Composition of Interfacial Layers in Complex Food Emulsions Before and After Aeration: Effect of Egg to Milk Protein Ratio

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ABSTRACT

Whipped emulsions were prepared at pilot scale from fresh milk, whole egg, and other ingredients, for example, sugars and stabilizers (starch, polysaccharides). Egg content was varied: 4 recipes were studied differing in their egg to milk protein ratio (0, 0.25, 0.38, and 0.68). Protein and fat contents were kept constant by adjusting the recipes with skim-milk powder and fresh cream. Emulsions were prepared by high-pressure homogenization and whipped on a pilot plant. Particle-size distribution determined by laser-light scattering showed an extensive aggregation of fat globules in both mix and whipped emulsions, regardless of recipe. Amount of protein adsorbed at the oil-water interface and protein composition of adsorbed layer were determined after isolation of fat globules. Protein load is strongly increased by the presence of egg in formula. Values obtained for the whipped emulsions were dramatically lower than those obtained for the mix by a factor of 2 to 3. Sodium dodecyl sulfate-PAGE indicated a preferential adsorption of egg proteins over milk proteins at the oil-water interface, regardless of recipe. This phenomenon was more marked in aerated than in unaerated emulsions, showing evidence for desorption of some milk proteins during whipping. Egg proteins stabilize mainly the fat globule surface and ensure emulsion stability before whipping. Air bubble size distribution in whipped emulsions was measured after 15 d storage. When the egg to milk protein ratio is decreased to 0.25, large air cells appear in whipped emulsions during storage, indicating mousse destabilization. The present work allows linking the protein composition of adsorbed layers at the fat globule surface to mousse formula and mousse stability.

(Key words: whipped emulsion, milk and egg protein, adsorption, oil-water interface)

Abbreviation key: CLSM = confocal laser scanning microscopy.

INTRODUCTION

Dairy foams represent a large number of aerated food products such as ice cream, whipped cream, aerosol cream, and mousse. Elaboration of such complex foamed emulsions is still mostly empirical and frequently based on industrial know-how. The difficulty in maintaining and explaining the properties of such products lies in the wide range of compounds (fats, proteins, emulsifiers, hydrocolloids, and colloidal particles) interacting to stabilize the final emulsion (Leser and Michel, 1999). Whipped cream consists of an oil-in-water emulsion that is subsequently foamed, that is, in which a dispersion of air bubbles is created. Stabilization of a whipped cream relies on a combination of destabilization and structure-building mechanisms (Smith et al., 2000). It is now well established that successful foaming depends on controlled partial coalescence of fat globules and fat crystallization (Boode and Walstra, 1993). In the initial stage of whipping, air bubbles would be covered by a proteinaceous membrane, including caseins and whey proteins (Brooker, 1993). When the air is incorporated, the interfacial film surrounding the fat globule is ruptured because of applied shear during whipping (Stanley et al., 1996; Leser and Michel, 1999). Considering that milk fat contains triglycerides with a wide melting range (–40 to 40°C), a globule is composed of solid and liquid fat at refrigerated temperature (Walstra and Jenness, 1984; Goff, 1997). Therefore, despite collisions between fat globules during whipping and rupture of the interfacial layer, coalescence is not complete due to crystalline fat (Stanley et al., 1996; Goff, 1997). This results in the formation of an irregular network of partially coalesced fat globules, which are prone to adsorb at the air bubble surface (Leser and Michel, 1999). As aggregated fat globules adhere to the air-serum interface, the interfacial film stabilizing the fat globule is ruptured and fat comes in direct contact with the air, protruding slightly into the bubble (Anderson and Brooker, 1988). Stabilization of

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air bubbles is hence achieved by an interfacial network of aggregated fat globules. In nonhomogenized whipped cream, aggregated fat globules at the air bubble surface bridge with globules of adjoining air cells or adsorb onto more than one air cell to build a network entrapping the air bubbles and strengthen the final foam structure (Anderson and Brooker, 1988). Unlike nonhomogenized whipped cream (stabilized by partially coalesced fat globules), homogenized whipped cream rigidity is ensured by calcium bridges between caseins adsorbed at the air-serum interface (Anderson et al., 1977; Darling, 1982).

