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**Original article**

# Composition of fat protein layer in complex food emulsions at various weight ratios of casein-to-whey proteins

Stéphanie SOURDET[a], Perla RELKIN[a]*, Pierre-Yves FOSSEUX[b], Victoire AUBRY[b]

[a] Laboratoire de Biophysique des Matériaux Alimentaires, ENSIA, 1 avenue des Olympiades, 91744 Massy Cedex, France
[b] Nestlé Product Technology Center Beauvais, Z.I. n° 2, rue Charles Tellier, 60000 Beauvais, France

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**Abstract** – We studied three complex food emulsions which differed only by the weight ratio of casein-to-whey proteins (80:20, 60:40, 0:100). We considered the stability of the fat droplets against aggregation/coalescence, the amount of adsorbed proteins, and the protein composition of the interface. Our results indicated that coalescence was inhibited whatever the protein composition. However, aggregation between fat droplets was observed in the casein-free emulsion (0:100), where the amount of adsorbed proteins at the fat globule surface was the lowest. Quantitative SDS-PAGE experiments showed that preferential adsorption of caseins over globular proteins occurs only in the emulsion based on the intermediate (60:40) casein-to-whey protein ratio. Furthermore, the proportion of adsorbed proteins which were found in polymeric forms seemed to increase with decreasing casein-to-whey protein ratio. The fat globule stability, total fat protein load and proportion of adsorbed protein in the polymeric forms were discussed in terms of adsorption properties of milk proteins at the oil-water interface, in relation to the casein-to-whey protein ratio.

**Milk protein / emulsion / stability / protein adsorption / disulfide bond**

**Résumé** – **Composition de la couche protéique adsorbée à la surface des globules gras dans des émulsions alimentaires complexes contenant différents rapports caséine/protéines sériques.** Nous avons étudié trois émulsions alimentaires complexes qui ne diffèrent entre elles que par le rapport massique caséine/protéines sériques (80:20 ; 60:40 et 0:100). Cette étude a été réalisée en considérant la stabilité des gouttelettes lipidiques vis-à-vis de l'agrégation/coalescence, la quantité de protéines adsorbées par unité de surface de globule gras et la composition de l'interface. Nos résultats

* Correspondence and reprints
Tel.: (33) 1 69 93 50 29; fax: (33) 1 69 93 50 05; e-mail: relkin@ensia.inra.fr

ont montré l'absence de coalescence dans les trois émulsions, et la présence d'agrégats n'a été observée que dans l'émulsion (0:100) ne contenant pas de caséine. Dans ce dernier cas, la quantité de protéines adsorbées à la surface des globules gras est la plus faible. Une étude quantitative par électrophorèse sur gel de polyacrylamide a indiqué une adsorption préférentielle des caséines par rapport aux protéines sériques dans le cas de l'émulsion préparée à base du ratio intermédiaire (60:40) de caséine/protéines sériques. De plus, la proportion de polymères adsorbés à la surface de globules gras semble augmenter lorsque le rapport massique caséine/protéiques sériques diminue. La stabilité des émulsions, la charge protéique des globules gras et la proportion de polymères adsorbés sont discutées en fonction du rapport caséine/protéines sériques.

**Protéine laitière / émulsion / stabilité / adsorption des protéines / pont disulfure**

## 1. INTRODUCTION

The homogenization step in emulsion manufacture disrupts fat into small droplets and favors adsorption of surface active components, leading to droplet stabilization through different physico-chemical interactions or chemical bonds, depending on the interdroplet medium [15, 48]. The molecular mechanisms responsible for storage stability in model emulsions have already been studied through analysis of the droplet size distribution and protein composition of either the aqueous phase or the fat surface layer [5, 6, 17, 19, 21, 28, 46, 47, 49]. In complex emulsions [2, 3, 22–25] as well as in model emulsions [13, 17, 21, 47], competitive adsorption of proteins and emulsifiers for the oil-water interface was also reported. This molecular process has been shown to have some effects on emulsion ageing [1, 8, 23, 40]. On the other hand, storage stability of complex emulsions was shown to be related to interaction forces between proteins and polysaccharides, and thickening effects of non-adsorbing polysaccharides [11, 16].

