12°C, which led to the increase in the amount of liquid TG from about 66 to about 81 to 82%. This liquid phase could have favored structural rearrangements of TG molecules in relation to the monotropic polymorphism of TG, because mixed crystals can dissolve and transform into a new and more stable polymorphic form (Small, 1986). Furthermore, $T_{\text{ripening}} = 21^\circ\text{C}$ decreased the viscosity of liquid fat compared with 12°C and could have promoted fractionation of the fat. Figure 7B shows the DSC curve of the high-melting point peak formed in Emmental cheese, which corresponded to the melting of 18 to 19% fat (Table 4). The cooling of the Emmental cheese from 21 to -5°C showed the formation of an exothermic peak corresponding to crystallization of TG molecules in the cheese (Figure 10B insert). The subsequent heating of the sample showed 2 well-separated endothermic

peaks, which means that the TG molecules that had crystallized on cooling melted separately on the subsequent heating (Figure 10B). Thus, 2 independent groups of TG were formed in Emmental cheese. The increase in the final melting temperature of TG molecules from $39.9 \pm 0.5^\circ\text{C}$ (12°C room) to $40.9 \pm 0.2^\circ\text{C}$ (21°C room) showed that crystalline structures with a higher compactness and thus, stability were formed in the warm room because of segregation of TG that form high-melting point crystals; that is, TG with saturated long-chain fatty acids. During 28 d of storage at 21°C, TG molecules had the time to incorporate into a crystal lattice, indicating that the polymorphism of TG depended on kinetic parameters. The storage of Emmental cheese in the 4°C room, $\Delta T_{\text{ripening}} = -17^\circ\text{C}$, led to an increase of the solid fat content from about 19% to about 53 to 54% (Table 4). The DSC melting curve showed the 3 endothermic peaks corresponding to LMP, MMP, and HMP fractions, as described by Timms (1980). The HMP fraction corresponded to the high-melting point peak formed for $T_{\text{ripening}} = 21^\circ\text{C}$ (Figure 7). The formation of the LMP and MMP peaks suggested that polymorphic evolutions of TG molecules occurred in Emmental cheese during storage at 4°C. The polymorphic transitions that occurred in the solid phase as a function of time were favored and accelerated by the amount of liquid fat phase in Emmental cheese at the temperature of ripening; that is, 12, 21, and then 4°C. The DSC melting curve of the cream stored 7 d at 4°C (Figure 8A) showed a different shape compared with the DSC curve recorded after storage of Emmental cheese 7 d with $T_{\text{ripening}} = 4^\circ\text{C}$ (Figure 7C). Thus, the thermal history influenced the types of crystals formed and the melting properties of fat. This result confirmed that storage of Emmental cheese at 21°C before storage at 4°C led to the segregation of high-melting point TG during the ripening process. Regarding the DSC melting curve of the cream stored at room temperature (Figure 8B), similar results were obtained for Emmental cheese stored in the 21°C room. Thus, the melting behavior of TG molecules crystallized in isothermal conditions at room temperature was similar in a complex matrix such as cheese and in fat globules.

On heating of Emmental cheese stored in the successive temperature-controlled rooms, no exothermic process was recorded (Figure 7). This result showed that there was no rearrangement of TG molecules through polymorphic transition to increase their stability on heating. Thus only the melting of stable crystals of TG molecules was observed. Only structural analysis, achieved with synchrotron radiation x-ray diffraction, would allow the identification of the molecular arrangement of TG molecules in the crystalline struc-

tures formed and to relate them to the thermal events. The DSC melting curves recorded for Emmental cheese in the 4°C room and at the end of ripening were similar to those of Famelart et al. (2002) and to the results obtained in Lopez et al. (2002b) for cream and AMF after 135 and 105 h of storage at 4°C, respectively. Lopez et al. (2002b) previously showed that the fat phase of cream and AMF is partially crystallized during long storage at 4°C and displays a complex polymorphism that is time and temperature dependent.

CONCLUSIONS

The examination of the physical properties of fat in complex food products, such as cheese, is of particular importance. The increased knowledge of the thermal properties and crystallization of fat might be of value to understand the functional and sensorial properties and for technical applications.