Pasteurization, homogenization, and emulsion aging are important stages in the manufacture of homogenized whipped cream and ice cream. After homogenization, the emulsion has to destabilize in a controlled way before whipping to produce a stable whipped cream (Leser and Michel, 1999). Low molecular weight emulsifiers such as mono- and diglycerides or sorbitan esters are commonly incorporated in dairy foams to control emulsion destabilization (Goff, 1997). Whereas small surfactants stabilize the interface via the Gibbs-Marangoni mechanism, proteins form a thick interfacial layer protecting the emulsion droplet against coalescence through steric and electrostatic repulsion. When low molecular weight surfactants are used together with proteins, competitive adsorption of 2 components at the interface makes mixed protein-surfactant films weaker than those obtained in the presence of protein only (Courthaudon et al., 1991). This causes some emulsion instability by reduced steric and electrostatic repulsion (Leser and Michel, 1999). Gelin et al. (1996) noted that small surfactants further displace proteins from the fat globule surface during the cold aging stage of ice cream manufacturing. This lowers steric stabilization by proteins and makes fat globules more prone to partial coalescence during whipping (Boode and Walstra, 1993).

Anderson and Brooker (1988) established the key role of interactions between fat globules and those between fat globules and the air-serum interface during manufacturing of whipped emulsion. The oil-water interface would condition emulsion stability before whipping and, consequently, foam functionality (Anderson and Brooker, 1988). It was then suggested that the amount of proteins adsorbed at the fat globule surface would be indicative of the extent of fat destabilization during whipping and, consequently, of foam quality (Goff, 1997; Bolliger et al., 2000). Segall and Goff (1999) suggested that better understanding of the relationship between the protein load, the adsorbed protein type, and the emulsion stability would allow to elaborate dairy foams with improved functional characteristics through handling of the interfacial film. This implies that food manufacturers would assess the impact of

formulation changes on foam quality without extensive pilot plant work (Bolliger et al., 2000). Foam stability would be predicted from properties of the fat-serum interface.

The aim of the present work was to characterize the adsorbed layer at the fat globule surface and link its properties to emulsion whippability and stability of whipped emulsions. The present study concerns the effect of partial substitution of skim milk/fresh cream with fresh whole egg on protein composition of oil-water interfacial layers in emulsions, unaerated or aerated. Data are analyzed by considering the effect of egg to milk protein ratio on protein composition of interfacial layers. When fresh whole egg is incorporated in the formula, other interfacial active compounds than proteins are incorporated, such as egg yolk phospholipids and lipids, in the form of lipoproteins. However, we paid particular attention to the protein composition of mix and whipped emulsions, because proteins play a key role at the fat-serum and air-serum interfaces in emulsification, whipping, and water-holding capacity (Phillips, 1981; Goff, 1997). Fresh milk and whole egg were the 2 distinct sources of proteins used in this work. Milk proteins, that is, caseins and whey proteins, are widely used to stabilize food emulsions and foams and, in particular, whipped creams and ice creams. Egg-white proteins exhibit many functional properties. Mine (1995) emphasized notably the excellent foaming properties of egg white and its capacity for coagulating because of heating. Egg yolk is commonly incorporated in many processed foods because of its exceptional emulsifying properties: low and high-density lipoproteins (LDL and HDL) are responsible for formation and stabilization of egg yolk-based oil-in-water emulsions (Kiosseoglou, 1989; Martinet, 2003). Goff (1997) reiterated that egg yolk was initially used in ice creams as an emulsifier, while presently used surfactants are mono-, diglycerides, and sorbitan esters. In our study, no additional small emulsifier was added to the recipes.

Our study is original because whipped emulsions were prepared not only from milk but also from whole egg: no similar work has been previously related in the literature. In the present work, adsorbed layers at the fat globule surface were studied; amount and nature of protein adsorbed at the oil-water interface were determined in emulsion and in whipped emulsion as a function of egg to milk protein ratio.

MATERIALS AND METHODS

Preparation of Mix and Whipped Emulsions

Emulsions were prepared according to industrial formulas and processes. The different ingredients used for emulsion preparation were fresh skim milk and cream

Table 1. Composition of the 4 recipes used for the manufacture of mix and whipped emulsions.

