

Protein-stabilized oil-in-water emulsions and low-fat dairy stirred gels

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Summary

The evolution of the oil-water interface was investigated throughout the preparation of stirred acid gels, which were models of stirred yoghurts. Stock emulsions were prepared (200 g.kg⁻¹) and diluted to the final values of 15 g.kg⁻¹ or 5 g.kg⁻¹ by a solution of milk proteins. Gels were obtained by addition of glucono- δ -lactone and stirred. Evolution of the protein load and composition of the interface was investigated at 10, 25 and 43 °C. The protein load at the oil-water interface (mg.m⁻²) was similar regardless of the nature and level of fat, and the liquid versus gel state of the continuous phase. It was reduced upon storage at 10 °C, but tended to increase at higher temperatures. The caseins / whey proteins ratio (Cas/WP) was higher at the interface of fresh emulsions (fat content, 200 g.kg⁻¹) than in skimmed milk. It decreased in low-fat matrices stored at 10 °C, becoming generally not significantly different from that of skimmed milk. At the interface of stirred gels, it also decreased significantly with the temperature of storage. Re-organization of interfacial casein micelles and secondary adsorption of WP were involved. The protein load and composition at the interface of the final products could not be simply extrapolated from that of the original concentrated emulsions.

Keywords

whey proteins; caseins; milk fat; emulsion; acid gel; oil-water interface

Acidified milk products are produced throughout the world, with yoghurt being one of the most popular. In such products, the fat is dispersed as milk fat droplets and can be primarily described as an oil-in-water emulsion, and secondly as a gelled emulsion after the acidification process. Although competition between the adsorption of individual proteins has been widely studied at the oil-water interface [1–4], little is known about the competition taking place in complex products where proteins are in excess in the continuous phase of the emulsions. Changing the dispersed phase of dodecane into medium chain triacylglycerols or into sunflower oil modified the structure of bovine serum albumin at the interface [5]. When soybean oil was replaced by milk fat, the protein load at the interface of the caseinate-stabilized emulsion decreased during storage at temperatures below 15 °C [6]. Authors explained the changes in terms of different solid-fat contents of milk fat and soybean oil, without mention of their difference of triacylglycerol composition.

The objective of the present work was to evaluate the impact of the modifications of the composition and organization of emulsions and stirred gels on the oil-water interface during their preparation. These matrices have been designed to model stirred yoghurts, from milk to final products. The composition of the matrices was modified by varying the nature and level of the emulsified fat. Using two fractions of anhydrous milk fat allowed us to modify greatly the solid fat content, with limited modifications of the fatty acid composition. The role of the organization of the continuous phase (i.e. liquid or gelled) was evaluated by comparison of the results on emulsions and stirred acid gels.

MATERIALS AND METHODS

Materials

Lactalis (Laval, France) provided the low-melting-point fraction (LMP) of anhydrous milk fat and the high-melting-point fraction (HMP) of anhy-

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drous milk fat. The triacylglycerol (TAG) composition was determined by reverse-phase HPLC as described by ROBINSON and MACGIBBON [7]. Two columns (Altima C18, 5 μ m, 4.6 mm \times 250 mm: Alltech, Deerfield, Illinois, USA) were used for the separation of TAG, which were detected and quantified with an evaporative light-scattering detector (Sedex 55; Sedere, Alfortville, France). The results were reported as % of the total peak area. The melting point of each milk fat fraction was calculated from the melting curves determined with a differential scanning calorimeter (DSC121; Setaram, Caluire, France) calibrated with indium and gallium. The melting point of LMP was 20 °C, and LMP contained 54.0% of low-melting-point TAG, 40.7% of medium-melting point TAG and 5.3% of high-melting-point TAG. The melting point of HMP was 48 °C and HMP contained 28.2% of low-melting-point TAG, 45.1% of medium-melting-point TAG and 26.7% of high-melting-point TAG. Ingredia Dairy Ingredients (Saint Pol-sur-Ternoise, France) supplied the skimmed milk powder (low-heat grade), which contained 50.3% (w/w) lactose and 34.9% (w/w) proteins. Roquette (Lestrem, France) supplied the glucono- δ -lactone (GDL, LysactoneR). Commercial sucrose (Beghin Say-Tereos, Lille, France) and mineral water (Danone, Volvic, France) were purchased from a local supermarket. Buffer salts (purities >99%) and other chemicals (Analytical grade) were purchased from Sigma-Aldrich (Saint Quentin Falavier, France).