Numerous studies [22, 23, 24, 40] which have been previously performed on the stability of complex emulsions considered the influence of the fat volumic fraction, total concentration of different proteins (skim milk powder, caseinate or whey protein concentrate) or non-proteic emulsifiers (mono- and di-glycerides, polysorbate 80, lecithin). The process variables such as homogenization pressure and temperature were also studied [42, 43], but little attention was paid to the effects of the protein structural state (micellar, globular, random coil) on the physico-chemical characteristics and stability of complex emulsions.

Globular whey proteins such as β-lactoglobulin (β-Lg), bovine serum albumin (BSA) and immunoglobulin G (IgG) contain both disulfide bonds and free sulfhydryl groups. α-Lactalbumin (α-La), another globular whey protein, contains disulfide bonds but no free thiol group [30, 35]. Among caseins (the major protein component of milk) only $\alpha_{s2}$- and κ- caseins contain disulfide bonds but no free thiol group. Heating solutions of globular whey proteins at temperatures higher than 75 °C may lead to denaturation [14, 38, 39, 41, 44], with consequences on surface hydrophobicity [4, 29] and the formation of polymers via a SH/S-S interchange reaction between whey proteins themselves [20, 33], and also between caseins and whey proteins [12, 26, 27]. On the other hand, heating milk in mixture with polysaccharides such as carrageenan, at a temperature higher than that of carrageenan transconformation contributes to associative interactions [32]. Besides these mechanisms which can take place in the continuous phase, previous studies performed on simple emulsions indicated the adsorption at the oil-water interface of complexes between milk proteins and anionic polysaccharides [11], and the

development at the oil-water interface of disulfide linkages between whey protein monomers and other protein molecules [10, 18, 34, 36].

In the present study we investigated some adsorption properties of milk proteins to the oil-aqueous interface in three complex emulsions which only differed by the weight ratio of casein-to-whey proteins. The aim of the study was to show how casein replacement by whey proteins could have effects on the stability of complex food emulsions.

## 2. MATERIALS AND METHODS

### 2.1. Manufacture of emulsions

The composition of the complex emulsions (all in weight proportion) consisted of 3% milk proteins, 9% hydrogenated palm kernel 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, sodium alginate, guar gum and carrageenan. The three emulsions 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 either a skim milk powder (SMP), a whey protein isolate (WPI) or a mixture of SMP and WPI powders. The physico-chemical characteristics of the SMP and WPI powders used to prepare the three emulsions are reported in Table I. The weight percentages of dry matter and ashes were obtained by heating the milk powders at 130 °C during a period needed to reach a constant weight, and at 550 °C for 5 h, respectively. The lactose content was obtained by application of the Boehringer Lactose/D-Galactose kit (Boehringer Mannheim, Meylan, France). Total nitrogen and non-protein nitrogen were determined by Kjeldahl analysis (AOAC, 1974). The relative proportions of caseins, β-lg, α-la, and other whey proteins (minor whey proteins) were determined by SDS-PAGE, according to Laemmli [31], in reducing conditions. The electrophoresis experiments were performed using PhastSystem apparatus (Pharmacia Biotech, Orsay, France), following the manufacturer's instructions, with a 12.5% w/w gel concentration (Pharmacia Biotech), and a molecular weight standard (Low Molecular Weight Electrophoresis Calibration Kit / Pharmacia) for identification of protein bands. 70 μL of the phase containing protein was added to 1 mL of Tris/HCl (10 mmol·L$^{-1}$) buffer (pH 8) containing EDTA (1 mmol·L$^{-1}$). Then, this protein solution was heated for 5 min in a

**Table I.** Chemical characteristics of skim milk powder (SMP) and whey protein isolate (WPI) used to prepare the emulsions.

| | SMP | WPI |
|---|---|---|
| pH | 6.64 | 6.72 |
| % Dry matter | 93.0 ± 0.4 | 94.9 ± 0.0 |
| % Ashes | 8.0 ± 0.2 | 4.0 ± 0.2 |
| % Lactose | 53.2 ± 0.5 | 0.1 ± 0.0 |
| % Protein[1] | 30.4 ± 2.2 | 87.1 ± 2.7 |

[1] (total nitrogen – non protein nitrogen) × 6.38.

