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THERMAL TRANSITIONS AND FAT DROPLET STABILITY IN ICE-CREAM MIX MODEL SYSTEMS
Effect of milk fat fractions

*A. Bazmi and Perla Relkin**

Ecole Nationale Supérieure des Industries Alimentaires, UMR 1211, Laboratoire de Biophysique des Matériaux Alimentaires
1 Avenue des Olympiades, 91 744 Massy, France

We studied thermal transitions and physical stability of oil-in-water emulsions containing different milk fat compositions, arising from anhydrous milk fat alone (AMF) or in mixture (2:1 mass ratio) with a high melting temperature (AMF–HMT) or a low melting temperature (AMF–LMT) fraction. Changes in thermal transitions in bulk fat and emulsion samples were monitored by differential scanning calorimetry (DSC) under controlled cooling and reheating cycles performed between 50 and –45°C (5°C min^{-1}). Comparison between bulk fat samples and emulsions indicated similar values of melting completion temperature, whereas initial temperature of fat crystallization (T_{onset}) seemed to be differently affected by storage temperature depending on triacylglycerols (TAG) composition. After storage at 4°C, T_{onset} values were very similar for emulsified and non-emulsified AMF–HMT blend, whereas they were lower (by approx. 6°C) for emulsions containing AMF or mixture of AMF–LMT fraction. After storage at –30°C, T_{onset} values of re-crystallization were higher in emulsion samples than in bulk fat blends, whatever the TAG fat composition. Light scattering measurements and fluorescence microscopic observations indicated differences in fat droplet aggregation-coalescence under freeze-thaw procedure, depending on emulsion fat composition. It appeared that under quiescent freezing, emulsion containing AMF–LMT fraction was much less resistant to fat droplet aggregation-coalescence than emulsions containing AMF or AMF–HMT fraction. Our results indicated the role of fat droplet liquid-solid content on emulsion stability.

Keywords: *DSC, emulsion, fat coalescence, freezing, milk fat, solid fat content*

Introduction

In milk fat, triacylglycerols account for 95–96%. They are composed by 64% saturated fatty acids, including mainly palmitic (26.1%) and stearin (13.3%) fatty acids. Among unsaturated fatty acids, oleic acid (25.5%) is the most abundant [1, 2]. For preparation of milk whipped-creams or ice creams, lipid ingredient comes mostly from milk fat (in United States) or vegetable fats (palm oil or palm kernel oil) in other countries. These lipids are constituted by a wide diversity of fatty acids and triacylglycerols (TAG), each characterized by its own melting temperature. Their chemical and physical properties may be modified by fractionation or hydrogenation, leading to different characteristic melting profiles and growing-up of solids [3]. Fractionation of anhydrous milk fat is commonly used in milk fat industry and the resulting fractions are used as value-added ingredients for their nutritional, flavoring or physical effects on food products, such as butter-like products, chocolate, bakery. The milk fat fractions containing high proportion of saturated or unsaturated TAG, which are characterized by a high (HMT) or low (LMT) melting temperature were shown to have effects on ice cream quality [4]. On the other hands, studies performed on model or real food emulsions, showed differences between temperature values needed for initiation of fat crystallization in bulk fat and in emulsions. The temperature differences were explained by differences in fat droplet size, distribution of catalytic impurities, adsorbed emulsifier types, freeze-thaw cycles, and thermo-mechanical treatments [5–10]. Crystallization behaviour in bulk and dispersed fat droplets may be monitored by using various physical techniques, such as dilatometry, ultrasonic velocity measurements, X-ray diffraction and differential scanning calorimetry, nuclear magnetic resonance [2, 7–13]. Particularly, it was observed that fat droplet crystallisation in protein-stabilized emulsions behave differently depending on adsorbed molecules and physical stability against aggregation-coalescence [7, 9, 10, 12, 14]. Differences in growing of fat crystals in protein-stabilized emulsions were explained by differences in molecule composition of adsorbed layers around fat droplets.