Experimental design

Our initial objective was to prepare model systems with protein and fat contents close to that of commercial dairy products, in a single-step process. Preliminary trials revealed that emulsions prepared with a protein concentration of 45 g·kg⁻¹ and a fat content of 15 or 50 g·kg⁻¹ possessed a droplet size distribution below 0.3 μ m. This did not allow to separate the oil droplets from casein micelles by centrifugation and, thus, to characterize the interface. Consequently, stock emulsions were first prepared with a protein concentration of 20 g·kg⁻¹ and a fat content of 200 g·kg⁻¹. Stock emulsions were then diluted with solutions containing the required protein and sucrose concentrations to obtain the final protein concentration of 45 g·kg⁻¹, a fat content of 15 g·kg⁻¹ or 50 g·kg⁻¹ and a lactose + sucrose concentration of 150 g·kg⁻¹. These low-fat emulsions were then acidified at 43 °C with GDL, and the resulting gels stirred. The stability of the fat droplets in the matrices was evaluated throughout preparation and after storage at 10, 25 or 43 °C by measurement of the droplet size distribution. The protein load at the oil-water interface and

protein composition of the interface layer were determined throughout the preparation. Each matrix was prepared and analysed at least in triplicate.

Preparation of dairy matrices

Emulsions

Recombined skimmed milk (protein content, 20 g·kg⁻¹) was prepared by adding skimmed milk powder to mineral water. The mixture was stirred overnight at 4 °C to ensure complete dispersion and hydration of the proteins, and then heated until the temperature reached 90 °C (i.e. 20 min). The anhydrous milk fat fractions were heated at 70 °C, and this temperature held for 30 min to erase their thermal history. The milk fat fraction (200 g·kg⁻¹) and recombined milk were premixed at 70 °C for 1 min at 250 Hz using a rotor-stator system (Polytron PT 6100; Kinematica, Littau, Switzerland) equipped with a 12 mm head. The coarse emulsions were then recirculated for 10 min at 70 °C through a high-pressure valve homogenizer (A0812W-A-CD; Stansted Fluid Power, Stansted, United Kingdom), set at 2 MPa, to obtain the stock emulsions with a fat content of 200 g·kg⁻¹.

Dilution solutions were prepared with skimmed milk powder and sucrose as described previously and then heated at 90 °C for 20 min. The dilution solution had a protein content of 50 g·kg⁻¹, a sucrose content of 80 g·kg⁻¹ to prepare emulsions with a fat content of 15 g·kg⁻¹, and a protein content of 56 g·kg⁻¹, a sucrose content of 100 g·kg⁻¹ to prepare emulsions with a fat content of 50 g·kg⁻¹ fat. These emulsions were stirred overnight at 10 °C before the acidification process.

Acid gels

The emulsions were acidified at 43 °C with glucono- δ -lactone as an acid precursor. The GDL concentration was adjusted for each matrix to reach a final pH of 4.6 within 4 h. It was 1.55% (w/v) for emulsions with a fat content of 15 g·kg⁻¹ and 1.65% (w/v) for emulsions with a fat content of 50 g·kg⁻¹. To slow down the hydrolysis rate and better mimic the pH decrease during the fermentation, GDL was added in two stages. First, 0.285% (w/v) GDL was added and the emulsions were stirred until a pH of 6.0. Then, the remaining GDL was added and the matrices were kept in a temperature-controlled incubator at 43 °C for 4 h. The resulting gels were stored overnight at 10 °C and thereafter stirred for 2 min with a rotor-stator system (Polytron PT 6100) equipped with a 12 mm diameter head working at 50 Hz. The stirred gels were stored at 10, 25, or 43 °C overnight before analysis. All samples were prepared at least in triplicate.

Determination of the droplet size distribution

The droplet size distribution was measured at ambient temperature using a static laser ($\lambda = 658$ nm) light-scattering analyser (Saturn DigiSizer 5200; Micromeritics Instrument Corporation, Atlanta, Georgia, USA). The refractive indices of the fat fractions and continuous phase (water) were set up at 1.458 and 1.331, respectively [8]. Before measurement, emulsions were diluted (1:1 v/v) with 35 mmol·l⁻¹ ethylene diamine tetra acetic acid (EDTA), which pH was adjusted to 7.0 with NaOH, to dissociate casein micelles and aggregates [8]. To determine the droplet size distribution of stirred acid gels, samples were diluted (1:5 v/v) with 6 mol·l⁻¹ urea, 100 mmol·l⁻¹ EDTA, 20 mmol·l⁻¹ imidazole buffer pH 7.0 [9] and stirred for 30 min prior to measurement, as described above. The mean droplet size was characterized by the volume-surface average diameter, d_{32} , of the fat droplets defined by:

$$d_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (1)$$

where n_i is the number of droplets of diameter d_i .

The specific surface area, SSA (m²·cm⁻³ lipid phase), was calculated according to:

$$SSA = \frac{6\Phi}{d_{32}} \quad (2)$$

where Φ is the fat mass fraction.