Ingredients	Egg/milk protein ratio			
	0	0.25	0.38	0.68
Skim milk/fresh cream	75.9	73.0	73.1	66.4
Skim milk powder	3.4	1.6	0.9	0
Sugars	19.1	17.5	16.1	19.5
Stabilizer mixture	1.6	1.6	1.6	1.6
Fresh whole egg	0	6.2	8.3	12.5
Milk protein (%)	3.5	2.8	2.6	2.0
Egg protein (%)	0	0.7	1.0	1.4
Total protein (%)	3.5	3.5	3.5	3.5
Total fat (%)	6.6	6.6	6.6	6.6
Total dry matter (%)	34.5	34.5	34.5	34.5

(respectively containing 0.5 g/L and 400 g/kg of milk fat), fresh whole egg (containing 115 g of fat/kg), sucrose, lactose, starch, and stabilizers consisting of a mixture of carrageenans and xanthan gum. Various amounts of fresh whole egg were incorporated to prepare the emulsions, while the total protein, fat, and DM contents (g/100 g) were kept constant at 2.4, 6.6, and 34.5%. As the egg content was increased in the recipe, the milk and cream contents were reduced to balance proteins and fat brought by fresh whole egg. Proportions of other ingredients were adjusted so that finally, only the egg to milk protein ratio varied. Thus, 4 different recipes were tested corresponding to egg to milk protein ratios of 0, 0.25, 0.38, and 0.68, respectively. The formula of each recipe is summarized in Table 1. All the ingredients were mixed together, sterilized in a plate exchanger, cooled, and then homogenized. The sterilized mix was cooled to 10°C and stored before whipping. Whipping was performed in a dynamic aerator (Mondomix, Nederhorst Den Berg, The Netherlands) with a constant overrun of 30% obtained by adjusting pump flow and shear rate. To study the influence of whipping on interfacial composition of fat globules, samples were collected before and after whipping. Thus, we distinguished the mix for each recipe, obtained after homogenization (not whipped), and the whipped emulsions.

Particle Size Measurements

Particle size distributions of mix and whipped emulsions were measured by laser-light scattering using a Mastersizer S (Malvern Instruments SA, Orsay, France). To disrupt aggregates of oil droplets, emulsion samples were dispersed in a dissociating medium consisting of a 1% (wt/vol) SDS solution (1 h, room temperature) and then measurements were carried out at room temperature (Tomas et al., 1994). The refractive index of the milk fat was 1.4564, and the imaginary part of

the refractive index (due to adsorption) was settled at 0.001. The average particle size was expressed as $d_{4,3}$ (the volume-weighted mean diameter) rather than $d_{3,2}$ because large particles were present in the different emulsions.

Furthermore, particle size distributions were measured after sample incubation with pronase (EC 3.4.24.31), a broad-spectrum mixture of endo- and exo-proteinases. We added the mixture of proteases to help disruption of emulsion-droplet aggregates by hydrolyzing proteins of interacting-droplets interfacial layers. Mix and whipped emulsions were diluted 1 in 40 (wt/wt) with 100 mM Tris-HCl, pH 7.5, 10 mM CaCl₂, 1% SDS buffer. After dilution of pronase in pure water (20 mg/mL), 1 mL of the enzyme solution was added to approximately 20 mL of substrate preparation. Mixture was incubated 24 h at 37°C under slight stirring and finally injected in the Mastersizer for size measurement. Analyses were performed in duplicate for each sample.

Isolation of Fat Globules from Mix and Whipped Emulsions

The method described by Patton and Huston (1986) was adapted to isolate and wash off fat globules from mix and whipped emulsions. Each sample was diluted 1 in 4 with 100 mM imidazole-HCl buffer (pH 6.7) containing 50% (wt/vol) sucrose. Concurrently, 10 mL of 100 mM imidazole-HCl buffer (pH 6.7) containing 35% (wt/vol) sucrose was placed in a 15-mL centrifuge tube. Approximately 4 g of the diluted sample was carefully deposited underneath the 10 mL of the 35% sucrose solution using a syringe. The tube was then centrifuged (3000 × *g*, 2 h, 28°C) to ensure creaming of fat globules. After centrifugation, 3 phases were distinguishable in the tube: a thick layer of cream at the top, a clear intermediate phase, and a cloudy yellowish phase at the bottom of the tube. The creamed fat globules were concentrated in the upper layer, whereas the nonadsorbed constituents and the aqueous phase of the emulsion remained in the lower phase. The intermediate phase was considerably depleted in material as shown by protein content assays. Immediately after centrifugation, the tube was frozen and stored at -20°C prior to analysis.