The aim of the present work, was to study thermal behavior of fat droplets in ice-cream mixes differing by their milk fat triacylglycerol composition, as

* Author for correspondence: relkin@ensia.fr

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Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

varied by using either anhydrous milk fat alone, or in mixture with a low melting or high melting temperature fraction, with consideration on emulsion physical stability under quiescent storage at 4 or –30°C.

Experimental

Materials

Emulsion preparation

The emulsions consist in (all in mass%) 10% milk fat samples, 10% non fat milk solids, 15% sucrose and 0.5% of a commercial blend of polysaccharide stabilizers (locust bean gum, sodium alginate, guar gum and carrageenan) and mono- and di-glycerides (Cremodan SE30-Danisco). To prepare emulsions differing by their milk fat TAG composition, we used either anhydrous milk fat (AMF) alone, or in mixture (at 2:1 mass proportion) with a high melting (HMT) or a low melting (LMT) temperature fraction. All the powder ingredients were dry blended and dispersed in hot water (70°C), and mixed under stirring for 10 min with melted milk fat samples. These premixes were pasteurized at 80°C for 10 min and then they were coarsely mixed in an Ultra Turax® high-speed mixer (T25basic, IKA labortechnik, Germany) at 11000 rpm for 1.5 min, and passed through a twin-stage valve homogenizer (Niro Soavi S.P.A., GEA, Italy) at 110/40 bar for a higher reduction of fat droplet size. The three emulsions were quenched to 4°C, and aged at this temperature for 24 h. Then, samples of these emulsions were stored at two different temperatures (4 or –30°C) for several weeks, and then brought to 20°C and gently stirred before sampling for characterization.

Methods

Thermal transition in bulk fat and emulsion samples

Differential scanning calorimetry (DSC7, Perkin Elmer Corporation, USA) was used to monitor physical state transformations in various fat samples (AMF, AMF–HMT, and AMF–LMT), in either bulk or emulsified phase. Thermal transitions were evaluated by DSC experiments in temperature ranges lying between 50 to –45°C, through quantification of energy released (or absorbed) during cooling (or heating) cycles at 5°C min^{-1} [10]. Temperature calibration was performed using pure indium and lauric acid (C12), and samples of milk fat or emulsions were weighted (~10 mg) and sealed in aluminum pans, with an empty pan as the reference. Emulsions samples (30 mL) were stored for several weeks, in glass bottles (3 cm diameter) either at 4 or –30°C, thawed at room temperature, and then gently stirred before sampling for analysis.

Particle size measurement

Emulsions, stored at 4 or frozen at –30°C, were analysed for their fat droplet-size distributions and deter-