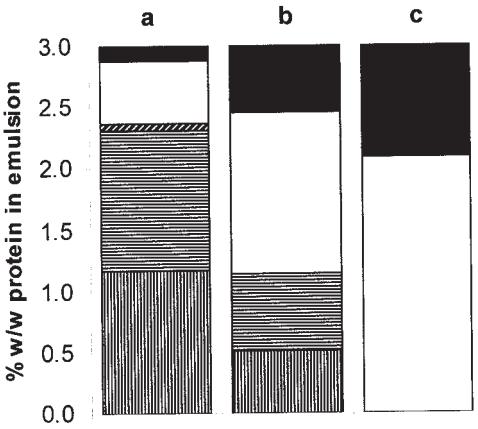


**Figure 1.** Concentration of each protein type obtained from integration of the colour intensity curve about PAGE in reducing conditions: (**a**) proteins present in the SMP, (**b**) proteins present in the SMP/WPI mixture, (**c**) proteins present in the WPI. α-Lactalbumin ■, β-lactoglobulin □, κ-casein ▨, β-casein ▤, $\alpha_{s1,2}$-casein ▥.

boiling water bath while stirring, before being cooled in an ice-bath. After the electrophoretic separation the gels were stained using 0.1% Coomassie blue (Phastgel Blue R-350). The corresponding electrophoretic patterns were scanned (Image Scanner, Pharmacia), and relative protein concentrations were determined densimetrically (Image Master, Pharmacia Biotech, Orsay, France). Results obtained from SDS-PAGE in non-reducing conditions indicated the absence of polymers in the milk powders used to formulate the emulsion. The concentration of each protein component in the milk powders (determined by SDS-PAGE in reducing conditions) are reported in Figure 1. Particularly, these results indicated the following casein-to-whey protein weight ratios: 80:20 (SMP), 60:40 (SMP/WPI mixture) and 0:100 (WPI).

The proportion of powders (milk protein samples, lactose, milk ultrafiltrate) used to formulate the 3 emulsions was determined using the physico-chemical characteristics of SMP and WPI powders reported in Table I. The WPI and SMP/WPI mixture had a lower salt and lactose content than the skim milk powder. The total solid non-fat content of the three emulsions was kept constant using milk ultrafiltrate and lactose to match the organic and mineral content carried out by SMP.

The manufacture of the emulsions was performed in a pilot plant. First, the powders (emulsifier/stabilizer, protein source, sucrose and milk ultrafiltration permeate) were dispersed in distilled water heated to 65 °C, then fat (pre-melted at 40 °C) and glucose syrup were added (feeding tank: 65 °C-15 min upon stirring). After pre-heating (72 °C-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-30 s), and then aged for 24 h (4 °C), before fat globule size analysis.

### 2.2. Fat globule size distribution

Emulsion samples were brought from 4 °C to room temperature before the determination of their fat globule size distribution. We used a Mastersizer apparatus (MS

1000-Malvern Instruments, Orsay, France). The emulsion samples were either directly dispersed in Milli-Q water or dispersed in 1% w/w SDS solution before the measurements. The emulsion dilution factor was approximately 1:2000. As previously demonstrated [47], the dispersion in 1% w/w SDS solution, which was used to dissociate the non-covalently bound aggregates of fat droplets, was shown to have no effect on the size distribution of the most stable emulsions. In the present study, we compared the values of $d_{0.5}$ (volume average median diameter) obtained after dispersion of the emulsions in distilled water or SDS solution, and we used the values of fat specific surface area, SSA ($m^2 \cdot mL^{-1}$) obtained after dispersion in SDS solution. Those parameters were calculated by the Malvern software, from the droplet size distributions carried out on the independent model basis. The presentation factor was selected after measuring the refractive index (ABBE Atago 3T) of the dispersed fat relative to water (1.08), and after measuring the absorbance at 633 nm (spectrophotometer Varian Cary 100, Les Ulis, France) of the dispersed phase (0.1). Analyses were performed on three different samplings.