Protein load at oil-water interface

The oil droplets were washed from unabsorbed proteins according to the method described by PATTON and HUSTON [10] as modified later [11]. Emulsions or stirred gels were diluted (1:5 v/v) with a dissociation buffer of pH 7.0 (6 mol·l⁻¹ urea, 100 mmol·l⁻¹ EDTA, 20 mmol·l⁻¹ imidazole) and stirred gently for 30 min. The mixtures (2 g) were then diluted by 2 g of a sucrose solution (500 g·kg⁻¹). As EDTA dissociates both casein micelles and protein aggregates, oil droplets from the stock emulsions (with a fat content of 200 g·kg⁻¹) were also processed without the addition of EDTA. Then, samples were carefully deposited with a syringe at the bottom of centrifuge tubes containing 10 ml of a sucrose solution (150 g·kg⁻¹). The tubes were centrifuged at 43 °C for 2 h at 1500 g. At this temperature, fats were kept liquid to prevent coalescence and the partial desorption of proteins from the interface [12]. After centrifugation, the tubes were frozen at -20 °C, and the creamed oil droplets carefully scraped with a spatula. Subsequently, a solution containing SDS (10 g·kg⁻¹) was added to displace the proteins from the oil droplet

interface. After 1 h at room temperature, samples were centrifuged at 50 °C for 1 h at 3000 g. The lower phase contained the desorbed proteins from the interface, which were quantified [13]. The interfacial protein concentration or protein load (Γ , mg·m⁻² interface) was calculated as follows:

$$\Gamma = \frac{[proteins]_{ads}}{SSA} \quad (3)$$

where $[proteins]_{ads}$ is the concentration of the adsorbed proteins in emulsions or stirred gels (mg·cm⁻³), and SSA was calculated according to eq (2).

Protein composition of the oil-water interface

The protein composition of the oil-water interface was analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) from oil droplets washed as described earlier. The washed droplets were mixed with an equal volume of the dissociation buffer consisting of 0.5 M Tris-HCl (pH 6.8), 0.05% (w/v) bromophenol blue, 10% (w/v) glycerol, 10% (w/v) β -mercaptoethanol and 4% (w/v) SDS. Electrophoreses were run on polyacrylamide gels (stacking: 3.5%, resolving: 12%) with a migration buffer consisting of 0.1 M Tris-HCl (pH 8.25), 0.1 M tricine and, 0.1% (w/v) SDS. Proteins were stained with a Coomassie Brilliant Blue solution, containing 0.05% (w/v) Coomassie blue R-250, 25% (w/v) ethanol and 10% (w/v) acetic acid. The electrophoresis gels were scanned on an imaging densitometer Bio-Rad GS710, and the surface of each band was determined with Quantity one 4.1 software (both Bio-Rad, Ivry-sur-Seine, France). The sum of the surfaces of bands corresponding to β -lactoglobulin and α -lactalbumin, major proteins of whey (WP), and those corresponding to α_s , β and κ casein, caseins (Cas), were calculated. The results were expressed as caseins to whey proteins ratio (Cas/WP).

Statistical analysis

Nine determinations were obtained for the average droplet diameter and for protein load at the interface of low-fat matrices. Three measurements were conducted for the protein load at the interface of stock emulsions and for Cas/WP. Results were subjected to multifactor analysis of variance (MANOVA) to examine the effect of the nature of fat, the temperature, the oil mass fraction and the structure (emulsion or stirred acid gel). If significant effects were found, multiple range tests were performed to determine which means were significantly different according to the Fischer's Least Significant Differences (LSD). The significance

level applied throughout the study was $p < 0.05$. Statistical analyses were performed with Statgraphics Plus version 3.0 software (Statistical Graphics Corporation, Rockville, Maryland, USA).

RESULTS

Evolution of fat droplets during preparation and storage of emulsions

Droplet size distribution

The droplet size distributions of the freshly prepared stock emulsions (Fig. 1A) were similar within the experimental error regardless of the nature of the emulsified fat. In the experimental conditions, the volume-surface mean diameters (d_{32}) were $1.0 \mu\text{m}$ and $1.1 \mu\text{m}$ for emulsions prepared with the LMP and HMP fractions, respectively. The standard deviation was $0.1 \mu\text{m}$ for both emulsions ($n = 9$). After dilution of the emulsions and enrichment of the continuous phase in proteins to obtain the low-fat emulsions, the droplet size distributions remained monomodal (Fig. 1B). The droplet size distributions were similar within the experimental error regardless of the nature of the emulsified fat. The volume-surface mean diameter (d_{32}) ranged from 1.0 to $1.1 \mu\text{m}$ for emulsions prepared with LMP fraction and from 1.1 to $1.2 \mu\text{m}$ for emulsions prepared with HMP fraction, with standard deviations of $0.1 \mu\text{m}$, similar to stock emulsions ($n = 9$). The emulsions were stable under quiescent conditions; neither coalescence, nor flocculation or droplet size variation were observed after 24 h at 10, 25, or 43 °C and up to 72 h (data not shown).