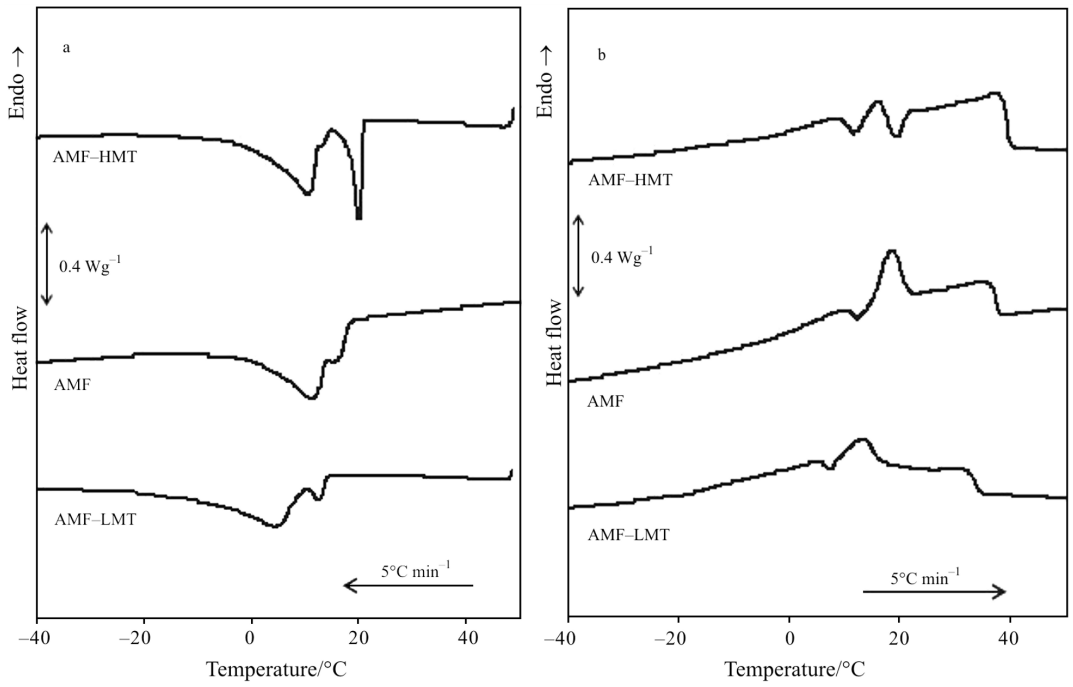

Fig. 1 DSC curves (5°C min^{-1}) obtained in bulk milk fat blends in the absence of SE30 (emulsifier-stabilizer mixture). a – cooling curves from 50 to –45°C, b – heating curves from –45 to 50°C after 5 min holding time at –45°C. AMF (anhydrous milk fat); LMT–HMT (low and high melting temperature fractions)

Table 1 Calorimetric parameters (5°C min^{-1}) of different blends of milk fat in bulk phase. (Cooling from 50 to –45°C and re-heating after 5 min holding at –45°C)

Sample		Fat crystallization				Fat melting		Supercooling/ °C
		T_{onset}/°C	$T_{peak\ 1}$/°C	$T_{peak\ 2}$/°C	ΔH/J g^{-1}	T_{end}/°C	ΔH/J g^{-1}	$\Delta T = T_{end} - T_{onset}$
Bulkfat without SE30	AMF	18.1±0.2	15.6±0.3	7.4±0.1	–53±0.5	38.9±0.3	97±0.9	21±0.5
	AMF–HMT	21.7±0.2	20.2±0.2	10.8±0.1	–58±0.9	40.9±0.4	100±1.3	19±0.6
	AMF–LMT	15.5±0.4	12.8±0.8	5.1±0.5	–48±0.7	35.8±0.5	90±1.7	20±0.9
Bulkfat with SE30	AMF	20.5±0.5	16.4±0.2	8.2±0.1	–55±1.0	40.9±0.1	95±1.7	20±0.6
	AMF–HMT	21.5±0.4	20.1±0.1	10.9±0.1	–58±0.6	41.1±0.2	104±0.4	21±0.6
	AMF–LMT	17.3±0.5	13.8±0.3	5.6±0.1	–49±0.4	38.1±0.4	89±1.1	21±0.8

mination of their mean volume-diameter $D_{4,3}$. This size parameter was deduced from integrated laser light scattering measurements (Malvern Mastersizer -MS 1000-Malvern Instruments, Orsay, France), on the basis of an independent model. After storage, the emulsions were brought to room temperature, and gently stirred before sampling and dilution in distilled water to approx. 1:1000. The presentation factor was selected after measuring the refractive index (ABBE Atago 3T) of dispersed fat relative to water (1.08) and absorbance (0.1) at 633 nm (spectrophotometer Varian Cary 100, Les Ulis, France). Changes in $D_{4,3}$ values were used for evaluation of structural stability against flocculation/coalescence in the various emulsions, before and after application of freeze-thaw procedure [15].

Epi-fluorescence microscopic observations

Emulsion fat droplets were observed by epi-fluorescence-microscopy (Olympus BX51TF, Japan), after staining using Nile Blue reactant (Sigma-Aldrich, France) as a fluorescent probe. The various emulsions were diluted to 0.01 mass% in distilled water (Millipore) and observed ×400 magnification.