To the authors' knowledge, this is the first time that a dynamic study is performed as a function of time and temperature to characterize the thermal properties of fat and to estimate the solid fat content, *in situ* in cheese. It has been shown that the supramolecular organization of fat dispersed in Emmental cheese (e.g., fat globules, aggregates of fat globules, or free fat) alters both its crystallization and melting properties. Furthermore, the temperature of ripening greatly influences the solid fat content and the type of crystals present in Emmental cheese. This study showed, using DSC, that $55.7 \pm 3.5\%$ of the fat phase dispersed in Emmental cheese is crystallized at the end of ripening at 4°C. As may be the case in many foods, the fat phase is partially crystallized at temperatures below 41°C. Thus, fat inclusions and fat globules are a mixture of crystals and oil between their storage temperature and 41°C. Furthermore, the present study suggested that the fat phase displays a complex polymorphism within a water-dispersed and highly complex processed food product such as Emmental cheese.

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REFERENCES

Ali, A. R., and P. S. Dimick. 1994. Melting and solidification characteristics of confectionary fats: Anhydrous milk fat, cocoa butter

- and palm kernel stearin blends. *J. Am. Oil Chem. Soc.* 71:803–806.
- Bertram, H. C., L. Wiking, J. H. Nielsen, and H. J. Andersen. 2005. Direct measurement of phase transitions in milk fat during cooling of cream – A low-field NMR approach. *Int. Dairy J.* 15:1056–1063.
- Chaland, B., F. Mariette, P. Marchal, and J. de Certaines. 2000. ¹H nuclear magnetic resonance relaxometric characterization of fat and water states in soft and hard cheese. *J. Dairy Res.* 67:609–618.
- Dalgleish, D. G. 1984. Measurement of electrophoretic mobilities and zeta-potentials of particles from milk using laser Doppler electrophoresis. *J. Dairy Res.* 51:425–438.
- Famelart, M.-H., Y. Le Graet, F. Michel, R. Richoux, and A. Riaublanc. 2002. Evaluation des méthodes d'appréciation des propriétés fonctionnelles des fromages d'Emmental de l'ouest de la France. *Lait* 82:225–245.
- Foubert, I., B. Vanhoutte, and K. Dewettinck. 2004. Temperature and concentration dependent effect of partial glycerides on milk fat crystallization. *Eur. J. Lipid Sci. Technol.* 106:531–539.
- Gresti, J., M. Burgaut, C. Maniongui, and J. Bezard. 1993. Composition of molecular species of triacylglycerols in bovine milk fat. *J. Dairy Sci.* 76:1850–1869.
- Hagemann, J. W. 1988. Thermal behavior and polymorphism of acylglycerols. Pages 9–95 in *Crystallization and Polymorphism of Fats and Fatty Acids*. N. Garti and K. Sato, ed. Marcel Dekker, New York, NY.
- Hinrichs, J., and H. G. Kessler. 1997. Fat content of milk and cream and effects on fat globule stability. *J. Food Sci.* 62:992–995.
- IDF/FIL. 1981. Catalogue of cheeses. Document 141. International Dairy Federation, Brussels, Belgium.
- IDF/FIL. 1986. Fromage et fromage fondu: Détermination de la teneur en matière grasse. Standard 5B. International Dairy Federation, Brussels, Belgium.
- IDF/FIL. 1987. Lait, crème et lait concentré non sucré, détermination de la matière sèche, standard 21B. International Dairy Federation, Brussels, Belgium.
- Jensen, R. G., and D. S. Newburg. 1995. Bovine milk lipids. Pages 543–575 in *Handbook of Milk Composition*. R. G. Jensen, ed. Academic Press, Inc., New York, NY.
- Keenan, T. W., and D. P. Dylewski. 1995. Intracellular origin of milk lipid globules and the nature and structure of the milk lipid globule membrane. Pages 89–130 in *Advanced Dairy Chemistry*. Vol. 2: Lipids. P. F. Fox, ed. Chapman and Hall, London, UK.
- Lambelet, P. 1983a. Comparison of NMR and DSC methods for determining solid content of fats: Application to milk fat and its fractions. *Lebens. Wiss. Technol.* 16:90–95.
- Lambelet, P. 1983b. Comparison of NMR and DSC methods for determining solid fat content of fats: Application to cocoa butter and its admixtures with milk fat. *Lebens. Wiss. Technol.* 16:200–202.
- Lavigne, F. 1995. Polymorphisme et transitions de phases des triglycérides. Applications aux propriétés thermiques et structurales de la matière grasse laitière anhydre et ses fractions. PhD thesis, Univ. Paris VII, Paris XI and ENSIA, France.
- Lopez, C. 2005a. Focus on the supramolecular structure of milk fat in dairy products. *Reprod. Nutr. Dev.* 45:497–511.
- Lopez, C., C. Bourgaux, P. Lesieur, S. Bernadou, G. Keller, and M. Ollivon. 2002a. Thermal and structural behavior of milk fat. 3. Influence of cooling rate and droplet size on cream crystallization. *J. Colloid Interface Sci.* 254:64–78.
- Lopez, C., C. Bourgaux, P. Lesieur, and M. Ollivon. 2002b. Crystalline structures formed in cream and anhydrous milk fat at 4°C. *Lait* 82:317–335.
- Lopez, C., B. Camier, and J.-Y. Gassi. 2006a. Development of the milk fat microstructure during the manufacture and ripening of Emmental cheese observed by confocal laser scanning microscopy. *Int. Dairy J.* doi:10.1016/j.idairyj.2005.12.015
- Lopez, C., N. Karray, P. Lesieur, and M. Ollivon. 2005b. Crystallization and melting properties of dromedary milk fat globules studied by X-ray diffraction and differential scanning calorimetry.