### 2.3. Cream separation from emulsions

The separation process of fat globules from the aqueous phase in the various emulsions was carried out by performing three successive centrifugation steps (Sigma 3K12 centrifugation) at 15 000 *g* for 60 min. Preliminary assays performed at 15 000 *g* for 60 min at 4 °C or 20 °C did not allow total cream separation, particularly for emulsions containing casein. The centrifugations were performed at 40 °C, temperature at which the fat is in liquid state. This choice was made on the basis of a previous study [45], where it was demonstrated that dispersed fat particles which are in liquid state do not coalesce. Consequently, centrifugation was performed at 40 °C to minimize overestimation of the depleted proteins which could result from disruption of protein film layers around the fat droplets. After each centrifugation step, the aqueous phase (without the cream layer) was sucked off using disposable syringes and needles, weighed and replaced by water to wash the separated cream.

### 2.4. Analysis of adsorbed protein

In most of the studies previously performed on protein-stabilized emulsions [6, 18, 22, 37, 47], the percentage of adsorbed proteins ($P_{ads}^{L}$) was determined through determination of depleted proteins (serum phase obtained after one centrifugation step). In the present study, we also considered the proteins which were desorbed from the washed cream by SDS molecules ($P_{ads}^{SDS}$). The last method has been previously applied to simple emulsions stabilized by caseinate or whey proteins [9, 28, 45, 46] and to ice cream model mixes [3]. In the present study, the final washed cream layer was dispersed in 5% w/w SDS with (reducing conditions) or without (non-reducing conditions) 2-mercaptoethanol (2.5% w/w), stirred at room temperature to desorb proteins [13, 28], and then centrifuged in the conditions described above. The resulting aqueous phase (without cream) which contained the SDS-desorbed proteins ($P_{ads}^{SDS}$) was submitted to Kjeldahl analysis.

The protein surface concentration (expressed in $mg \cdot m^{-2}$ of fat globule surface) was calculated following the expression:

$$\Gamma = (d \times P_{ads} \times P_0 \times 1000) / (SSA \times \phi) \quad (1)$$

where d is the fat density, $P_{ads}$ is the weight percentage of adsorbed proteins (as determined after desorption by SDS ($P_{ads}^{SDS}$) or after depletion using one centrifugation step of the initial emulsion ($P_{ads}^{L}$)), $P_0$ is the weight percentage of protein in the initial

emulsions, SSA is the specific surface area ($m^2 \cdot mL^{-1}$), as calculated by the Malvern software from the monomodal fat globule distribution obtained after emulsion dispersion in 1% w/w SDS solution, and $\phi$ is the fat weight fraction.

The SDS-depleted protein solutions were analyzed by electrophoresis, following the method described above. This procedure was used to identify the nature of proteins which were depleted by SDS from the three emulsions' washed cream layers. The results obtained from SDS-PAGE experiments performed in reducing and non-reducing conditions were used to calculate the relative concentration of protein polymers which were desorbed from the extensively washed cream layers.

## 3. RESULTS AND DISCUSSION

### 3.1. Fat globule size distribution

The particle size distributions obtained after emulsions' dispersion in distilled water were monomodal for the SMP and SMP/WPI emulsions, and bimodal (Fig. 2) for the WPI one. The values of the corresponding $d_{0.5}$ parameter are shown in Figure 3. They clearly indicated the presence of much larger dispersed particles in the case of casein-free emulsion. The second peak, observed when the WPI emulsion was dispersed in water, disappeared (Fig. 2) after dilution in SDS solution. The corresponding $d_{0.5}$ value ($0.51 \pm 0.04$ µm) was very close to that observed ($0.59 \pm 0.06$ µm, Fig. 3) for the emulsions containing casein, when dispersed in distilled water. The differences between the two methods of observation of particle size distribution in the WPI emulsion suggested that hydrophobic interactions may be responsible for the existence of the second population of particles, which probably results from aggregation. After aggregate dissociation under the effect of SDS, the three emulsions showed a monomodal fat globule size distribution, with similar values of specific surface area (Tab. II). The stability against aggregation of the emulsions containing casein could be due to specific adsorption properties of casein molecules. Casein molecules could provide steric stabilization by protruding further into solution, contrary to WPI which could destabilize the emulsion by bridging flocculation [10, 15]. Britten and Giroux [5] studied the emulsifying properties of whey protein and casein blends (1% w/v total protein concentration, pH 7) through the change in densimetric profile of the corresponding simple emulsions (30% soya oil). They observed