Results and discussion

Thermal behavior of bulk fat samples

Crystallization and melting behavior of bulk fat samples (AMF, alone or in mixture with LMT and HMT fraction, at 2:1 mass proportion) was determined under cooling and re-heating cycles at 5°C min^{-1}. The corresponding heat flow curves (Fig. 1a and b) differed, depending on TAG composition of milk fat samples, as provided by AMF and HMT or LMT fractions. Samples containing AMF and its blends with LMT or HMT fractions indicated the presence of two distinguishable (or three) peaks of crystallization (or melting). Heat value released (or absorbed) upon cooling (or re-heating) of these fat samples was the lowest for the sample containing the LMT fraction. This result is due to a lower ratio of saturated-unsaturated TAG in AMF–LMT fat samples [2, 3]. Furthermore, results reported in Table 1 showed that addition of Cremodan SE30 (emulsifier and stabilizers blend) led to higher T_{onset} (crystallization) and T_{end} (melting) values in AMF and AMF-LMT samples, but no change for thermal transitions in AMF–HMT sample. Again, fat TAG composition and presence of additives, such as emulsifiers and polysaccharides con-

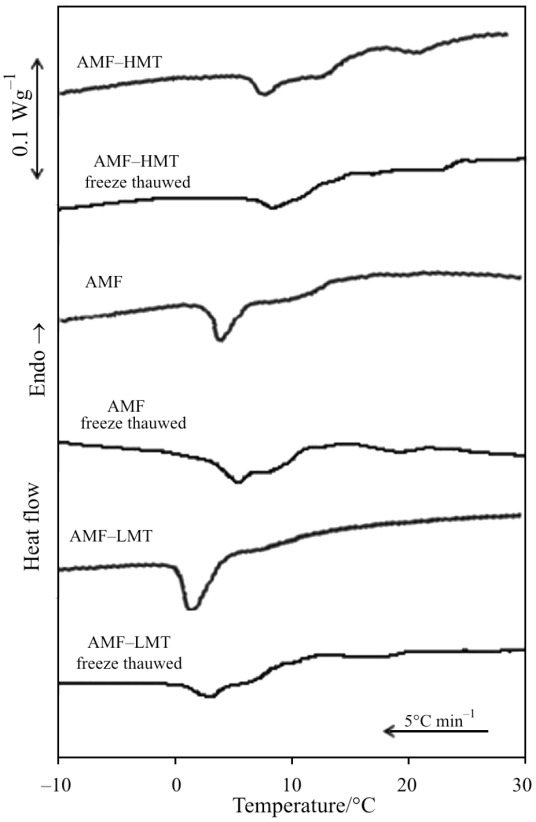

Fig. 2 Cooling DSC curves (from 50 to –45°C at 5°C min^{-1}) obtained from emulsions stored at 4°C, or freeze-thawed after storage at –30°C. AMF (anhydrous milk fat); LMT–HMT (low and high melting temperature fractions)

tained in the SE30 blend, seemed to affect differently the nucleation and crystallization rate, depending on the proportion of high and low melting temperature triacylglycerols.

Thermal behavior of emulsified fat samples

Heat flow curves observed during cooling steps of emulsions, which were stored at 4 or –30°C, are shown in Fig. 2. Calorimetric parameters evaluated from DSC cooling and re-heating curves are reported in Table 2.

Initial temperature of crystallization, T_{onset} observed from emulsions stored at 4°C (Table 2) was very close to that observed from corresponding bulk fat samples, only for the emulsion containing AMF–HMT fraction. It was lower (by approx. 6°C) for the other emulsions containing AMF in the absence or presence of LMT fraction. As previously demonstrated [6–9], differences in T_{onset} values between bulk fat samples and emulsions could indicate different nucleation mechanisms of fat crystallization, homogeneous nucleation mechanism occurring at a lower temperature than heterogeneous one. Following this hypothesis, nucleation of fat crystallization could be homogeneous in emulsions containing AMF in the absence or presence of LMT fraction, and heterogeneous in emulsion containing AMF–HMT fraction. Supercooling temperature values (ΔT) calculated from T_{end} (fat melting completion upon re-heating) and T_{onset} (temperature of initial heat flow release upon cooling) are also reported in Table 2. This parameter seemed to be dependent on TAG composition, in relation with the proportion of saturated/unsaturated fatty acids, as provided by different milk fat samples. Our results indicated that ΔT value is the lowest in the emulsion containing AMF–HMT fraction, where the proportion of saturated TAG was the highest.