- Comparison with anhydrous dromedary milk fat. *Eur. J. Lipid Sci. Technol.* 107:673–683.
- Lopez, C., F. Lavigne, P. Lesieur, C. Bourgaux, G. Keller, and M. Ollivon. 2005c. Thermal and structural behavior of anhydrous milk fat: 3. Influence of cooling rate. *J. Dairy Sci.* 88:511–526.
- Lopez, C., F. Lavigne, P. Lesieur, G. Keller, and M. Ollivon. 2001a. Thermal and structural behavior of milk fat: 1. Unstable species of anhydrous milk fat. *J. Dairy Sci.* 84:756–766.
- Lopez, C., F. Lavigne, P. Lesieur, G. Keller, and M. Ollivon. 2001b. Thermal and structural behavior of anhydrous milk fat: 2. Crystalline forms obtained by slow cooling. *J. Dairy Sci.* 84:2402–2412.
- Lopez, C., P. Lesieur, C. Bourgaux, G. Keller, and M. Ollivon. 2001c. Thermal and structural behavior of milk fat: 2. Crystalline forms obtained by slow cooling of cream. *J. Colloid Interface Sci.* 240:150–161.
- Lopez, C., P. Lesieur, G. Keller, and M. Ollivon. 2000. Thermal and structural behavior of milk fat: 1. Unstable species of cream. *J. Colloid Interface Sci.* 229:62–71.
- Lopez, C., M.-B. Maillard, V. Briard-Bion, B. Camier, and J. A. Hannon. 2006b. Lipolysis during ripening of Emmental cheese considering organisation of fat and preferential localisation of bacteria. *J. Agric. Food Chem.* (accepted)
- Marangoni, A. G., and R. W. Lencki. 1998. Ternary phase behavior of milk fat fractions. *J. Agric. Food Chem.* 46:3879–3884.
- Marangoni, A. G., A. J. Wright, S. J. Narine, and R. W. Lencki. 2000. Comment on the use of direct pulsed nuclear magnetic resonance solid fat content measurements in phase behavior studies of lipid mixtures. *J. Am. Oil Chem. Soc.* 77:565–567.
- Michalski, M.-C., V. Briard, and F. Michel. 2001. Optical parameters of milk fat globules for laser light scattering measurements. *Lait* 81:787–796.
- Michalski, M.-C., B. Camier, V. Briard, N. Leconte, J.-Y. Gassi, H. Goudédranche, F. Michel, and J. Fauquant. 2004a. The size of native milk fat globules affects physico-chemical and functional properties of Emmental cheese. *Lait* 84:343–358.
- Michalski, M.-C., M. Ollivon, V. Briard, N. Leconte, and C. Lopez. 2004b. Native fat globules of different sizes selected from raw milk: Thermal and structural behavior. *Chem. Phys. Lipids* 132:247–261.
- Norris, R., and M. W. Taylor. 1977. Comparison of NMR and DSC methods for the estimation of solid fat content. *N.Z. J. Dairy Sci. Technol.* 12:160–165.
- Prentice, J. H. 1987. Cheese rheology. Pages 299–344 in *Cheese Chemistry, Physics and Microbiology*. Vol. 1: General aspects. P. F. Fox, ed. Elsevier Appl. Sci. Publ., London, UK.
- Relkin, P., M. Fabre, and E. Guichard. 2004. Effect of fat nature and aroma compound hydrophobicity on flavor release from complex food emulsions. *J. Agric. Food Chem.* 52:6257–6263.
- Rowney, M., P. Roupas, M. Hickey, and D. W. Everett. 1998. Milkfat structure and free oil in Mozzarella cheese. *Aust. J. Dairy Technol.* 53:110.