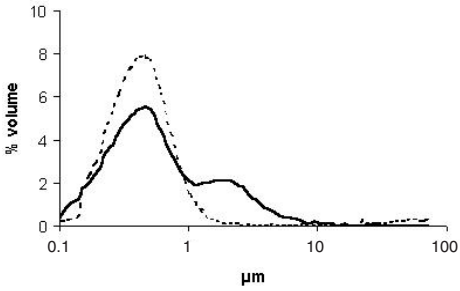


**Figure 2.** Fat globule size distribution for the WPI-based emulsion (dispersion in distilled water —— or 1% w/w SDS – – – –).

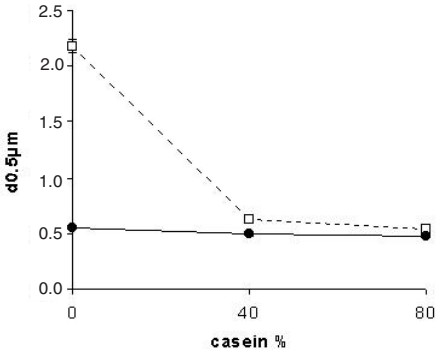


**Figure 3.** Volume average median diameter ($d_{0.5}$) as a function of casein percentage in each emulsion. Emulsion dispersed in distilled water (□) or in 1% w/w SDS solution (●).

**Table II.** Effects of the weight ratio of casein-to-proteins on the percentage of adsorbed proteins, as determined either from depleted proteins after one centrifugation ($P_{ads}^{L}$) or after desorption from the washed cream layer by SDS molecules ($P_{ads}^{SDS}$), and on the corresponding $\Gamma^{L}$ and $\Gamma^{SDS}$ values, specific surface area (SSA) values, as determined by Malvern software by light scattering from emulsions dispersed in 1% SDS solution, and percentage of intermolecular disulphide-bound proteins (% polymers) which were desorbed by SDS molecules from the washed cream layers of the various emulsions (SMP = skim milk powder, WPI = whey protein isolate).

| Casein:whey protein weight ratio | $P_{ads}^{L}$ | $P_{ads}^{SDS}$ | $\Gamma^{L}$ $mg \cdot m^{-2}$ | $\Gamma^{SDS}$ $mg \cdot m^{-2}$ | SSA $m^2 \cdot mL^{-1}$ | Polymers % |
|---|---|---|---|---|---|---|
| 80:20 (SMP) | 50.0 ± 1.7 | 6.5 ± 0.5 | 10.1 ± 0.4 | 1.3 ± 0.1 | 14.7 ± 0.2 | 15 ± 1 |
| 40:60 (SMP/WPI) | 43.7 ± 1.0 | 3.2 ± 0.3 | 10.2 ± 0.2 | 0.8 ± 0.1 | 13.7 ± 0.6 | 27 ± 2 |
| 0:100 (WPI) | 25.7 ± 0.8 | 1.3 ± 0.1 | 4.4 ± 0.2 | 0.2 ± 0.0 | 12.8 ± 0.2 | 40 ± 1 |

higher creaming and coalescence upon storage for emulsions containing casein. But destabilization decreased when monoglycerides (0.5% w/v) were added or when the protein solutions were heated (80 °C for 30 min) before emulsion formation. The absence of coalescence in our complex emulsions whatever the casein-to-whey protein ratio could be due to the presence of polysaccharides, which may contribute, via a higher viscosity of the continuous phase [11, 16], to a reduction of droplets meeting probability.