Emulsions, which were submitted to storage at –30°C, presented a much lower supercooling value, ΔT (by ~4 to 10°C), compared to emulsions stored at 4°C. The re-crystallization T_{onset} values found in frozen-thawed emulsions were higher (by ~3°C) than for bulk fat samples, whatever the lipid composition. Previous studies showed that upon freeze-thawing cycles, both water and fat crystals could have effects on fat droplet crystallization [17]. This could explain the results found in the present study, whatever the TAG composition.

Table 2 Calorimetric parameters (5°C min^{-1}) of different emulsions containing different blends of milk fat and aged either at 4°C for 24 h or stored at –30°C for several weeks (Cooling from 50 to –45°C and re-heating after 5 min holding at –45°C)

Emulsion	Sample	Fat crystallization			Fat melting		Supercooling/°C
		T_{onset}/°C	$T_{peak\ max}$/°C	ΔH/J g^{-1}	T_{end}/°C	ΔH/J g^{-1}	$\Delta T = T_{end} - T_{onset}$
Aged emulsion	AMF	11.8±0.3	4.0±0.5	–18.0±1.7	36.0±0.5	54.3±8.2	24.2±0.8
	AMF–HMT	22.8±0.3	8.3±0.4	–19.7±2.8	40.9±0.6	56.7±1.3	18.0±0.9
	AMF–LMT	9.8±0.0	1.8±0.1	–18.0±3.5	34.9±0.6	25.8±3.6	25.0±0.6
Freeze-thawed emulsion	AMF	21.4±0.7	5.5±0.1	–25.2±4.1	36.5±0.4	46.4±4.4	15.0±1.1
	AMF–HMT	25.1±0.6	8.6±0.1	–18.0±2.2	39.2±0.7	56.6±0.1	14.1±1.3
	AMF–LMT	20.2±0.1	3.0±0.1	–22.7±2.4	35.6±0.3	26.2±4.9	15.4±0.4

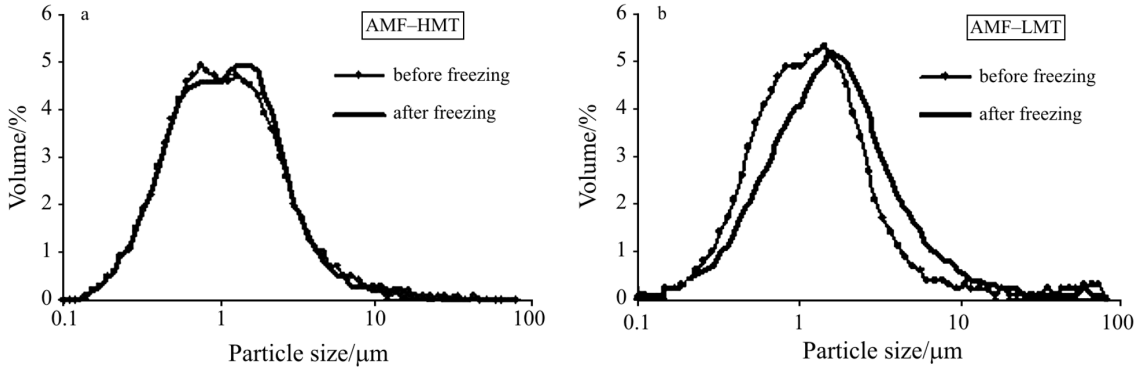

Fig. 3 Example of particle size distributions of fat droplets observed from emulsions, before and after freeze-thaw a – anhydrous milk fat and its high melting temperature fraction (AMF–HMT), and b – anhydrous milk fat and its low melting temperature fraction (AMF–LMT), before and after freezing

