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FAT CRYSTALLIZATION IN COMPLEX FOOD EMULSIONS
Effects of adsorbed milk proteins and of a whipping process

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Abstract

Crystallization of fat droplets in complex emulsions, which differed only by the initial structure of proteins, was studied by differential scanning calorimetry, before and after application of a whipping process. Upon cooling at 5 or 1°C min^{-1}, the temperature needed to initiate fat crystallization was lower, and one more distinguishable crystallization peak was detected in emulsions containing caseins, in comparison with the emulsion containing pure whey proteins. Furthermore, the whipping process was accompanied by more protein depletion from the fat droplet surface, less resistance to coalescence, and a lower supercooling effect in the emulsion based on pure whey proteins.

Keywords: DSC, emulsion, fat crystallization, partial coalescence, whipped emulsion

Introduction

As with many food systems, the structural properties of emulsions determine their stability and sensory properties [1]. Being thermodynamically unstable, the resistance to physical changes, such as flocculation/coalescence of fat globules in oil/water emulsions, is achieved by addition of small surface active molecules and proteins, and also thickening polysaccharides [2]. The properties of the interface layer around the fat globules are due to different physico-chemical interactions or chemical bonds, depending on the interdroplet medium agents [3]. Besides these properties, fat crystallization has been demonstrated to play a role in emulsion stability [4]. Numerous techniques such as dilatometry [5], ultrasonic velocity measurements [6], X-ray diffraction and differential scanning calorimetry [8–11] have been used to study the physical state transitions in emulsions. For a long time, previous studies have been performed on hydrocarbon-in-water and triglycerides emulsions [5, 6, 12], and on emulsified milk fat [8, 10, 11]. They focused on the mechanism of crystallization in finely dispersed fats, and emulsifiers adsorbed at droplets interface were considered

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to act as catalytic impurities, with distinction between bulk heterogeneous and surface heterogeneous nucleation, and to a mechanism of a secondary nucleation [13]. In most of these studies, the droplet size and the structure of emulsifier hydrophobic chains, and the nature of fat (pure triglycerides, *n*-paraffins, milk fat) were observed to have effects on the degree of supercooling and on the crystallization rate of finely dispersed fat.

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_{S_1}, α_{S_2}, β and κ-caseins) only α_{S_2}- and κ-caseins contain disulfide bonds but no free thiol group. Whey proteins (β-lactoglobulin, β-lg; bovine serum albumin, BSA; and immunoglobulin G, IgG) have a globular tertiary structure, and they contain both disulfide bonds and free sulfhydryl groups, whereas α-lactalbumin (α-la) contains disulfide bonds but no free thiol group. These differences in structural properties are reflected by different adsorption properties at the solution- and oil and gas interfaces. Particularly, caseins and whey proteins adsorb around fat globules in simple [14, 15] and complex emulsions [16, 17], but surface concentration of caseins is higher than for whey proteins.

Recently [17] we have studied the protein composition of the interface layer around the globule fats in complex food emulsions, as affected by milk protein types (total milk proteins, pure whey proteins, and a mixture of skim milk powder and whey proteins), its effects on globule fat crystallization and melting [18]. In the present work, we focused on the crystallization behaviour, of two other complex emulsions based either on pure whey proteins or on a mixture of whey proteins and micellar caseins, paying attention to the effect of a whipping process on the resistance to coalescence.

Materials and methods

The manufacture of the emulsions was performed on a pilot plant, as described previously [17]. The emulsions consisted (all in mass proportion) of 2.25% milk proteins, 9% hydrogenated palmkernel oil, 5.3% lactose, 0.8% mineral ions from milk permeate ultrafiltrate, 14% sucrose, 3% glucose syrup (dextrose equivalent 40), and 0.5% stabilizer/emulsifier mixture composed of mono- and diglycerides, locust bean gum, guar gum and carrageenan. They were based on the same milk solid non fat content, but they differed by the nature of the milk protein powder used in the formulation. We used an isolate of micellar caseins (MC) and a whey protein isolate (WP) to prepare emulsions E100 (stabilized by 100 WPI) and E80 (stabilized by 80% WPI and 20% CSN). After pre-heating (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), pasteurized (86°C for 30 s). Samples of these emulsions were aged for 24 h (4°C), and other samples were submitted to a whipping process in a scrapped-surface heat exchanger at –4°C. These whipped emulsions (WE 100, and WE 80), were then stored at –30°C until characterization.

Structural parameters, such as fat globule size distribution, amount and nature of adsorbed proteins were determined in emulsions (E100 and E80), and in whipped

products (WP100 and WP80). We used Mastersizer apparatus (MS 1000-Malvern Instruments, Orsay, France), Kjeldahl method, and SDS-PAGE analysis (PhastSystem apparatus combined to Image Scanner and Image Master software, Pharmacia Biotech, Orsay, France), as previously described [17].