- Samuelsson, E. G., and J. Vikelsoe. 1971. Estimation of the amount of liquid fat in cream and butter by low resolution NMR. *Milchwissenschaft* 26:621–625.
- Sehat, N., J. K. G. Mossoba, M. M. Yurawecz, M. P. Roach, J. A. G. Eulitz, K. Morehouse, and K. M. Youh Ku. 1998. Identification of conjugated linoleic acid isomers in cheese by gas chromatography, silver ion high performance liquid chromatography and mass spectral reconstructed ion profiles. Comparison of chromatographic elution sequences. *Lipids* 33:963–971.
- Shi, Y., C. M. Smith, and R. W. Hartel. 2001. Compositional effects on milk fat crystallization. *J. Dairy Sci.* 84:2392–2401.
- Singh, A. P., D. J. McClements, and A. G. Marangoni. 2002. Comparison of ultrasonic and pulsed NMR techniques for determination of solid fat content. *J. Am. Oil Chem. Soc.* 79:431–437.
- Söderberg, I., L. Hernqvist, and W. Buchheim. 1989. Milk fat crystallization in natural milk fat globules. *Milchwissenschaft* 44:403–406.
- Small, D. M. 1986. The physical chemistry of lipids. From alkanes to phospholipids. Pages 347–382 in *Handbook of Lipid Research*. D. J. Hanahan, ed. Plenum Press, New York, NY.
- Steffen, C., P. Eberhard, J. O. Bosset, and M. Rueegg. 1993. Swiss-type varieties. Pages 83–110 in *Cheese: Chemistry, Physics and Microbiology*. Vol. 2. 2nd ed. P. F. Fox, ed. Chapman and Hall, London, UK.
- ten Grotenhuis, E., G. A. van Aken, K. F. van Malssen, and H. Schenck. 1999. Polymorphism of milk fat studied by differential scanning calorimetry and real-time x-ray powder diffraction. *J. Am. Oil Chem. Soc.* 76:1031–1039.
- Timms, R. E. 1980. The phase behavior and polymorphism of milk fat, milk fat fractions, and fully hardened milk fat. *Aust. J. Dairy Technol.* 35:47–53.
- Tunick, M. H., J. J. Basch, B. E. Maleeff, J. F. Flanagan, and V. H. Holsinger. 1989. Characterization of natural and imitation Mozzarella cheeses by differential scanning calorimetry. *J. Dairy Sci.* 72:1976–1980.
- Tunick, M. H. 1994. Effects of homogenization and proteolysis on free oil in Mozzarella cheese. *J. Dairy Sci.* 77:2487–2493.
- Tunick, M. H., and E. L. Malin. 1997. Differential scanning calorimetry of water buffalo and cow milk fat in Mozzarella cheese. *J. Am. Oil Chem. Soc.* 74:1565–1568.
- Ulberth, F., R. G. Gabernig, and F. Schrammel. 1999. Flame ionization detector response to methyl, ethyl, propyl, and butyl esters of fatty acids. *J. Am. Oil Chem. Soc.* 76:263–266.
- Walstra, P., and R. Jenness. 1984. Rheological properties. Pages 290–300 in *Dairy Chemistry and Physics*. John Wiley & Sons, New York, NY.
- Walstra, P., and E. C. H. van Beresteijn. 1975. Crystallization of milk fat in the emulsified state. *Neth. Milk Dairy J.* 29:35–65.
- Wiking, L., H. C. Bertram, L. Bjorck, and J. H. Nielsen. 2005. Evaluation of cooling strategies for pumping of milk – Impact of fatty acid composition on free fatty acid levels. *J. Dairy Res.* 72:476–481.
- Wright, A. J., R. W. Hartel, S. S. Narine, and A. G. Marangoni. 2000. The effect of minor components on milk fat crystallization. *J. Am. Oil Chem. Soc.* 77:463–475.