Effect of storage temperature on emulsion stability

Examples of fat droplet size distributions, observed from emulsions containing AMF in presence of LMT or HMT fraction, which were stored at 4°C or submitted to freeze-thaw process (–30°C), are shown in Fig. 3. They showed that after storage at 4°C, fat droplet size distribution presented two distinguishable peaks (at approx. 0.9 and 1.8 μm), whatever the lipid composition. However, after the freeze-thaw step, fat droplet size distribution observed for AMF–LMT emulsion (Fig. 3b) presented one principal peak located at a higher size (at approx. 2 μm). At the contrary, freeze-thaw step seemed to have no significant effect on fat droplet size in emulsions containing AMF in the absence or presence of HMT fraction (Fig. 3a). Mean volume diameter values ($D_{4,3}$), evaluated from emulsions differing by their milk fat TAG composition or storage conditions are reported in Table 3. The increase in $D_{4,3}$ value observed for AMF–LMT emulsion stored at –30°C, relative to emulsion storage at 4°C could be due to fat droplet aggregation-coalescence. This emulsion behavior, under the freeze-thaw procedure applied in this study, was not observed in emulsions containing AMF or mixture of AMF–HMT fraction. Water crystallization in emulsions may be accompanied by more solute concentration in the aqueous continuous phase. This could lead under freeze-thaw cycles, to a higher fat droplet close packing and instability by aggregation-coalescence mechanism [16]. Our results (Fig. 4) indicated that fat droplet coalescence could be the highest in the emulsion containing AMF–LMT fraction, where the liquid-solid fat content was the highest. At the contrary fat droplets in the emulsions containing AMF and AMF–HMT fraction, where the liquid-solid fat content was lower, appeared to be more stable against coalescence.

Table 3 Effect of storage temperature on average volume-diameter ($d_{(4,3)}$ in μm) of fat droplets in the emulsions prepared using anhydrous milk fat alone (AMF), or in mixture with high melting milk fat fraction (HMT) or with low melting milk fat fraction (LMT)

	AMF–HMT	AMF	AMF–LMT
Storage at 4°C	1.85±0.01	1.70±0.00	1.85±0.06
Storage at –30°C	1.72±0.02	1.70±0.04	3.00±0.05

Thus, quiescent storage of emulsions at –30°C seemed to have different effects on both DSC re-crystallization curves and fat droplet aggregation-coalescence, depending on their milk fat composition. T_{onset} values, initial temperature of exothermic heat flow deviation observed under cooling of frozen-thawed emulsions were much less affected in the emulsion where AMF was partially replaced by a HMT fraction. In parallel, light scattering measurements and