Quantification of Proteins Adsorbed at the Oil-Water Interface

To quantify protein adsorbed at the fat droplet surface, each of the 3 phases previously described were analyzed. The frozen tube was cut out to separate each phase and to collect separately the creamed layer, the

intermediate phase, and the dense lower phase. Each of the 3 phases was thawed and carefully weighed, and protein content was determined according to the method of Markwell et al. (1978). Protein content was directly measured in the intermediate and lower phases, whereas protein desorption was first required in the creamed layer. In a centrifuge tube, 0.5 mL of thawed cream was diluted with 2 mL of 1% (wt/vol) SDS solution. The mixture was vigorously stirred, incubated for 1 h at room temperature to ensure complete desorption of protein adsorbed at the fat globule surface, and centrifuged ($3000 \times g$, 1 h, 28°C). The creamed layer concentrating the fat globules free of protein was discarded. The solution of desorbed proteins corresponding to the lower phase was analyzed for protein content according to the method of Markwell et al. (1978). Finally, the amount of protein adsorbed at the fat droplet surface (F_{ADS} in %) was calculated as follows:

$$F_{\text{ADS}} = \frac{P_{\text{C}}}{P_{\text{C}} + P_{\text{IP}} + P_{\text{LP}}} \times 100$$

where P_{C} , P_{IP} , and P_{LP} represent the mass of protein (mg) in the cream, the intermediate phase, and in the lower phase, respectively.

Identification of Adsorbed Proteins by SDS-PAGE

Protein analysis by SDS-PAGE was carried out in the solution obtained after protein desorption by SDS as described previously. Protein samples were half diluted with a dissociation buffer (0.125 M Tris-HCl (pH 6.8), 4% SDS, 20% glycerol, 10% β -mercaptoethanol, and bromophenol blue) and were boiled for 5 min at 100°C . Electrophoresis was run on a 10% acrylamide gel (stacking gel: 3.5% acrylamide) with a migration buffer containing 50 mM Tris, 0.4 M glycine, pH 8.8, and 0.1% SDS. Migration was performed at 60 V using a Mini Protean 3 System (Bio-Rad, Ivry-sur-Seine, France) with a Power Pac 300 power supply (Bio-Rad). Proteins were subsequently stained with a Coomassie blue solution (0.05% Coomassie blue, 25% ethanol, and 10% acetic acid). The destaining solution contained 7% acetic acid and 40% ethanol. Gels were scanned with a GS710 imaging densitometer (Bio-Rad) and analyzed with Quantity One 4.1 software (Bio-Rad). Sodium dodecyl sulfate-PAGE low range standards (Bio-Rad) were used to estimate molecular weights and identify proteins on the gels. Two standard curves were established from milk or egg proteins and correlated the optical density to the quantity of proteins in a single band. They allowed us to quantify the different proteins adsorbed at the fat globule surface (Figure 1).

Confocal Laser Scanning Microscopy

Images were recorded on a confocal laser scanning microscope (CLSM; Leica, Rueil-Malmaison, France) equipped with an inverted microscope and with 2 lasers, Ar and He/Ne (excitation wavelengths: 488 and 633 nm, respectively; detected emission wavelengths above 490 and 640 nm, respectively). Labeling of proteins and fat was performed using Nile Blue and Nile Red. A 0.2% (wt/vol) solution of dye in ethanol was prepared and 2 drops of the solution were spread at the bottom of a glass dish. A mousse sample ($\sim 1 \text{ cm}^3$) was cut off with a razor blade and carefully loaded over the dye solution without additional pressure to avoid deformations or displacement of air bubbles. Samples were incubated 10 min before observation to allow dye diffusion into the mousse. A small cool pack was set at the top of the sample to control temperature during imaging.

Air Cell Measurement

Mousse was loaded onto a glass slip between 2 microscope glass slides using a Pasteur pipette and overlaid with a glass coverslip. This technique allowed observation of samples with similar thickness. Imaging was carried out using a Leica DM IRB inverted optical microscope (Leica). Images were acquired using a 3 CCD video camera system (Sony, Paris, France) and processed with Visilog software, version 5.4 (Noesis, Orsay, France). Two slides were prepared and observed for each sample and 5 images were recorded for each slide. The bubble size range accessible by this method is 25 to $1000 \mu\text{m}$. In practice, the method is reliable up to $170 \mu\text{m}$ (gap between the receiving glass and the cover slide) due to deformation of bubbles of larger size.