Protein load at oil-water interface

The protein load at the oil-water interface was compared in stock and low-fat emulsions to ensure that it was not being modified by the dilution of oil droplets by a concentrated solution of proteins. As the separation of oil droplets from stirred gels had to be performed with EDTA to dissociate the casein network, oil droplets from the emulsions were also separated with the same procedure, and the results were compared to those obtained without EDTA. The protein load at the interface of freshly prepared stock emulsions (fat content, $200 \text{ g}\cdot\text{kg}^{-1}$) was about $5.5 \text{ mg}\cdot\text{m}^{-2}$ (Tab. 1). It did not depend on the nature of the emulsified fat. A significant decrease in the protein load at the interface ($p < 0.05$) occurred upon storage. Thus, after 24 h of storage at 10 °C and subsequent separation without EDTA, the protein load fell to $2.4 \text{ mg}\cdot\text{m}^{-2}$ regardless of the nature of the emulsified fat. When oil droplets were centrifuged in the presence

of EDTA, the protein load was significantly lower ($p < 0.05$) than it was without EDTA ($1.8 \text{ mg}\cdot\text{m}^{-2}$). After 24 h of storage at 10 °C and subsequent centrifugation in the presence of EDTA, the protein load increased to approximately $2.5 \text{ mg}\cdot\text{m}^{-2}$. It was not significantly different from the values obtained after storage and when centrifugation was performed without EDTA, except for the protein load

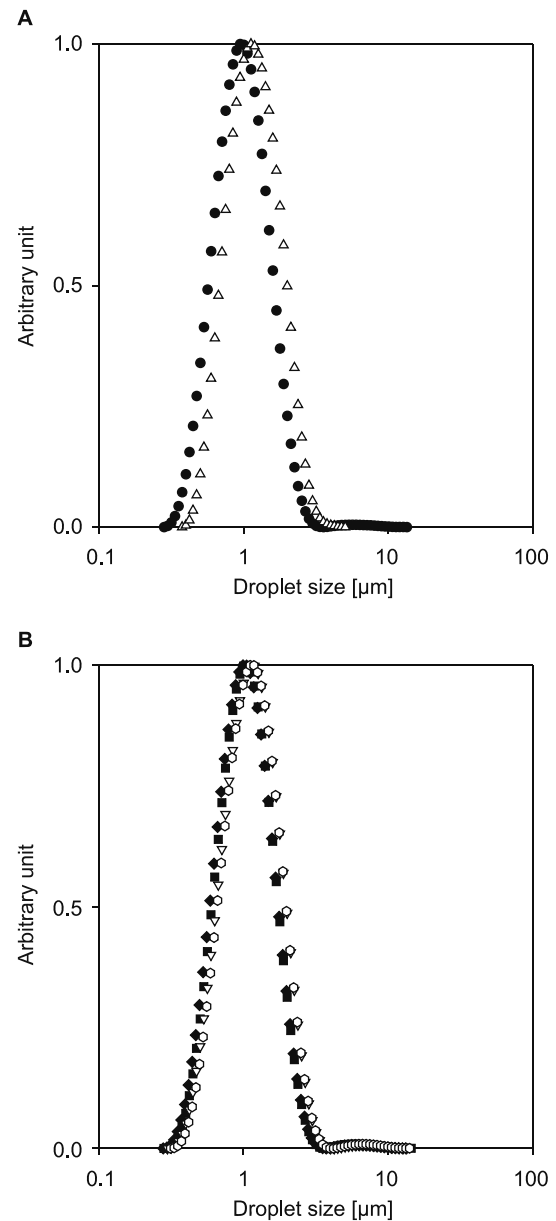


Fig. 1. Droplet size distributions of dairy emulsions.

A – Stock emulsions prepared with $200 \text{ g}\cdot\text{kg}^{-1}$ of the LMP fraction of milk fat (●) or with the HMP fraction of milk fat (△).

B – Low-fat emulsions prepared by dilution of the corresponding stock emulsions. (■) $15 \text{ g}\cdot\text{kg}^{-1}$ LMP, (◆) $50 \text{ g}\cdot\text{kg}^{-1}$ LMP, (▽) $15 \text{ g}\cdot\text{kg}^{-1}$ HMP, (○) $50 \text{ g}\cdot\text{kg}^{-1}$ HMP.

Tab. 1. Protein load ($\text{mg}\cdot\text{m}^{-2}$) at the oil-water interface in stock emulsions prepared with $200\text{ g}\cdot\text{kg}^{-1}$ of low-melting-point (LMP) or high-melting-point (HMP) fraction of anhydrous milk fat during storage at $10\text{ }^{\circ}\text{C}$.

Time of storage [h]	Milk fat fraction			
	LMP		HMP	
	– EDTA	+ EDTA	– EDTA	+ EDTA
0	5.56 ± 0.31^a	1.79 ± 0.02^d	5.45 ± 0.23^a	1.83 ± 0.03^d
24	2.39 ± 0.03^c	2.66 ± 0.04^b	2.41 ± 0.08^c	2.46 ± 0.08^c

Oil droplets were centrifuged without or with EDTA (80 mM), and the protein load determined in each condition.