The crystallization behavior of hydrogenated bulk palm kernel oil, their emulsified fat globules, before and after the whipping process was studied by performing cooling experiments at two different constant-rates (5°C min^{-1} with DSC7 Perkin Elmer; and 1°C min^{-1} with μ-DSC Setaram III). The samples (10 to 60 mg) were loaded into one of the calorimeter pans, while the other (reference pan) was empty (DSC7) or contained undecane (μ-DSC). After a melting step from 25 to 50°C, the samples were cooled in the calorimeters up to –40°C (bulk fat) or –10°C (emulsions). To study the effects of the amount and nature of adsorbed proteins on the crystallization behavior of emulsified fat, we determined the following calorimetric parameters: initial crystallization temperature (T_i), temperature of maximum rate of crystallization (T_{max}), fractional completion of the reaction (x) as a function of cooling temperature. These last parameters were deduced from pattern analysis of the heat flow evolution (dh/dt) upon cooling, and on the basis of the following assumptions:

$$A_T = x_T \int_{T2}^{T1} (dh/dt)dT = x_T \Delta_{cal} H \quad (1)$$

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 base-line sample which was drawn between the initial and final deviations of the heat flow. In this study, x_T was assumed to represent the fractional completion of the reaction.

Results and discussion

Crystallization behaviour

The major fatty acid components of the vegetable fat used in this study are C12:0 (32%), C14:0 (11%), C16:0 (24.7%), and C18:1 (21%). The curves represented in Fig. 1 showed the pattern of heat released upon cooling bulk fat samples, from 45°C to either –40°C at 5°C min^{-1} (DSC7), or to –10°C at 1°C min^{-1} (μ-DSC). On cooling at 1°C min^{-1}, one broad crystallisation peak appeared between approx. 20 and 7°C, with a maximum deviation of heat flow located at T_{max}=12.7°C. On cooling at 5°C min^{-1} a sharp peak (T_{max}= 14.4°C) was observed between 16 and 12°C, was followed by a broader peak (T_{max}= 7.5°C) extending up to –10°C. The first peak could correspond to crystallisation of high melting triglyceride fat components, and the second which was observed only upon cooling at the faster rate (5°C min^{-1}) could correspond to crystallisation of lower melting fat triglyceride components [19]. The crystallization curves of emulsified fat samples containing either pure whey proteins (E100) or a mixture of 20% caseins and 80% whey proteins (E80) are shown in Fig. 2 and Fig. 3, respectively. The end temperature of fat melt-

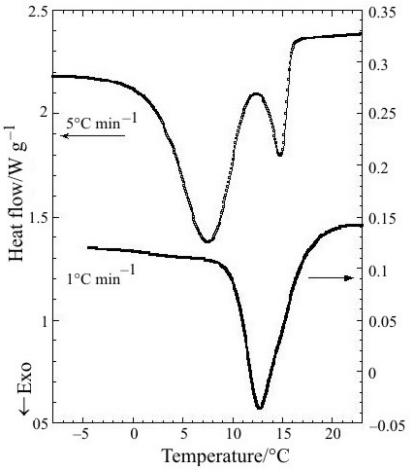

Fig. 1 Example of DSC curve of palm kernel hydrogenated fat (bulk fat) as obtained upon cooling from 45 to –40°C (at 5°C min^{-1}) and from 45 to –10°C (at 1°C min^{-1})

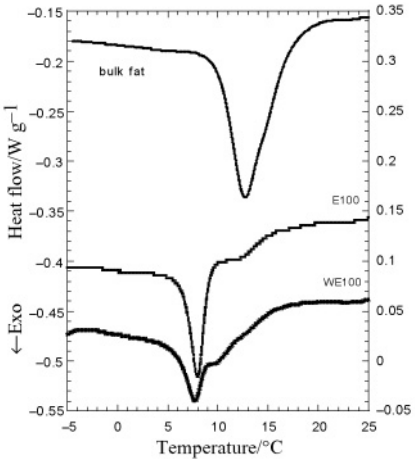

Fig. 2 DSC curves of bulk fat, emulsion (E100) and whipped emulsion (WE100) samples based on pure whey proteins and cooled from 45 to –10°C (at 1°C min^{-1})