RESULTS AND DISCUSSION

Fat Globule Size Distribution

Figure 2 shows particle size distributions in mix and whipped emulsions after dispersion in 1% SDS (by weight) solution. Regardless of recipe, the mixes were found to be bimodal emulsions with at least 80% volume of the oil phase within the size range of 1 to $104 \mu\text{m}$ (whipped emulsions gave similar results). This latter population could possibly represent very large particles or result from aggregation of material in the emulsions, including fat globules. In this case, as SDS is inefficient in disrupting aggregates, it suggests that globule surface would not be accessible to SDS. Moreover, samples were observed with a light microscope after staining of proteins with brilliant Coomassie blue (results not shown). Very large aggregates were detected, involving nonadsorbed proteins and many fat globules. With the

Figure 1. A. Sodium dodecyl sulfate-PAGE of milk proteins (std: protein standards). Various amounts of fresh-milk proteins were applied to the gel; bands were identified and quantified to build a standard curve. B. Standard curve correlating the optical density (OD) to the protein mass in each lane. C. SDS-PAGE for protein identification and quantification in a whipped emulsion prepared from an egg to milk protein ratio of 0.25. After isolation of fat globules, SDS-PAGE was performed on the cream layer (Cr), the intermediate phase (Ip), and the lower phase (Lp).

purpose of elucidating instability and aggregate formation in heated whey protein stabilized oil-in-water emulsions, Euston et al. (2000) studied the aggregation rate of emulsions at various protein concentrations. They concluded that adsorbed proteins at the fat globule surface aggregate with nonadsorbed heat-denatured whey proteins free in the aqueous phase, resulting in heat-induced instability of whey protein stabilized emulsions. The aggregate structure was even considered as a clump of oil droplets held together by a glue of denatured whey proteins, correlating our results.

To disrupt protein aggregates in mix and whipped emulsions, samples were incubated with pronase, a nonspecific protease consisting of a mixture of endo- and exoproteinases. After incubation with the enzyme,

mix and whipped emulsions were still found to be bimodal (Table 2). However, $d_{4,3}$ was decreased for all samples, the average particle size being reduced to half its original value in some recipes. Large particles initially present in mix and whipped emulsions probably resulted from aggregation involving proteins. Nevertheless, the action of pronase was limited: some large particles persisted in emulsions after incubation with the enzyme. Some coalescence cannot be excluded. Otherwise, the presence of other ingredients can explain the difficulty encountered in disrupting aggregates. We should reiterate the presence of different types of polysaccharides in the emulsions: starch, xanthan gum, and carrageenans (γ , κ , and ι). Starch and xanthan gum could account for the formation of a gel-like structure, limiting the enzyme action, and contributing to the aggregation of fat globules and proteins. Yang et al. (2003) studied the gelation of starch-based dairy food models and showed in particular that partial replacement of whey protein isolate by egg white proteins in presence of starch and sucrose increases the gel strength. In other respects, Yoshida et al. (1998) showed that xanthan/water systems gel at concentrations above 0.5 wt% after annealing at 40°C and subsequent cooling. According to Bryant and McClements (2000), opacity, gelation rate, and final rigidity of a heated-denatured whey protein isolate solution are increased when as little as 0.1 wt% xanthan is added. Although xanthan concentration used in this work was less than 0.5 and even 0.1 wt%, the formation of xanthan aggregates could not be excluded, as suggested by Euston et al. (2002) in a previous work about the effect of polysaccharides on droplet aggregation in heated whey protein-stabilized emulsions. The formation of a gel-like net-

Figure 2. Particle-size distribution of mix in 1% (wt/vol) SDS solution for 4 different recipes.

Table 2. Average particle sizes ($d_{4,3}$, μm) in mix and whipped emulsions before and after incubation with pronase in 1% (wt) SDS solution.