### 3.2. Amount of adsorbed protein and fat protein load

The Kjeldahl method was applied to determine the protein concentration in the three aqueous phases (without cream) obtained in the three successive centrifugation steps and that obtained after desorption by SDS molecules from the washed cream. We observed a correct protein recovery for all the 3 emulsions, in agreement with Hunt and Dalgleish [28] who used SDS-PAGE to check the protein recovery. Our results, reported in Table II, indicated that the percentage of adsorbed proteins was higher for the emulsions which contained casein than for the WPI emulsion. Considering that the amount of adsorbed protein is either equal to $P_{ads}^{L}$ or $P_{ads}^{SDS}$ (as defined above) we may conclude that the amount of adsorbed proteins increases with the casein-to-whey protein weight ratio. This result is similar to that previously shown for model emulsions [5, 6, 21], despite the presence in our complex emulsions of mono- and di-glycerides, polysaccharides, glucose and sucrose. On the other hand, the higher value of $P_{ads}^{L}$ than $P_{ads}^{SDS}$ could be explained by the fact that $P_{ads}^{L}$ takes into account both tightly and loosely adsorbed proteins (i.e. all the layers of adsorbed proteins on the fat globules' surface), as reported by Shimizu et al. [46] from simple milk protein-based emulsions. However, evaluation of the percentage of adsorbed proteins using $P_{ads}^{SDS}$ values considers only tightly adsorbed proteins which are desorbed by SDS molecules, from extensively washed creams. These proteins probably correspond to the first protein layer(s) which is (are) adsorbed at the interface. Light-scattering experiments performed after the dispersion of the emulsions in distilled water indicated a mechanism of fat globule aggregation in the WPI emulsion (Fig. 2), contrary to the emulsions containing casein. As previously reported in simple emulsions [5, 28], this mechanism could be related to the lower adsorption property of whey proteins, when compared to caseins. Indeed, due to its size,

the probability that a protein meets a fat globule in turbulent flow is higher for a casein micelle than for a whey protein [49]. Therefore, as the three emulsions are based on the same total protein concentration, the trend in the proportion of adsorbed proteins depends on the casein-to-whey protein ratio. It could be explained either by a higher competitive displacement of whey proteins by surfactant molecules than caseins, or differences in the probability that a protein meets a fat globule, or the thicker films formed at the interface by casein micelles than dissociated proteins, or preferential adsorption of caseins over whey proteins [5, 22, 28].

The fat globule protein load was calculated using Equation (1). The SSA values determined for the three emulsions did not differ very much (Tab. II). Therefore, the values of $P_{ads}{}^{L}$ and $P_{ads}{}^{SDS}$, and their corresponding values $\Gamma^{L}$ and $\Gamma^{SDS}$ follow a similar trend. Other results (Fig. 4) concern the similar values found for $\Gamma^{L}$, from the two emulsions containing casein. These results indicated that emulsions containing SMP reached the maximum $\Gamma^{L}$ value (10 mg·m$^{-2}$) when in mixture with WPI. For emulsions containing WPI and no casein, the protein load was only half this last value. However, the amount of tightly adsorbed protein increased linearly with casein content ($R^2 = 0.9997$). The results of $\Gamma^{SDS}$ suggest that the amount of tightly adsorbed proteins on fat droplets is six times higher for SMP than for WPI. Unlike $\Gamma^{L}$, $\Gamma^{SDS}$ did not reach a maximum value. Both the variation in $\Gamma^{L}$ towards a plateau value and the linear increase in $\Gamma^{SDS}$ as a function of casein concentration in the three emulsions cannot be explained by their respective SSA values, which did not differ very much (Tab. II). In previous studies, Britten and Giroux [5] showed that the interfacial pressure of mixed WPI/sodium caseinate at various ratios (ranging from 0:100 to 100:0) remained the same as long as caseinate was present in the mixture. In parallel, they also reported that the fat protein load in the corresponding emulsions increased as the caseinate proportion increased from 20 to 100%. These last results, combined with those obtained in the present study, indicate that two systems (simple emulsions and complex food emulsions), which are quite different by their composition, may bring the same kind of information, at least when they are both stabilized by similar milk proteins.