ing (results not shown) in non-emulsified and emulsified samples (approx. 36°C) was invariably much higher than the onset temperatures of crystallisation (14.7°C for E100, 10°C for E80, and 20°C for bulk fat), in similar conditions of heat-treatment (from 45°C to –10°C, at 1°C min^{-1}). Thus the supercooling effect seemed to be higher for the emulsion containing caseins (E80) than for the emulsion containing only whey proteins (E100), and for bulk fat. Furthermore, upon cooling at both 1 or 5°C min^{-1}, the emulsion E80 presented two distinguishable exothermic peaks (Fig. 3), whereas the emulsion E100 presented a crystallization heat flow pattern with a major peak which seemed to be preceded by a slight shoulder (Fig. 2). This difference is probably due to differences in the protein types used to prepare the two emulsions, but not to the difference in the cooling

rate. The crystallization temperature domain lying between 15 and 5°C, seemed to be independent on the protein types used to prepare each emulsion. However, the fractional completion of reaction in this temperature region, calculated by using Eq. (1), was higher than 80% for E100 which did not contain caseins, and lower than 50% for E80 which contained 20% caseins (Fig. 4a). The overall heat of reactions released upon cooling up to –10°C and adsorbed upon melting were in the following order E100<E80<fat, indicating that less solid fat content was developed in fat droplets stabilized by pure whey proteins than by mixture of whey proteins and caseins.

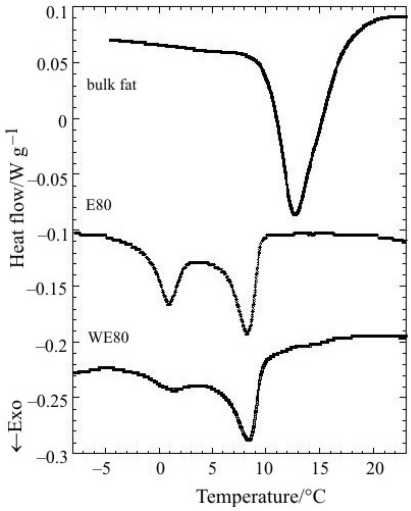

Fig. 3 DSC curves of bulk fat, emulsion (E80) and whipped emulsion (WE80) samples based on pure whey proteins, and cooled from 45 to –10°C (at 1°C min^{-1})

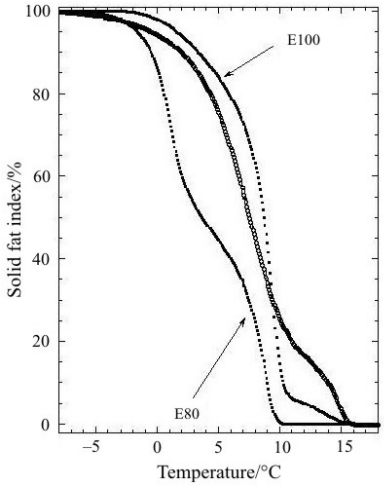

Fig. 4a Evolution of solid fat index, as a function of temperature for bulk fat and emulsion samples based on pure whey proteins (E100) and mixture of 80% whey proteins and 20% caseins (E80), and cooled from 45 to –10°C (at 5°C min^{-1})

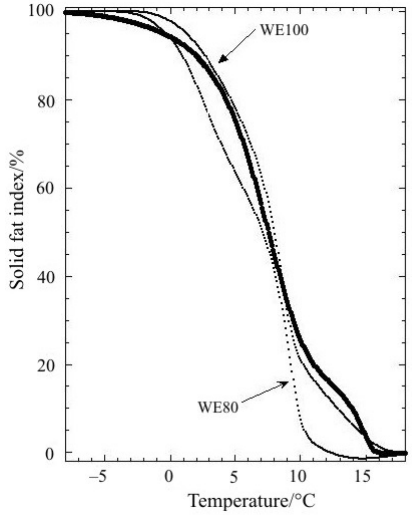

Fig. 4b Evolution of solid fat index as a function of temperature for bulk fat and whipped emulsion samples based on pure whey proteins (WE100), and on mixture of 80% whey proteins and 20% caseins (WE80), and cooled from 45 to –10°C (at 5°C min^{-1})

When whipped emulsions were cooled at 1°C min^{-1}, the heat flow pattern shown in Fig. 2 indicated an increase in the shoulder height located at the left of the main crystallization peak for the WE100. However, the peak located at the left side of the main crystallization peak observed for E80 sample became less significant, in comparison with the corresponding non-whipped emulsion (Fig. 3). In the same way, the curves shown in Fig. 4b which were obtained upon cooling at 5°C min^{-1}, indicated less difference between the onset temperature of crystallization between the whipped emulsions and non-emulsified fat sample, particularly for WE100. Thus both the protein types used to stabilize the emulsions, and the further whipping process at –4°C, have effects on the DSC heat flow patterns observed upon cooling.