Egg/milk protein ratio	SDS		SDS + pronase	
	Mix	Whipped emulsion	Mix	Whipped emulsion
0	53.3 $\pm$ 2.5	...	23.3 $\pm$ 1.1	...
0.25	43.0 $\pm$ 2.0	51.9 $\pm$ 2.4	37.9 $\pm$ 1.8	32.8 $\pm$ 1.5
0.38	52.8 $\pm$ 2.5	52.7 $\pm$ 2.5	23.1 $\pm$ 1.1	27.3 $\pm$ 1.3
0.68	54.2 $\pm$ 2.6	57.2 $\pm$ 2.7	25.4 $\pm$ 1.2	38.1 $\pm$ 1.8

work involving starch and xanthan would trap proteins and oil droplets and consequently promote their aggregation. Moreover, Capron et al. (1999) investigated the aggregation and gelation of mixed solutions of β -lactoglobulin and κ -carrageenan. Addition of κ -carrageenan increases the rate of heat-induced aggregation in β -lactoglobulin solutions. Considering our emulsions, the presence of carrageenans could increase the aggregation rate of nonadsorbed proteins and, as inferred by Euston et al. (2002), it could probably increase the aggregation rate of whole emulsions.

Part of Proteins Adsorbed at the Fat Droplet Surface

Figure 3 represents the part of protein adsorbed at the oil-water interface in mix and in whipped emulsions vs. egg to milk protein ratios used for emulsion manufacture. The quantity of protein adsorbed at the oil-water interface is much lower in whipped emulsions than in mix, regardless of the recipe. Precisely, there is between 2.4 and 3.4 times less protein adsorbed at the fat globule surface in whipped emulsions than in

mix for egg to milk ratios of 0.68 and 0.25, respectively. Moreover, protein load is dramatically increased in mix and whipped emulsions when egg is incorporated in the formula. It is interesting to note the very low value measured in the mix made with milk only (no egg) and to remember that this emulsion was not whippable.

These results clearly display an important amount of protein adsorbed at the oil-water interface before whipping, except when no egg was incorporated. The interfacial film surrounding the fat globules would be relatively thick in emulsions containing egg. Whipping induces protein desorption from the oil-water interface and would consecutively thin the interfacial layer at the fat globule surface. This phenomenon has already been described; in particular, Barford et al. (1991) specified that shear forces applied during whipping and freezing process of ice cream manufacture favors protein desorption from the fat globule surface.

According to the literature, fat crystallization and partial coalescence of fat globules are key factors in producing stable whipped emulsions. Emulsions have to be partly destabilized to ensure successful whipping; Melsen and Walstra (1989) and Goff (1997) indicated notably that homogenization of milk fat with caseins and whey proteins produces an emulsion too stable to be whipped. The protein depletion at the oil-water interface has to be sufficient to weaken the interfacial film surrounding the fat globules and allow partial coalescence to occur (Bolliger et al., 2000). In the present work, whipping reduces protein load at the fat globule surface. Shear forces applied as air is incorporated into mix allow proteins to be pulled off the globule surface and destabilize the mix toward partial coalescence. Because whole egg was incorporated in the recipes, we have to mention the possible role of nonprotein constituents at the oil-water interface: egg yolk phospholipids may modify the composition of fat globule surface.

In mix containing egg, roughly half the protein present is adsorbed at the oil-water interface before whipping, and this value falls by roughly one-third after whipping. In the mix containing no egg, almost no protein (8.8 %) is adsorbed at the oil-water interface before whipping. Therefore, whipping cannot cause that dra-

Figure 3. Part of protein adsorbed (%) at the fat globule surface in mix and whipped emulsions as a function of egg to milk protein ratio used for emulsion manufacture.

Figure 4. Quantification of egg and milk protein adsorbed at the fat globule surface. Egg to milk protein ratio at the oil-water interface in mix and whipped emulsions is represented as a function of egg to milk protein ratio used for emulsion manufacture.

matic protein desorption from the oil-water interface encountered in mixes containing egg. This means that during aeration of mix, no protein can be released from the oil droplets migrating toward the newly formed air-water interface to stabilize the surface of air bubbles.

