Thermal Behavior of Fat Droplets as Related to Adsorbed Milk Proteins in Complex Food Emulsions. A DSC Study

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ABSTRACT: The thermal behavior of hydrogenated palm kernel oil-in-water emulsions, which differed in their milk-protein composition, was studied in parallel with other characteristic parameters such as the aggregation/coalescence of fat droplets, and the proportion of adsorbed proteins at the oil/water interface. DSC was applied to monitor the crystallization and melting behavior of nonemulsified and emulsified fat samples. Comparison between nonemulsified and emulsified fat samples showed that in emulsified samples the initial temperature of fat crystallization and the temperature of the completion of melting were invariably lower and slightly higher, respectively. Furthermore, in complex food emulsions the supercooling temperature needed to initiate fat crystallization and the variation in its growth rate in the cooling experiment were dependent on the amount and nature of the adsorbed proteins. Our results indicate that the total replacement of milk proteins by whey proteins affected the fat crystallization behavior of emulsified fat droplets, in parallel with changes in their protein surface coverage and in their physical stability against fat droplet agglomeration.

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In emulsions, the resistance to physical changes, such as flocculation/coalescence of fat globules, is related to properties of the interfacial layer around the fat globules, different physicochemical interactions or chemical bonds, and the interdroplet medium (1–3). In addition to these properties, fat crystallization has been demonstrated to play a role in emulsion stability (4,5). Numerous techniques such as dilatometry (6,7), ultrasonic velocity measurements (8,9), X-ray diffraction, and DSC (10–15) have been used to study physical state transitions in emulsions. Studies performed on hydrocarbon and TG oil-in-water emulsions, on emulsified milk, or on natural fat have focused on the crystallization mechanism in dispersed fats. They showed that the adsorbed emulsifiers might act as catalytic impurities, leading to a distinction between bulk heterogeneous and surface heterogeneous nucleation, and to a mechanism of secondary nucleation (16). More recently, Hindle *et al.* (9) showed that the kinetics of the secondary nucleation process in supercooled *n*-hexadecane and cocoa butter oil-in-water emulsions could be mediated by droplet–droplet collisions. In most of the previous studies, the droplet size, the structure of the hydrophobic chains of the emulsifier, and the nature of the fat were observed to have effects on the degree of supercooling and on the crystallization rate of finely dispersed fat, but without considering the possible effect of the composition of the fat protein layer on the crystallization behavior.

Milk proteins are largely used to improve the kinetic stability of food emulsions (3). Caseins (the major protein component of milk) have a micellar structure, and among their molecule components (α_{s1}-, α_{s2}-, β-, and κ-caseins) only α_{s2}- and κ-caseins contain disulfide bonds but no free thiol group. Whey proteins [β-lactoglobulin (β-lg) BSA, and immunoglobulin G (IgG)] contain both disulfide bonds and free sulfhydryl groups, whereas α-lactalbumin (α-la) contains disulfide bonds but no free thiol group; they have a globular tertiary structure that is characterized by the location of hydrophilic and ionizable amino acid groups on the surface and by hydrophobic ones mainly buried in the interior.

Recently, we studied the effect of the weight ratio of casein-to-whey proteins on the physical stability and fat crystallization in complex food emulsions that differed in their protein content (17,18). A different physical stability and different trends of fat crystallinity were observed in emulsions containing either a whey protein isolate (WPI) or a mixture of WPI and micellar caseins (17). In emulsions where skimmed milk powder (SMP) was totally or partially replaced by whey proteins, we observed aggregation between fat droplets only in the emulsion based on whey proteins but no coalescence whatever in the protein composition (18). Furthermore, determination of the composition of the fat protein layers in those emulsions indicated that a high proportion of proteins might be adsorbed in polymeric forms, particularly in the emulsions containing WPI.

In the present manuscript, we report on the fat crystallization behaviors in those last emulsions, which were stored for several weeks at –30°C.