Fat globule size distribution

The fat globule size distributions observed after dispersion in distilled water of the two emulsions indicated a bimodal shape, with a shoulder located at approximately 0.8 μm and a principal peak at approx. 2 μm. When the two emulsions were dispersed in SDS solutions, the globule size distributions became monomodal, indicating that the physical change in emulsion structure upon storage, was due to aggregation of fat globules and not to coalescence. After aggregate dissociation under the effect of SDS molecules, the $D_{4,3}$ values (Figs 5a and 5b), decreased to a similar value (0.81±0.02 μm for E100 and 0.87 ±0.07 μm for E80) whatever the emulsion protein composition.

The fat globule size distributions of the whipped emulsions, were also bimodal when observed after dispersion in distilled water. But, contrary to the non-whipped emulsions, after dispersion in SDS solutions the fat globule size distribution re-

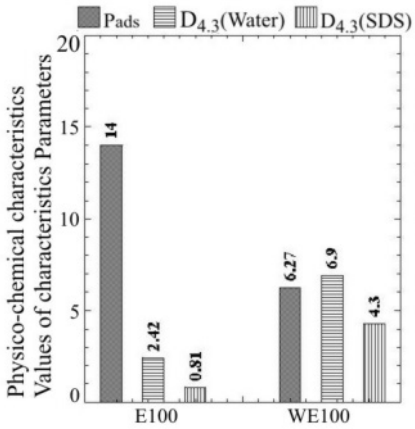

Fig. 5a Physico-chemical characteristics of fat droplets in emulsion E100, and whipped emulsion WE100 based on pure whey proteins. P_{ads} is the protein coverage in $mg_{protein}\ g_{fat}^{-1}$, $D_{4,3}$ (water) and $D_{4,3}$ (SDS) are the average volume-surface diameters (in μm) of fat droplets observed after dispersion in distilled water and in SDS solution (see text)

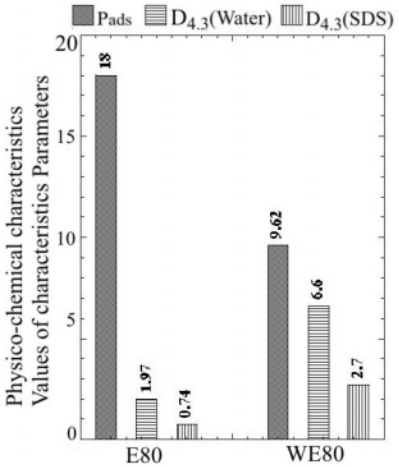

Fig. 5b Physico-chemical characteristics of fat droplets in emulsion E80, and whipped emulsion WE80 based on mixture of 80% whey proteins and 20% caseins. P_{ads} is the protein coverage in $mg_{protein}\ g_{fat}^{-1}$, $D_{4,3}$ (water) and $D_{4,3}$ (SDS) are the average volume-surface diameters (in μm) of fat droplets observed after dispersion in distilled water and in SDS solution (see text)

mained bimodal for both the two emulsions. The $D_{4,3}$ values of WE100 and WE80 were much more higher than those of E100 and E80, suggesting that whipping process in our conditions could induce coalescence or covalent bridging between fat droplets. Furthermore, one of these mechanisms (or both) seemed to be more favoured in the absence of caseins (WE100) than in their presence (WE80). It is worthy to note that the development of solid fat content upon cooling up to –10°C emulsions

without caseins (WE100), was lower than for WE80 by approximately 20%. In parallel, the average droplet sizes in the corresponding whipped emulsions increased in the order WE100>WE80.

Amount of adsorbed proteins

After a centrifugation step performed at 5000 g for 20 min, the cream layers separated from the aqueous phases of the two emulsions were analysed for their protein and fat contents by using Kjeldahl and Monjonnier methods, respectively, as described in [17]. The mass ratio of adsorbed proteins-to-cream fat (and the $D_{4,3}$ value) is higher (and lower) for E80 than E100 emulsion (Fig. 5a). As previously demonstrated [17], these results indicated that although the presence of mono- and di-glycerides and polysaccharides, caseins are more surface active than whey proteins. Under the whipping process used in this study, the amount of adsorbed proteins, P_{ads}, relative to 1 g of fat (Fig. 5b) decreased to half of the values found before application of the whipping process, and consequently the values of $D_{4,3}$ increased, indicating less resistance to coalescence. It is noteworthy again that this trend is more significant for whipped emulsions without caseins.

Likely in simple emulsions, we observed in the present study that whey proteins have less surface adsorption properties at the oil/water interface when alone, than when in mixture with caseins. These preferential adsorption properties of caseins over whey proteins are accompanied by i) a higher resistance to flocculation/coalescence in emulsions containing caseins, by ii) a higher supercooling effect needed to initiate fat droplet crystallisation in emulsions and whipped emulsions containing caseins, and iii) similar trend of fat crystallization in bulk fat and in casein-free whipped emulsions, where the highest fat droplet coalescence was observed.

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