Results are mean \pm standard deviation ($n = 3$). Within a row or a column, values with different superscript letters are significantly different ($p < 0.05$).

at the interface of the emulsion prepared with the low-melting-point fraction of milk fat.

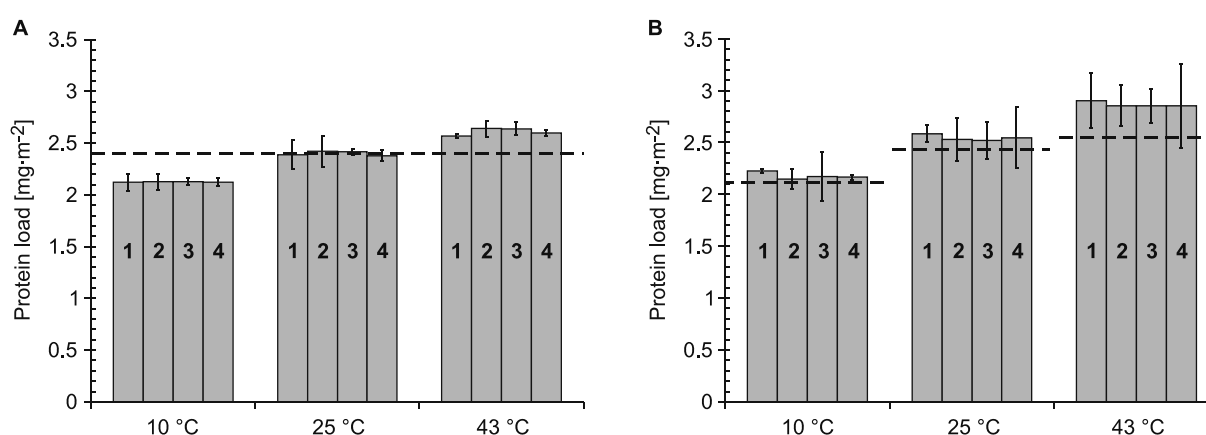
The protein load at the interface of low-fat emulsions ranged from $2.1\text{ mg}\cdot\text{m}^{-2}$ to $2.9\text{ mg}\cdot\text{m}^{-2}$ after 24 h of storage, and increased with the temperature of storage (Fig. 2A). The variations were not related to the nature or amount of the emulsified fat. As compared to the corresponding stock emulsions stored at $10\text{ }^{\circ}\text{C}$, low-fat emulsions stored at $10\text{ }^{\circ}\text{C}$ had a significantly lower protein load, a similar protein load after storage at $25\text{ }^{\circ}\text{C}$ and, finally, a significantly higher protein load after storage at $43\text{ }^{\circ}\text{C}$. The increase was moderate: 11% between $10\text{ }^{\circ}\text{C}$ and $25\text{ }^{\circ}\text{C}$, and 8% between $25\text{ }^{\circ}\text{C}$ and $43\text{ }^{\circ}\text{C}$.

Protein composition of oil-water interface

Because of the complexity of the composition of milk proteins, the results are reported as the ratio between the cumulated surfaces of electrophoretic bands corresponding to major milk

proteins, namely, caseins and whey proteins. The ratio was designated Cas/WP. In heated recombined skimmed milk, the Cas/WP was 3.7 (Tab. 2). In freshly prepared stock emulsions, the Cas/WP of the adsorbed proteins increased greatly, reaching 4.6 to 5.2 depending on the sample. When the separation of oil droplets was performed in the presence of EDTA, the Cas/WP of the adsorbed proteins decreased to about 4 regardless of the nature of the emulsified fat. This value was not significantly different from the ratio observed in skimmed milk powder.

The values of Cas/WP observed in low-fat emulsions stored at $10\text{ }^{\circ}\text{C}$ for 24 h were not significantly different (Tab. 3), regardless of the nature of the fat and fat content. After 24 h of storage at $25\text{ }^{\circ}\text{C}$, Cas/WP decreased significantly to about 3.4. It fell to 3.0 at $43\text{ }^{\circ}\text{C}$, indicating that whey proteins were adsorbed preferentially to caseins at the oil-water interface.

**Fig. 2.** Protein load at oil-water interface of emulsions and stirred gels.

A – Protein load at oil-water interface of emulsions stored at $10\text{ }^{\circ}\text{C}$, $25\text{ }^{\circ}\text{C}$ or $43\text{ }^{\circ}\text{C}$.

B – Protein load at oil-water interface of stirred gels.

1 – $15\text{ g}\cdot\text{kg}^{-1}$ of the LMP fraction of milk fat, 2 – $15\text{ g}\cdot\text{kg}^{-1}$ HMP fraction of milk fat, 3 – $50\text{ g}\cdot\text{kg}^{-1}$ of the LMP fraction of milk fat, 4 – $50\text{ g}\cdot\text{kg}^{-1}$ HMP fraction of milk fat.