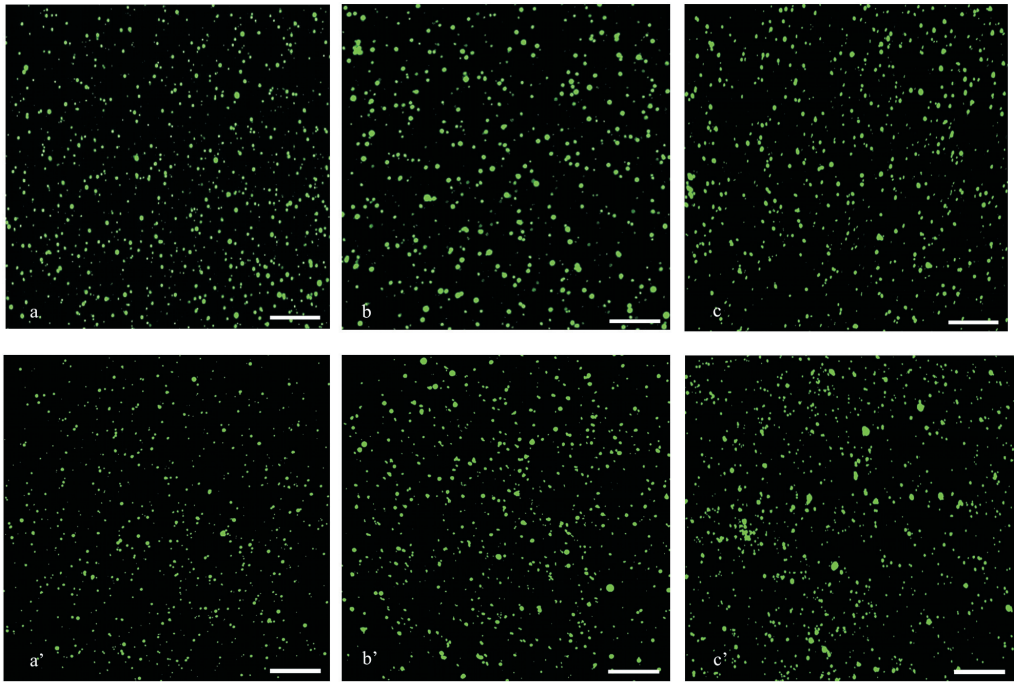

Fig. 4 Epi-fluorescence micrographs observed from emulsions containing different milk fat blends AMF (anyhydrous milk fat); LMT (low melting temperature fraction), HMT (high melting fraction), (labeling with Nile Blue, bright areas correspond to fat droplets), bar scale= 20 μm. a – AMF aged emulsions, b – AMF–HMT aged emulsion, c – AMF–LMT aged emulsion, a' – AMF freeze-thawed emulsion, b' – AMF–HMT freeze-thawed emulsion, c' – AMF–LMT freeze-thawed emulsion (400×)

fluorescence microscopic observations indicated a much higher effect on fat droplet aggregation-coalescence under freeze-thaw procedure in the emulsion where AMF was partially replaced by a LMT fraction. These results indicated that crystalline aqueous and fat phases in emulsions behave differently upon freeze-thaw procedure, depending on milk fat TAG composition. Coupling of DSC measurements with time-resolved synchrotron X-ray diffraction at both small and wide angles [17–19] could help to explain this behaviour, particularly through characterization of polymorphic transitions which could be responsible for emulsion stability or instability, under freeze-thaw procedure.

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References

1 P. H. Laakso, K. V. V. Nurmela and D. R. Homer, J. Agric. Food Chem., 40 (1992) 2472.
2 L. Lavigne, Ph.D. Doctorat thesis-ENSIA-Massy (1995) p. 360.
3 M. L. Herrera, M. de León Gatti and R. W. Hartel, Food Res. Int., 32 (1999) 289.
4 A. M. Abd El-Rahman, S. A. Madkor, F. Ibrahim and A. Kilara, J. Dairy Sci., 80 (1997) 1926.
5 P. Walstra and R. Jenness, Milk Fat Globules, In Dairy Chemistry and Physics, (1984) p. 254.
6 K. Boode, C. Bispernink and P. Walstra, Colloids Surf., 61 (1991) 55.
7 D. J. McClements, S. R. Duncan, J. B. German, C. Simoneau and J. E. Kinsella, J. Food Sci., 58 (1993) 1148.
8 C. Lopez, C. Bourgaux, P. Lesieur, G. Keller and M. Ollivon, J. Coll. Int. Sci., 254 (2002) 64.
9 P. Relkin, A. Ait-Taleb, S. Sourdet and P.-Y. Fosseux, J. Am. Oil Chem. Soc., 80 (2003) 741.
10 P. Relkin, S. Sourdet and P.-Y. Fosseux, J. Therm. Anal. Cal., 71 (2003) 183.
11 E. Dickinson, M. I. Coller, D. J. McClements, S. Peasgood and M. J. W. Povey, J. Chem. Soc. Faraday Trans., 86 (1990) 1147.
12 J. N. Coupland, Curr. Opin. Coll. Int. Sci., 7 (2002) 445.
13 D. Kalnin, G. Garnaud, H. Amenitsch and M. Ollivon, Food Res. Int., 35 (2002) 927.
14 P. Relkin and S.Sourdet, Food Hydrocoll., 19 (2005) 503.
15 S. Sourdet, P. Relkin, V. Aubry and P.-Y. Fosseux, Lait, 82 (2002) 567.
16 P. Thanasukarn, R. Pongsawatmanit and D. J. McClements, Colloids Surf., A: Physicochemical and Engineering Aspects, 246 (2004) 49.
17 T. Awad and K. Sato: In Physical Properties of Lipids; A. Marangoni, S. Narine, Eds; Marcel Dekker: New York 2002, Chapter 2, pp. 37–62.
18 D. Kalnin, O. Schafer, H. Amenitsch and M. Ollivon, M. Cryst. Growth Des., 4 (2004) 1283.
19 P. Relkin, F. Mariette, D. Kalnin, C. Michon and M. Ollivon, Science des Aliments, 25 (2005) 413.

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