MATERIALS AND METHODS

Emulsion preparation. The emulsions were manufactured at a pilot plant, as previously described (17,18). The emulsions consisted (all in weight proportions) of 3% milk proteins, 9% hydrogenated palm kernel oil, 5.3% lactose, 0.8% mineral

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ions from milk permeate ultrafiltrate, 14% sucrose, 3% glucose syrup (dextrose equivalent 40), and 0.5% stabilizer/emulsifier mixture composed of MG and DG, locust bean gum, guar gum, and carrageenan. They were based on the same milk solid nonfat content, but they differed in the nature of the milk protein powder used in the formulation. We used an SMP and a WPI to prepare three emulsions: one based on 100% SMP, the second based on a 50% weight ratio mixture of SMP and WPI, and the third based on 100% WPI. After preheating (72°C for 1 min in a plate exchanger), the premix was homogenized (110 + 40 bar, 72°C) using a two-stage APV Gaulin homogenizer (Evreux, France) and pasteurized (86°C for 30 s). After aging 24 h at 4°C, emulsion samples were distributed into flat pouches (20 × 10 × 0.5 cm) and submitted to freezing pulsed air as the cooling agent, then stored at –30°C for several weeks. Before characterization, the mixes were defrosted at 4°C overnight and brought to lab temperature before sampling.

Emulsion characterization. Structural parameters such as fat globule size distribution and the amount of adsorbed proteins in the three complex emulsions were determined. Experiments were performed on a Mastersizer apparatus (MS 1000-Malvern Instruments, Orsay, France) by using the Kjeldahl method, as described previously (18). Light-scattering measurements and protein determinations were taken from three and two independent samplings, respectively.

The crystallization and melting behaviors of hydrogenated bulk palm kernel oil and its emulsified fat globules were monitored, as described elsewhere (17). Cooling and reheating experiments ($2°C \cdot min^{-1}$) were performed by using a DSC7 PerkinElmer apparatus (with Pyris software) with sealed aluminum pans. The sample (approx. 10 mg) and the reference (empty pan) were held at 50°C for 5 min to melt the fat crystals, cooled to –40°C (bulk fat) or –10°C (emulsions), and then reheated to 50°C after a holding time (5 min). The following calorimetric parameters were deduced from the thermograms: temperature of the completion of melting (T_{end}), initial crystallization temperature (T_{onset}), temperatures of peak heat flow deviation (T_p), and fractional completion of the reaction (x) as a function of the cooling temperature. This last parameter was deduced from a pattern analysis of the evolution of heat flow (dh/dt) upon cooling and on the basis of the following assumptions:

$$A_T = x_T \int_{T_2}^{T_1} (dh/dt)_T \, dT = x_T^* \, \Delta_{cal}H \qquad [1]$$

A_T, the apparent heat of crystallization at temperature T, was calculated from the partial area under the exothermal heat flow curve; $\Delta_{cal}H$, the calorimetric heat of crystallization, was calculated by using a straight line drawn between the initial and final deviations of the heat flow. In this study, x_T was assumed to represent fractional completion of the crystallization reaction (or index of crystallinity).

The calorimetric parameters extracted from DSC measurements were obtained from at least three different experiments. Experimental uncertainties for T_{onset} and T_p were less than 0.3°C, and $\Delta_{cal}H$ was within ±5%. To avoid crystallization of water in the emulsified samples, the cooling step was performed up to –10°C, and all the crystallization curves were analyzed between T_{onset} (initial temperature of the first heat flow deviation from the baseline) and –10°C.

RESULTS AND DISCUSSION

Fat globule size distribution. The particle size distributions observed after dispersion of the emulsion samples in distilled water presented two distinguishable peaks (Fig. 1), indicating that emulsions with increased proportions of WPI contained increasing proportions of larger-size particles. These results are reflected in the increased average volume diameter ($D_{4,3}$) of fat globules in emulsions with WPI content (Table 1). In our previous study (18), performed on the same emulsions but before the freezing step, we observed a slightly bimodal particle size distribution for only the casein-free emulsion. However, as in the present study, when the defrosted emulsions were dispersed in the SDS solution, fat droplets corresponding to the larger size classes disappeared in favor of the smaller size class (located at approx. 0.5 µm), as shown in Figure 1. These differences between the two methods of observation (distilled water or SDS solution) and between emulsions stored at either 4 or at –30°C suggest that the second population of particles could be composed of noncovalently bound aggregates of fat droplets (18), and also that storage at –30°C caused more aggregation of fat droplets (higher values of $D_{4,3}$ in distilled water) than storage at 4°C but no coalescence [no significant change in values of the emulsion specific surface areas (SpA), which were determined after dispersion in the SDS solution].