Partition of Egg and Milk Proteins at the Oil-Water Interface

Protein composition of adsorbed layers was determined by SDS-PAGE. We quantified milk and egg proteins present at the oil-water interface; the results were expressed in terms of egg to milk protein ratios at the fat globule surface. These ratios were compared with those initially used for emulsion manufacture (Figure 4). Results show that whatever the formula, the egg to milk protein ratio is higher at the fat globule surface than in the initial ingredient mixture. This observation applies to the mix as well as to the whipped emulsions. We concluded that there is preferential adsorption of egg proteins over milk proteins at the fat globule surface in mix and whipped emulsions, whatever the recipe. Furthermore, the preferential adsorption of egg proteins at the oil-water interface is emphasized in the mousses because egg to milk protein ratio at the fat globule surface is higher in whipped emulsions than in mix. It is noteworthy that egg to milk protein ratios at fat globule surface are respectively 2 times and 4 times higher in mix and in whipped emulsions than in the recipes. For instance, from a 0.25 ratio in the recipe, it becomes 0.49 in mix, and 0.96 in whipped emulsions.

At first, the preferential adsorption of egg proteins at the oil-water interface in the mix shows evidence for competition between milk and egg proteins for the fat

globule surface during homogenization. Egg proteins would exhibit better emulsifying activity than milk proteins. Aluko et al. (1998) and Mine and Keeratiurai (2000) studied the competitive adsorption between whey or caseinate proteins and egg yolk lipoproteins in oil-in-water emulsions. In emulsions made with various ratios of whey protein isolate to low- or high-density lipoprotein, egg yolk lipoproteins predominate at the oil droplet surface. Whey proteins are systematically displaced from the oil-water interface, the displacement level increasing with the concentration of yolk lipoproteins (Aluko et al., 1998). Moreover, low-density lipoproteins displace β -casein, whereas high-density lipoproteins completely desorb all caseinate proteins in emulsions stabilized by caseinate-lipoprotein mixtures (Mine and Keeratiurai, 2000). In the present study, prevalence of egg proteins at the fat globule surface in the mix would confirm the previous results and the higher emulsifying activity of egg proteins. Nevertheless, we could not elucidate the mechanisms leading to the preferential adsorption of egg proteins. Indeed, as the amount of protein adsorbed at the fat globule surface increases with the egg content in mix, the preferential adsorption of egg proteins could result from milk protein displacement or from additional binding of egg proteins to milk proteins present at the interface.

Secondly, we considered the preferential adsorption of egg proteins in whipped emulsions. We concluded previously (according to the results obtained from Figure 4) that protein desorption from the fat globule surface occurred during the whipping process. Consequently, since the preferential adsorption of egg proteins at the oil-water interface is stronger in mousses than in mix, this clearly results from a desorption of milk proteins from the fat globule surface during whipping. The egg proteins predominate at the fat globule surface after homogenization and aging, and this phenomenon is enhanced during whipping due to milk protein desorption.

Finally, although part of milk proteins adsorb at the fat globule surface, we could raise the question of the function of excess protein in the emulsions. Goff (1997) specified that excess protein remaining in the serum contributes to overrun and texture of the whipped emulsions. During whipping, newly formed air bubbles are initially stabilized by protein adsorption (Needs and Huitson, 1991). Anderson et al. (1987) indicated that β -casein, which leaves casein micelles when milk is cooled at low temperature, and to a lesser extent, whey proteins, stabilize the air bubbles initially. We could postulate that these previous observations should apply to the present study and that additional proteins, in particular egg white proteins, should initiate whipping by stabilizing the newly created air bubbles (Martinet

Figure 5. Scanning laser confocal micrographs of mousses prepared from egg to milk protein ratios of 0.68 (A), 0.38 (B), and 0.25 (C). Images show the typical structure of whipped emulsions with fat globules surrounding the air bubbles: ab = air bubble; fg = fat globule; pa = protein aggregate.

et al., 2003). However, Anderson and Brooker (1988) specified that the amount of protein stabilizing the air bubbles is quite small and that, consequently, normal cream protein content is in large excess compared with what is required to initiate the whipping process. Then most of nonadsorbed proteins would enhance viscosity in mix (Kinsella, 1984) and ensure texture and stability to the foam matrix.

Structure of Whipped Emulsions

Structure of whipped emulsions according to the egg to milk protein ratio as determined by CLSM is presented in Figure 5. Images show similar organization within mousses, regardless of recipe. Fat globules are mainly located at the air-water interface, coating the air bubbles. Furthermore, it seems that fat globules retain their original shape at the air bubble surface; particularly, no evidence for partial coalescence was made within whipped emulsions. Images do not display any network of aggregated fat globules either. Matrix constituents visible by CLSM were large protein aggregates filling space between air bubbles.