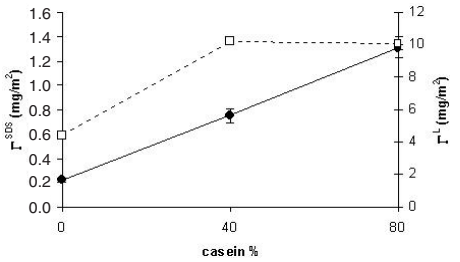


**Figure 4.** Fat protein load as determined by the SDS-desorption method ($\Gamma^{SDS}$: ●) or protein depletion after one centrifugation ($K^{L}$: □) as a function of casein percentage in each emulsion.

### 3.3. Nature of the adsorbed proteins

SDS-PAGE experiments in reducing conditions and non-reducing conditions were applied to solutions of SMP, SMP/WPI and WPI powders used to prepare the emulsions. They were also applied to protein solutions obtained after desorption by SDS molecules from the washed creams. The corresponding electrophoretic bands were used to characterize the protein composition of the milk powders used to formulate the emulsions. The concentration of each protein component was deduced from the area under each densimetric peak, relative to the total peak areas. The concentration of each milk protein which was desorbed by SDS molecules from the cream layer of the corresponding emulsions are reported in Figure 5.

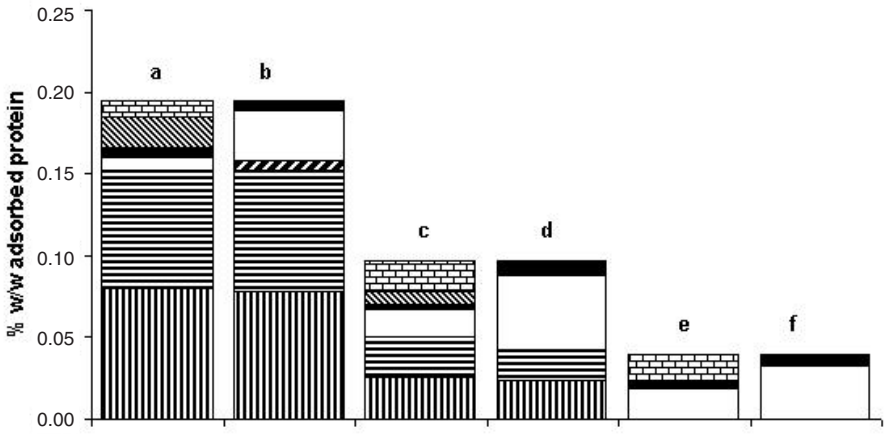


**Figure 5.** Concentration of each protein type obtained from integration of the colour intensity curve about PAGE: (**a**) adsorbed protein in non-reducing conditions for the SMP emulsion, (**b**) adsorbed protein in reducing conditions for the SMP emulsion, (**c**) adsorbed protein in non-reducing conditions for the SMP/WPI emulsion, (**d**) adsorbed protein in reducing conditions for the SMP/WPI emulsion, (**e**) adsorbed protein in non-reducing conditions for the WPI emulsion, (**f**) adsorbed protein in reducing conditions for the WPI emulsion. α-Lactalbumin ■, β-lactoglobulin □, κ-casein ▨, β-casein ▤, $\alpha_{s1,2}$-casein ▥, high molecular weight polymers ▦, ~80 kg·mol$^{-1}$ polymers ▧.