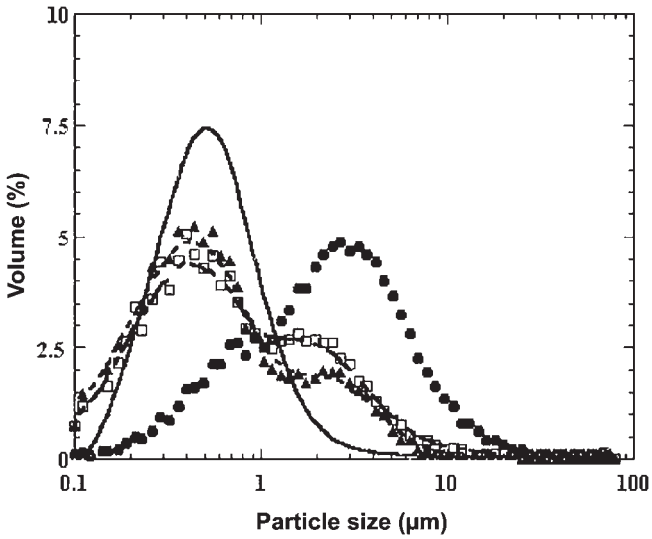

FIG. 1. Particle size distributions observed after dispersion in distilled water of emulsions containing skimmed milk powder (SMP) (▲), whey protein isolate (WPI) (●), or their mixture (SMP/WPI) (□). A monomodal size distribution was observed in all three emulsions after dispersion in 1% SDS solution (example of WPI emulsion, in bold curve).

TABLE 1
Effect of Storage Temperature on the Amount of Adsorbed Proteins at the Fat Droplet Surface (P_{ads}), the Average Volume Diameters ($D_{4,3}$), and the Specific Surface Area (SpA), as Determined by Light-Scattering Measurements After Dispersion in Distilled Water (for $D_{4,3}$ values) or SDS Solution (for SpA values)[a]

	P_{ads} (wt%)	P_{ads} (wt%)	$D_{4,3}$ (μm)	$D_{4,3}$ (μm)	SpA ($m^2 \cdot mL^{-1}$)	SpA ($m^2 \cdot mL^{-1}$)
Storage temperature	4°C	–30°C	4°C	–30°C	4°C	–30°C
SMP	1.50 ± 0.05	1.65 ± 0.05	0.77 ± 0.20	0.93 ± 0.06	14.7 ± 0.2	14.1 ± 0.2
SMP/WPI	1.40 ± 0.03	1.38 ± 0.05	0.82 ± 0.16	1.46 ± 0.05	13.7 ± 0.6	14.2 ± 0.3
WPI	0.65 ± 0.02	0.90 ± 0.04	1.2 ± 0.03	3.43 ± 0.05	12.8 ± 0.2	13.6 ± 0.5

[a]SMP, skimmed milk powder; WPI, whey protein isolate; SMP/WPI, mixture of SMP and WPI.

Amount of adsorbed proteins and fat globule protein load. The protein concentrations in the aqueous phases, which were separated from the cream layer after a centrifugation step, were used to determine the amount of adsorbed proteins at the fat droplet surface by their difference from the initial protein concentration. The results reported in Table 1 indicate that the amount of proteins, which were not depleted from the cream layer (considered as adsorbed proteins, P_{ads}), increased with the proportion of caseins. The decrease in the amount of adsorbed proteins with decreasing casein concentration was also observed previously in simple and complex emulsions (18–23). The results reported in Table 1 also indicate that storage at –30°C seemed to hinder protein depletion under the centrifugation step, particularly for the emulsion containing WPI. This could be explained by the formation of polymers between the adsorbed WPI monomers and the disulfide groups on other protein molecules, as previously observed in hydrocarbon emulsions stabilized by β-lg (24), and in our complex emulsions before the freezing step (18). The fat globule protein load (Γ), calculated by using the emulsion SpA obtained from light-scattering measurements in the SDS solution combined with the percentage of adsorbed proteins (%P_{ads}), increased from 6.5 to 11.3 $mg \cdot m^{-2}$ for emulsions stored at –30°C (instead of 4.4 to 10 $mg \cdot m^{-2}$ found after storage at 4°C), with decreasing concentration of WPI. This trend in the fat globule protein load is in good agreement with other results obtained from similar complex food emulsions (21–23).