Values are the means of 9 measurements (3 preparations in triplicate). Dashed lines represent the protein load at the interface of stock emulsions (in the case of emulsions and the corresponding protein load of emulsions in the case of gels).

Tab. 2. Ratio of adsorbed caseins to whey proteins (Cas/WP) in heated recombined milk and at the oil-water interface of freshly prepared emulsions containing 200 g·kg⁻¹ of the milk fat fractions.

Samples	Cas/WP
Heated recombined milk	3.7 ± 0.1 ^a
Oil-water interface of freshly prepared emulsions centrifuged with EDTA	
LMP	4.0 ± 0.2 ^a
HMP	3.8 ± 0.2 ^a
Without EDTA	
LMP	4.6-4.9
HMP	4.8-5.2

LMP – low-melting-point fraction of milk fat, HMP – high-melting-point fraction of milk fat.

Values reported for skimmed milk powder and with EDTA are mean ± standard deviation of 3 determinations. Within a column, values with the same superscript letters are not significantly different ($p > 0.05$). Values reported without EDTA were obtained from two independent experiments.

Tab. 3. Ratio of caseins to whey proteins (Cas/WP) adsorbed on fat droplets of emulsions and stirred gels after 24 h of storage at 10 °C, 25 °C or 43 °C.

Sample	Temperature of storage		
	10 °C	25 °C	43 °C
Emulsions 15 g kg ⁻¹ fat			
LMP	4.0 ± 0.3 ^{aA}	3.3 ± 0.2 ^{bA}	2.9 ± 0.1 ^{cA}
HMP	3.9 ± 0.2 ^{aA}	3.5 ± 0.2 ^{bA}	3.0 ± 0.1 ^{cA}
Emulsions 50 g kg ⁻¹ fat			
LMP	4.0 ± 0.1 ^{aA}	3.4 ± 0.1 ^{bA}	2.9 ± 0.1 ^{cA}
HMP	3.8 ± 0.4 ^{aA}	3.5 ± 0.1 ^{bA}	3.0 ± 0.1 ^{cA}
Stirred gels 15 g kg ⁻¹ fat			
LMP	4.0 ± 0.1 ^{aA}	3.4 ± 0.1 ^{bA}	3.0 ± 0.1 ^{cA}
HMP	3.8 ± 0.2 ^{aA}	3.5 ± 0.1 ^{bA}	3.0 ± 0.1 ^{cA}
Stirred gels 50 g kg ⁻¹ fat			
LMP	2.7 ± 0.1 ^{aB}	2.2 ± 0.0 ^{bB}	2.2 ± 0.1 ^{bB}
HMP	2.9 ± 0.1 ^{bB}	2.5 ± 0.1 ^{bC}	2.3 ± 0.0 ^{cB}

LMP - matrices prepared with the low-melting-point fraction of milk fat, HMP - matrices prepared with the high-melting-point fraction of milk fat.

Values are means of 3 determinations. Within a column, values with same superscript capital letters are not significantly different ($p > 0.05$). Within a row, values with same superscript small letters are not significantly different ($p > 0.05$).

Fat droplets and the interface in stirred gels

Droplet size distribution after gelation and storage

All stirred gels exhibited similar droplet size distributions ($p > 0.05$), regardless of the nature of the emulsified fat, the fat content and the temperature of storage. Gels contained fat droplets with volume surface mean diameters ranging from

1.2 µm to 1.4 µm and a standard deviation equal to 0.1 µm ($n = 9$). These results were similar to those for the low-fat emulsions and overlapped the corresponding distribution (Fig. 1). The dilution of stirred acid gels in a dissociating buffer had been used to characterize the evolution of fat droplet size during the Camembert cheese production [9]. A slight increase of the fat droplet size had been observed from the raw milk to the Camembert curd. Thus, it was obvious that the addition of GDL, the subsequent pH decrease and formation of the protein network did not induce coalescence of the fat droplet, which were stable upon storage at 10, 25 or 43 °C for 24 h and up to 72 h (data not shown).

Protein load of oil-water interface of stirred gels

Similar to the emulsions, the nature and amount of the emulsified fat had no significant effect on the protein load at the interface of stirred gels, which increased with the temperature of storage (Fig. 2B). When compared to the corresponding low-fat emulsions, the protein load at the interface of stirred gels was not significantly different, except for matrices stored at 43 °C. The extent of the increase of the protein load with the temperature of storage was moderate and close to values reported for low-fat emulsions: 14% between 10 °C and 25 °C, and 11% between 25 °C and 43 °C. In such complex matrices, the acidification and formation of the protein network did not deeply modify the protein load at the oil-water interface.