For SMP emulsion, comparison between the values reported in Figures 1a and 5b, showed that the relative proportion of caseins (mainly $\alpha_s$- and β-casein) and whey proteins (mainly α-La and β-Lg) seemed to adsorb in similar proportions to those in milk. This finding does not agree with the hypothesis of a preferential adsorption of caseins over globular whey proteins, but it agrees with previous studies on recombined milk [37]. The values obtained in non-reducing conditions (Fig. 5a) indicated that S-S polymers of approximately 80 kg·mol$^{-1}$, and of higher molecular mass can be found as components of the protein layer around the fat globules, in addition to milk monomeric proteins. These polymers may be composed of β-Lg and κ-casein, as indicated by the appearance of the corresponding bands in reducing conditions (Fig. 5b) in parallel with the presence of polymer bands in non-reducing conditions (Fig. 5a). For the SMP/WPI emulsion, which is based on a lower casein-to-whey protein ratio, comparison between the values reported in Figures 1b and 5d (reducing condition) showed a preferential adsorption of caseins over whey proteins. The concentration of polymers observed in non-reducing conditions (Fig. 5c) indicated that polymerization is higher (in percentage relative to the total adsorbed proteins) in SMP/WPI emulsion than in SMP emulsion. In regard to the trend observed in the electrophoretic band intensities corresponding to α-La and β-Lg monomers, the polymerization could occur mainly between α-La and β-Lg molecules.

A higher relative concentration of β-Lg molecules (Figs. 5e and 5f) seemed to be involved in polymerization for the casein-free emulsion than for the SMP/WPI and SMP emulsions. The concentration of polymers found by eletrophoretic analysis of desorbed proteins by SDS molecules from the three cream layers are reported in Table II. It seems that the increase in the

proportion of disulfide bonding in SDS-desorbed proteins increases with the whey protein proportion used to prepare the emulsions: the higher the whey protein proportion, the more disulfide and sulfhydryl groups and the more disulfide bond exchanges. For a long time, interactions between caseins and β-Lg via the formation of disulfide bonds between denatured whey proteins and caseins have been observed in heated milk protein solutions [35, 38]. Comparison between results obtained by electrophoretic analysis of protein composition of the cream layers which were separated from non-pasteurized emulsions (results not shown), and those obtained from pasteurized emulsions (present results) indicated higher protein load values for unpasteurized emulsions, but no significant difference in the proportion of disulfure bonding. Moreover, it was observed recently that time-dependent polymerization occurs in simple emulsions stabilized by whey proteins [7, 8, 18, 34, 36]. In these previous studies performed on simple model emulsions, it was demonstrated that flocs might be formed initially during homogenization by non-covalent bonding (e.g. hydrogen, ionic, hydrophobic or van der Waals bonds) between proteins adsorbed onto different droplets. However, it was also postulated that high molecular weight protein polymers could be formed at the oil-water interface with a time-dependent mechanism. In the present study, fat droplets did not form aggregates in emulsions containing casein, and fat globule flocculation was observed in the WPI emulsion (Figs. 2 and 3). These results may lead to the conclusion that the protein polymers (which were desorbed by SDS molecules from the cream layer) are not due to covalent bridging between protein molecules adsorbed on different droplets, and they are not formed under the effect of pasteurization treatment. Further time-dependent investigations of protein composition of the layer around fat globules in complex food emulsions are needed to confirm that the polymer formation at the oil/solution interface is time-dependent, as in simple emulsions.

## 4. CONCLUSION

The results obtained in this study showed that the fat globules in the casein-free emulsion were the least stable against aggregation and that they presented the lowest fat protein load. For the emulsion which contained an intermediate casein-to-whey protein ratio (60:40), the fat protein load ($\Gamma^{L}$) which was determined by using the depletion method (amount of proteins depleted from the cream layer, obtained after one centrifugation step) was close to that determined for the SMP emulsion in similar conditions. Furthermore, for both emulsions containing casein, the aggregation of fat globules was avoided. When we considered the fat protein load values calculated from the SDS-desorbed proteins from the extensively washed cream layer, we observed a linear increase in $\Gamma^{SDS}$ as a function of casein concentration. Comparison between the results obtained from SDS-PAGE in reducing and non-reducing conditions indicated a preferential adsorption at the oil-water interface of caseins over whey proteins only in the SMP/WPI emulsion. Also, the proportion of polymers observed from the washed cream seemed to arise from the protein layer around the fat globules.

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