Thermal behavior. We used a palm kernel fat (97.5% TG and 2.5% DG) composed mainly of the following FA: 12:0 (31.5%), 14:0 (11.3%), 16:0 (24.7%), 18:1 (20.5%), and 18:2 (3.9%) (Nestlé-PTC Beauvais, personal communication). The curves in Figure 2 were obtained upon cooling (a) and reheating (b) of a nonemulsified fat sample. They indicate that after melting of all the fat crystals, a supercooling (approx. 18°C) was needed to initiate crystallization (as detected through the first release of heat flow). The heat flow signal observed in the cooling experiment showed the presence of a shoulder located at 21.7°C, accompanied by a broad crystallization peak at 16.4°C. The shoulder could correspond to crystallization of high-melting TG and the major peak to crystallization of lower-melting components. The curves in Figure 3 were obtained upon cooling (a) and reheating (b) of the emulsion sample containing the mixture of SMP and WPI powders, at a 50% weight ratio. Temperatures for the completion of fat melting, and the melting enthalpy change values in nonemulsified and emulsified fat samples differed by less than 2°C and 15%, respectively (Table 2). Invariably, temperatures for the completion of fat melting were slightly higher for all three

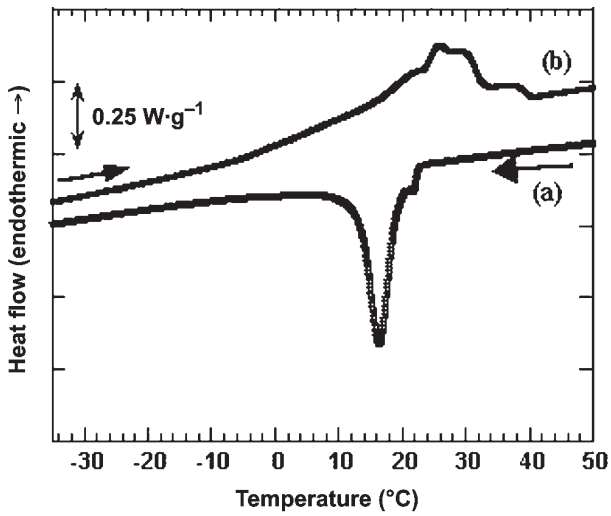

FIG. 2. Cooling (a) and heating (b) curves at $2°C \cdot min^{-1}$ obtained from a palm kernel fat sample used for an emulsion preparation.

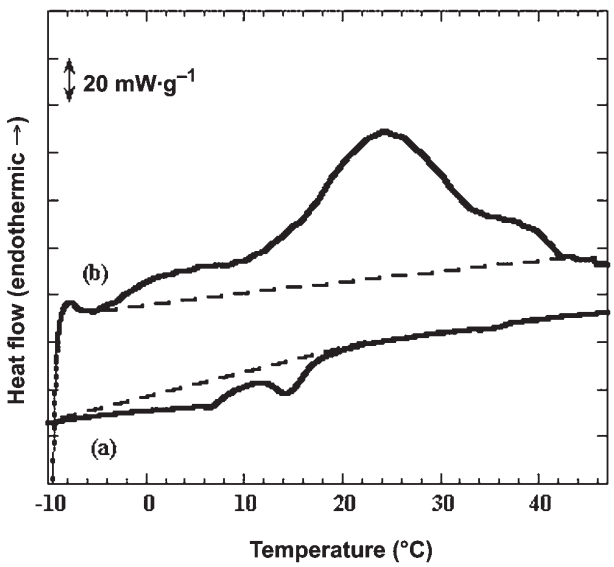

FIG. 3. Example of cooling (a) and heating (b) curves at $2°C \cdot min^{-1}$ obtained from an emulsion containing a mixture of SMP and WPI. See Figure 1 for abbreviations.