Composition of oil-water interface in stirred gels

Cas/WP at the oil-water interface of stirred gels containing 15 g·kg⁻¹ fat was not significantly different from the ratio observed in the corresponding emulsions (Tab. 3), regardless of the nature of the fat and the temperature of storage. Conversely, the values of Cas/WP were significantly lower in the stirred gels which fat content was 50 g·kg⁻¹, than in the corresponding emulsions. The observed values were below 3.0 and lower than the values of the recombined heated milk. This result indicates a preferential adsorption of whey proteins at the oil-water interface of stirred gels containing 50 g·kg⁻¹ fat when compared to other matrices.

DISCUSSION

From skimmed milk to dairy emulsions:
preferential adsorption of casein micelles

Cas/WP of 3.7 in heated recombined skimmed milk is close to the already reported values [15].

The protein load of $5.6 \text{ mg}\cdot\text{m}^{-2}$ at the interface of freshly prepared stock emulsions is in good agreement with the values found when skimmed milk powder was used as an emulsifier [15, 16]. In such conditions, a portion of casein adsorbed at the interface was present in the form of micelles, as revealed by electron microscopy [15]. The higher values of Cas/WP in the adsorbed layers of freshly prepared emulsions with a fat content of $200 \text{ g}\cdot\text{kg}^{-1}$, compared to the original skimmed milk powder, confirms the preferential adsorption of caseins when compared to whey proteins [15, 17, 18]. When the separation of droplets of stock emulsions was achieved in the presence of EDTA, the protein load at the interface was drastically reduced (Tab. 1) and Cas/WP at the interface decreased to reach values not significantly different from that of skimmed milk powder (Tab. 2). This suggests that some of the micelles constituting the adsorbed layer were disrupted, thereby decreasing the proportions of caseins at the interface. This probably results from the ability of EDTA to chelate calcium present in the casein micelles as colloidal calcium phosphate [14], which provokes micelle disorganization and leakage of casein molecules from the interface. This partial disruption of the micelle organization after the addition of EDTA has been already observed in emulsions, with a sharp decrease in the protein load at the interface to a final value of $2 \text{ mg}\cdot\text{m}^{-2}$ [15]. This value, very close to ours (Tab. 1), is also similar to the values reported for sodium caseinate-stabilized

emulsions [18–20]. Our results indicate that initially the caseins adsorb as casein micelles. Upon storage and gelation, the structure evolves and, finally, the adsorbed caseins are found as individual proteins and behave as caseinates. Fig. 3 is a schematic view of the evolution of the interface in dairy emulsions according to these assumptions.

Dissociation of protein aggregates and adsorption of whey proteins as temperature of storage increased

Evolution of protein load upon storage at low temperature or in the presence of EDTA can result from the dynamics of casein micelles, casein-whey protein and whey protein-whey protein aggregates, that led to progressive disruption of the aggregates adsorbed at the interface during storage and to a decrease in the protein load (Fig. 3). A similar phenomenon was observed for molecular, micellar and aggregated states of β -casein [21]. The adsorption of individual caseins or whey proteins is less sensitive to the ionic interactions with calcium and to the presence of EDTA (Fig. 3). A decrease in the protein load was also observed at low temperatures for dairy emulsions stabilized by sodium caseinates [6]. The authors noticed an increase in the protein load at the interface at a higher temperature of storage, in agreement with the present work, showing a slightly higher protein load at the interface when the low-fat emulsions were stored at 43°C , as compared to storage at 10°C . Protein-protein interactions tend to be favoured when the temperature increases, tightening the packing of

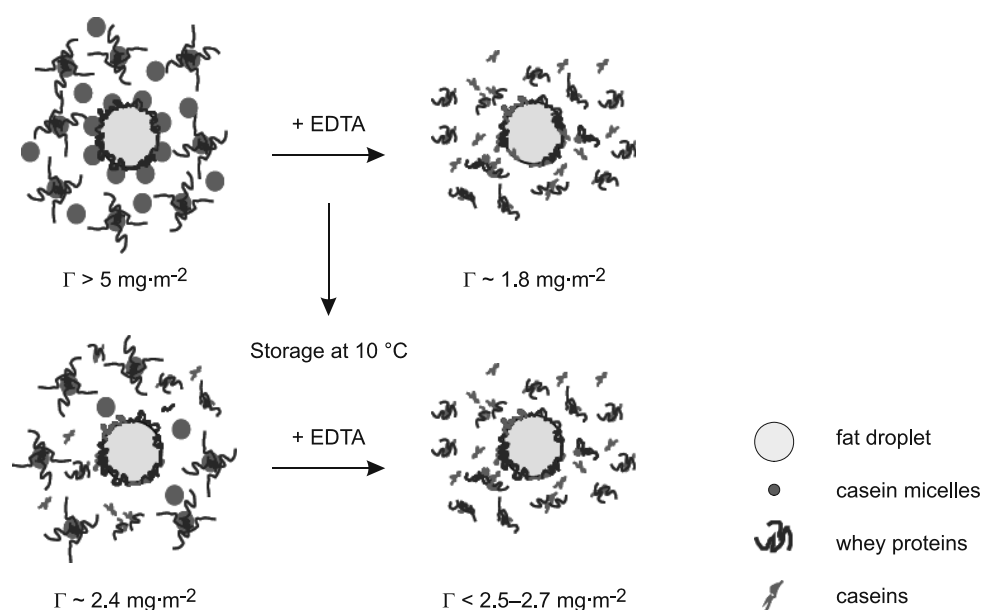


Fig. 3. Schematic drawing of the evolution of the adsorption of various proteins at the oil-water interface of emulsion during storage at 10°C or after droplet separation in the presence of EDTA.