In the present study, mousses observed by CLSM exhibit the typical structure of whipped emulsions described by several authors. Anderson and Brooker (1988) specified that all dairy foams containing significant amounts of fat exhibit similar surface features of air cells irrespective of whether emulsifiers or stabilizers were added or whether homogenization was carried out. Observation of mousses prepared in the present work confirms the conclusions of Anderson and Brooker: air bubbles are mainly stabilized by fat globules adhering to the air-water interface. However, unlike whipped creams or ice creams, mousses are not stabilized by a network of fat globules clumps and clusters (Anderson and Brooker, 1988). Various fat contents of these products would account for the differences ob-

served in their structure. Mousses prepared in the present work contain only 6.6% fat, whereas fat content of ice creams and whipped creams is at least 10 and 30%, respectively. The formation of a network from partially coalesced fat globules in mousses seems less likely than in whipped creams. This was precisely suggested by Goff (1997) about ice creams: cross linking of fat globules from one air cell to the next and thus forming an infrastructure is less likely in ice creams than in whipped creams due to the lower fat content of the previous ones.

Though mousses are not stabilized by a network of fat globules, partial coalescence of these could not be excluded; it is not evidenced by CLSM in the present study. This is probably due to the lower resolution of CLSM ($\sim 0.1 \mu\text{m}$) in comparison with electron microscopy ($\sim 0.1 \text{ nm}$), commonly used for foam imaging. We did notice that CLSM images show extensive protein aggregation within mousses, whatever the recipe, confirming the previous results of particle size measurements.

Stability of Whipped Emulsions

Air bubble size distribution in whipped emulsions after 15 d in storage at 4°C is represented in Figure 6 for the different egg to milk protein ratios (0.25, 0.38, and 0.68; emulsions prepared without egg were not whippable). We determined that mousses exhibit similar air bubble size distributions just after whipping, whatever the recipe (results not shown). Differences between samples appeared after 15 d storage at 4°C . As shelf life in such mousses is 28 d, these results constitute a valuable tool to evaluate overall quality of whipped emulsions, prepared from milk and egg, during storage. In mousses prepared with egg to milk protein ratios of 0.38 and 0.68, air bubbles with a diameter lower than $65 \mu\text{m}$ represent 82 and 85% of air volume,

Figure 6. Size distribution of air bubbles in whipped emulsions after 15 d storage at 4°C. Cumulative air volume fraction (%) was represented as a function of air bubble diameter (μm) for mousses prepared from egg to milk protein ratios: 0.25, 0.38, and 0.68 (emulsions manufactured without egg were not whippable).

respectively. In contrast, air bubble diameter in whipped emulsions prepared with a ratio of 0.25 reaches 135 μm (for 84% of air). Thus, large air cells appear during storage in mousses prepared with the lowest egg to milk protein ratio. These results clearly demonstrate the impact of the egg content on the macroscopic stability of whipped emulsions prepared from milk and whole egg. For an egg to milk protein ratio lower than 0.38, mousses destabilize during storage.

The presence of egg greatly influences the composition of interfacial layers in mix and in whipped emulsions, as well as the foam stability. Relationship between physicochemical properties of interfacial layers and foam properties is being investigated further.

CONCLUSIONS

This work constitutes an original study of complex whipped emulsions made with fresh milk and whole egg. Egg constituents contribute largely to structure building in whipped emulsions and consequently to structural and textural properties of final products. During homogenization, egg proteins preferentially adsorb over milk proteins at the oil-water interface and cover mainly the fat globule surface; therefore, they ensure mix stability before whipping. Shear forces applied during whipping cause significant desorption of milk proteins from the oil-water interface and thin the interfacial coating of fat globules. Partially destabilized fat globules adsorb at the air-serum interface and coat the air bubbles, stabilizing the mousse structure. A strong decrease in the egg content used for mousse

manufacture leads to destabilization of whipped emulsions during storage. The present study clearly correlates the emulsion formula (i.e., egg to milk protein ratio used for emulsion manufacture) to the protein composition of adsorbed layer at the fat globule surface and to the mousse stability, that is, to the shelf life of whipped emulsions.

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