TABLE 2
Calorimetric Parameters of Crystallization and Melting of Bulk Fat and Emulsified Fat Samples Differing by Their Protein Content[a]

Sample	Transition	T_{onset} (°C ± 0.3)	T_p1 (°C ± 0.3)	T_p2 (°C ± 0.3)	T_{final} (°C ± 0.3)	$\Delta_{cal}H$ (J·g^{-1} fat ± 5%)
SMP	Crystallization	20.3	15.2	6.7	(–10)	–19.6
	Melting	(–5)	24.2	—	42.5	133
SMP/WPI	Crystallization	19.5	14.5	6.3	(–10)	–19.4
	Melting	(–5)	24.1	—	42.5	134
WPI	Crystallization	23.6	17.1	7.7	(–10)	–20.7
	Melting	(–5)	24.0	—	41.8	110
Bulk fat	Crystallization	23.1	21.7*, 16.4	—	(–10)	–103
	Melting	(–5)	23.5*, 25.7	29.6*, 38*	41.1	138

[a]Temperature values in parentheses were fixed to calculate calorimetric heat of reactions ($\Delta_{cal}H$) between T_{onset} (temperature of the initial deviation of heat flow) and T_{final} (completion of temperature deviation). T_p are peak temperatures, and (T)* are shoulder temperatures. SMP/WPI is mixture of SMP and WPI (50 wt%). See Table 1 for abbreviations.

emulsions than for the bulk fat sample, as observed previously for milk fat when in bulk or as globules in recombined creams (14). In comparison with bulk fat [Fig. 2(a)], the temperature of the initial crystallization of fat droplets in emulsions containing caseins [Figs. 3(a) and 4] was lower (by 3 to 4°C), and that for emulsions without caseins was slightly higher (by approx. 0.5°C). Concerning the amount of heat released upon cooling at 2°C·min^{-1}, it was approx. 75% and less than 20% of the heat needed to melt fat crystals formed in bulk fat and dispersed fat droplets in emulsions, respectively (Table 2). When emulsions were submitted to a similar thermal history except for the cooling rate, which was very much lower (0.3°C·min^{-1}, instead of 2°C·min^{-1}), the heat released upon cooling was also approximately 20% of the heat absorbed upon reheating (results not shown). The difference between these released and absorbed heat values could be due to crystallization of some TG fractions or to the polymorphic transition of crystals formed during the isothermal holding step at –10°C. This explanation is supported by the slight shoulder seen at approx. 4°C in the reheating thermogram of the emulsions [Fig. 3(b)], which is not seen in the bulk fat melting thermogram [Fig 2(b)]. Application of other techniques, such as X-ray diffraction, is needed to confirm this hypothesis.

Plots of the solids (determined by applying Eq. 1) detected in our conditions during the cooling experiments at 2°C·min^{-1} indicated that besides differences in supercooling temperatures to initiate crystallization from premelted samples of nonemulsified and emulsified fat, the degree of crystallinity was dependent on the protein types and cooling temperature ranges (Fig. 5). In particular, one can see that, in comparison with the bulk fat, there is a slight anticipated crystallization of fat for the WPI emulsion but a delayed fat crystallization in emulsions containing caseins. However, in the three emulsions, the growth rate of fat crystallinity seemed to behave differently in the WPI emulsion and in emulsions containing caseins. After the initial increase in fat crystallinity, the growth rate seemed to be reduced more significantly in emulsions containing caseins, and then become more accelerated

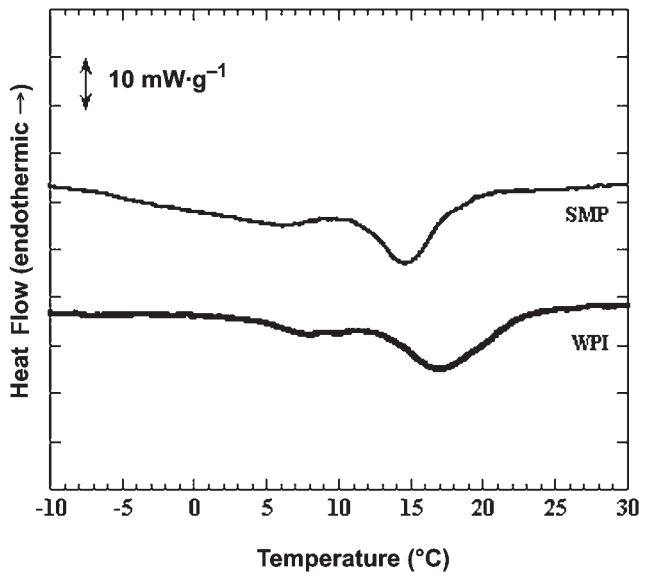

FIG. 4. Comparison of cooling curves at 2°C·min^{-1} for emulsions containing SMP or WPI. For abbreviations see Figure 1.