the adsorbed proteins and increasing the interfacial protein load [21]. Simultaneously, Cas/WP decreased and reached values significantly lower than Cas/WP in skimmed milk (Tab. 3). This pointed out a preferential secondary adsorption of whey proteins compared to caseins. Our results clearly indicate that the protein load at the oil-water interface of emulsions depends on the temperature of storage, which is probably related to the organization, or aggregation state, of the proteins.

Little effect of dilution on interface composition

Upon dilution of the stock emulsions with skimmed milk and sucrose solutions to decrease the fat mass fraction, the continuous phases of the emulsions were enriched in milk proteins. In such conditions, the secondary adsorption of proteins to the oil-water interface already has been observed [22]. In contrast, in our systems, the protein load at the interface was slightly reduced in low-fat emulsions stored at 10 °C as compared to stock emulsions, indicating that such secondary adsorption did not take place. At the same time, Cas/WP was not significantly different from that observed in the corresponding stock emulsions. This finding demonstrates that the dilution of the fat mass fraction with an enriched protein solution did not alter the composition of the interface.

No effect of physical state of the milk fat on oil-water interface of emulsions and stirred gels

The droplet size distribution was remarkably stable during the storage of stock and low-fat emulsions, regardless of the nature of the milk fat and the temperature of storage. It was also almost insensitive to dilution and acid gelation. In the same way, in our experimental conditions, the milk fat fraction did not modify the protein load or the Cas/WP at the interface in any of the matrices, despite the different physical state of the dispersed fat globules. According to the DSC melting curves, the LMP fraction of milk fat was totally liquid at 25 °C and 43 °C and contained about 22% solid fat at 10 °C while HMP fraction contained about 9, 50 and 73% solid at 43, 25 and 4 °C, respectively [23]. We can therefore conclude that, in the present dairy emulsions and stirred acid gels, the physical state of the dispersed fat did not quantitatively or qualitatively influence the protein adsorption at the oil-water interface.

Acid gelation of dairy emulsions slightly modifies the composition of the oil-water interface

As observed in the low-fat emulsions, the protein load at the oil-gel interface increased with the temperature of storage. The extent of increase was

in the same order as that of emulsions. Neither the nature of the fat nor its content modified the protein load at the interface of the gels. The evolution of the interface composition of the stirred gels exhibited similar trends for products containing 15 g·kg⁻¹ of fat as that for low-fat emulsions: in the gels stored at 10 °C, Cas/WP was not significantly different from that observed in the corresponding stock emulsions, but decreased when the temperature of storage increased. In stirred gels containing 50 g·kg⁻¹ of fat, the trends differed slightly. After storage at 10 °C, the Cas/WP was significantly lower than that of skimmed milk, with the difference increasing for higher temperatures of storage, pointing out a preferential adsorption of whey proteins at the interface in these matrices.

CONCLUSION

The nature of emulsified fat, i.e. the low- and high-melting-point fractions of anhydrous milk fat, or their liquid / solid ratio, did not modify the droplet size distribution and the stability of dairy emulsions and stirred acid gels designed to be models for stirred yoghurts. The protein load at the oil-water interface of the matrices was also insensitive to the nature and physical state of the emulsified fat and to the organization of the continuous phase (i.e. neutral liquid versus acid gel). The protein load was higher at the oil-water interface of the freshly prepared emulsions than in the recombined skimmed milk due to adsorption of casein micelles during emulsification. During the storage of the emulsions, the reorganization of interfacial proteins led to a decrease in the protein load, which was more or less pronounced according to the temperature, with simultaneous modifications in the casein : whey protein ratio at the interface. Disruption of the casein micelles and secondary adsorption of whey protein were likely involved in these changes. Our results highlight that, in complex dairy matrices, both the protein load and composition of the interface can evolve throughout dilution, storage or gelation, and that the interfacial composition of the final products cannot be simply extrapolated from that of the original concentrated emulsions.

Acknowledgments

The funding of this work by the French government, INRA, Danone, Nestlé, and Yoplait within the framework of the CANAL program “ARLE” (Interactions between aroma compounds-food matrices and packaging) and by the Région Pays de la Loire within the framework of the VANAM program “Interactions matrices-solutés” is gratefully acknowledged.

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Received 11 December 2008; revised 12 February 2009; accepted 27 February 2009.