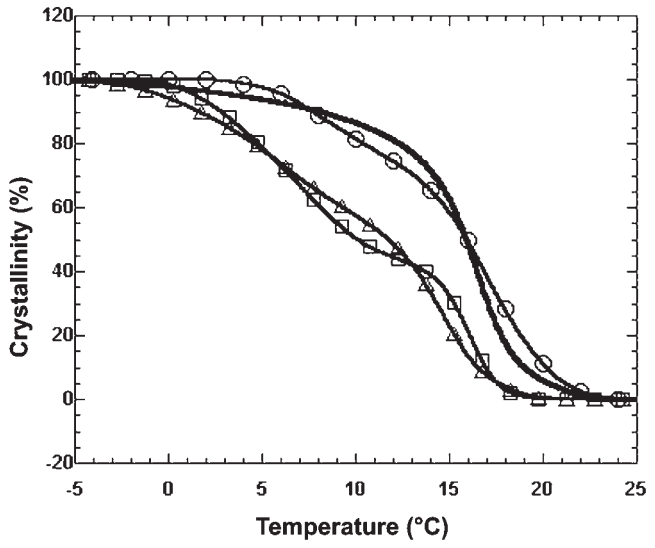

FIG. 5. Temperature dependence of crystallization in a bulk fat sample (bold curve) and in emulsions containing the WPI (○), the SMP (△), or SMP/WPI (□). For abbreviations see Figure 1.

in a third temperature range, than in the WPI emulsion. We observed a similar effect of protein type on the supercooling temperature for the crystallization of other complex emulsions, where we replaced SMP with WPI, either alone or in a mixture with micellar caseins (17). The anticipated (or delayed) fat crystallization occurring in a WPI emulsion (or emulsions containing caseins), compared to the bulk fat sample (Fig. 5), could be explained by a catalytic or noncatalytic action of adsorbed molecular species, as previously reported for small-M.W. emulsifiers in hydrocarbon and TG oil-in-water emulsions (5–7,11,12). Skoda and van den Tempel (6) demonstrated that in model emulsions, the degree to which nucleation is promoted increased with the degree of similarity between the molecular structure of the emulsifier and the crystallizing TG. They also assumed that micelles of MG could act as catalytic impurities for the crystallization of fats. However, in our case, the structure of whey proteins and caseins did not resemble that of TG. Furthermore, the results shown in Table 1 indicate that in our emulsion samples, despite the presence of MG and DG (known to compete with proteins for the interface), a significant amount of protein was adsorbed at the fat droplet surface. In emulsions containing caseins, there was a greater supercooling effect (Fig. 5), and after a first temperature range of fat crystallization, the increase in the degree of crystallinity seemed to be reduced more than in the WPI emulsion. In emulsions containing caseins, there were a lower level of fat droplet aggregation (but the average size of individual fat droplets was not significantly different) and larger amounts of adsorbed proteins than in the WPI emulsion (Table 1). That crystallization occurs in emulsified systems in distinguishable temperature regions (as shown in Fig. 5) was also observed previously in emulsions of TG stabilized by various emulsifier types (6,7) and in cocoa butter stabilized by either a small-M.W. emulsifier (Tween 80) or by caseins (9). In those studies, although the first increase in fat crystallization was attributed to the adsorbed emulsifiers, the following step (presenting a reduced crystallization rate) presumably arose from homogeneous nucleation, on account of the noncatalytic action of the adsorbed emulsifiers on finely dispersed fat droplets (5–8). The reduction in the crystallization rate after its sudden increase was also assumed to arise from droplet–droplet collision (9). From our results, which were obtained from a fat with a very wide range of different TG and milk proteins at different weight ratios and in combination with emulsifiers, sugars, and stabilizers, it appeared that the lowest difference between the fat crystallization behavior of nonemulsified and emulsified fat samples was observed for the WPI emulsion, where physical instability with respect to fat droplet aggregation (highest values of $D_{4,3}$) was the highest.

Our results showed that fat crystallization in emulsions is dependent on the nature of the adsorbed proteins, which influence the physical stability of fat droplets with respect to aggregation/coalescence. Replacement of SMP by WPI led to increased fat droplet agglomeration, a decreased amount of adsorbed proteins, and a different trend in fat crystallization in a controlled-cooling experiment compared to emulsions containing caseins. Further investigations are needed to clarify the role of the protein composition of fat layers on the crystallization mechanism of emulsified fats.

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