

Microfluidization of model dairy emulsions. II. Influence of composition and process factors on the protein surface concentration

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Summary – The influence of some composition variables (butter oil, sodium caseinate and monoglyceride contents) and process variables (pressure and temperature) on the relative adsorbed protein fraction (F_{ads} , %) and on the protein load (Γ , mg m⁻²) of fat globules was studied in a model dairy emulsion (oil-in-water produced by microfluidization). The amount of adsorbed proteins (F_{ads} and Γ) was evaluated immediately after emulsification by coupling the separation of oil and aqueous phases of the emulsion produced by centrifugation to the determination of protein content. The specific surface area (A_{sp}) of oil-water interface was obtained by photon correlation spectroscopy after the protein aggregates were dissociated by an appropriate buffer. A central composite experimental design was used to obtain two nonlinear multiple regression equations relating F_{ads} and Γ to sodium caseinate (0.5 to 3.9 wt%), butter oil (5.2 to 14.7 wt%) and monoglyceride (0.08 to 0.88 wt%) contents, and to the emulsification pressure (7.8 to 76.3 MPa) and temperature (35 to 100 °C). These two functions explained 92.7 and 90.6% of the variation in F_{ads} and in Γ , respectively, and made it possible to evaluate the independent influence of each experimental variable. The results indicate that the sodium caseinates (NaCas), as a group, seem much less easily adsorbed than monoglyceride (glycerol monostearate, GMS) molecules when the GMS:NaCas molecular ratio (R) is higher than 5: more numerous and possibly more surface active than proteins, monoglycerides probably settle at the interface quicker than proteins. A qualitative model is also presented to illustrate this possible competition and estimate its influence on the parameters that characterize protein adsorption (F_{ads} and Γ) and fat globule size (d_{vs}).

protein surface adsorption / protein load / protein-surfactant competition / microfluidization / milkfat globule / oil-in-water emulsion / emulsification

Résumé – Microfluidisation d'émulsions laitières modèles. II. Influence des facteurs de procédés et de formulation sur la concentration de protéines à l'interface. L'influence de certaines variables de composition (teneurs en huile de beurre, caséinates de sodium, et monoglycérides) et

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de procédé (pression et température) sur la fraction de protéines adsorbées à l'interface (F_{ads} , %) et sur la charge protéique (Γ , mg m^{-2}) des globules gras d'une émulsion produite par microfluidisation a été étudiée. L'évaluation de la quantité de protéines adsorbées (F_{ads} et Γ) a été réalisée juste après l'émulsification en associant la séparation des phases aqueuse et lipidique de l'émulsion par centrifugation à une détermination de la teneur en protéines. La surface spécifique (A_{sp}) des globules gras a été évaluée par spectroscopie de corrélation photonique après que les agrégats protéiques aient été dissociés dans un tampon approprié. L'utilisation d'un dispositif expérimental de type central composite a permis d'obtenir deux équations de régression multiple non linéaires reliant F_{ads} (%) et Γ (mg m^{-2}) aux teneurs en caséinates de sodium (0,5 à 3,9%), huile de beurre (5,2 à 14,7%) et monoglycérides (0,08 à 0,88%), à la pression (7,8 à 76,3 MPa) et à la température d'émulsification (35 à 100 °C). Ces deux fonctions expliquent respectivement 92,7% et 90,6% des variations de la fraction protéique adsorbée et de la charge protéique des globules gras dans l'intervalle de valeurs des paramètres considérés. Les résultats indiquent que les caséinates de sodium (NaCas), en tant que groupe, semblent moins facilement adsorbés que les monoglycérides lorsque le ratio moléculaire GMS:NaCas (R) est supérieur à 5: plus nombreux et peut-être plus tensioactifs que les protéines, les monoglycérides colonisent probablement l'interface plus rapidement que les protéines. Un modèle qualitatif représentant cette possible compétition et son influence sur les paramètres caractérisant l'adsorption protéique (F_{ads} et Γ) et la taille des globules gras (d_{vs}) est également proposé.

adsorption protéique en surface / charge protéique / compétition protéine-surfactant / microfluidisation / globule gras / émulsion huile-dans-l'eau / émulsification

ABBREVIATIONS

- A_{sp} : specific surface of fat globules ($\text{m}^2 \text{kg}^{-1}$);
 A_t : total surface area of fat globules (m^2);
 $[BO]$: butter oil concentration (wt%);
 cv : coefficient of variation of the volume-weighted size distribution (%);
 d_{vs} : volume-weighted average fat globule diameter (m);
 F : number of factorial points of the design or Fisher ratio (dimensionless);
 F_{ads} : adsorbed protein fraction (%);
 g : gravitational constant (9.81 m s^{-2});
 $[GMS]$: monoglyceride concentration (wt%);
 k : number of independent variables (dimensionless);
 M : total mass of fat globules (dimensionless);
 $[Prot]$: protein concentration (wt%);
 P : emulsification pressure ($\text{Pa} = \text{m}^{-1} \text{kg s}^{-2}$);
 r : rotor radius (m);
 R : GMS:protein molecular ratio (dimensionless);
 T : emulsification temperature (K);
 x : independent variable;
 x_{BO} : mass fraction of butter oil (dimensionless);
 x_{GMS} : mass fraction of monoglycerides (dimensionless);
 y : dependent variable;
 β : coefficients determined by the least squares method (dimension variable);
 ε : random component error (dimension variable);
 ϕ : volume fraction of the dispersed phase (dimensionless);
 ϕ_{cas} : volume fraction of the casein micelles in milk (dimensionless);
 γ : interfacial tension ($\text{N m}^{-1} = \text{kg s}^{-2}$);
 Γ : protein load (g m^{-2});
 ρ : mass density (kg m^{-3});
 $\rho_{Milk}^{20\text{ }^\circ\text{C}}$: mass density of milk (kg m^{-3});
 ρ_{BO} : mass density of butter oil (kg m^{-3});
 ρ_{GMS} : mass density of monoglycerides (kg m^{-3});
 $\sigma_{F_{ads}}$: variance of the adsorbed protein fraction (dimensionless);
 σ_Γ : variance of the protein load ($\text{g}^2 \text{m}^{-4}$).

INTRODUCTION

In food emulsions, there are mainly two classes of molecules that show a strong tendency to accumulate at the dispersed droplet surface during emulsification. These molecules are proteins and low molecular mass surfactants, respectively (Dickinson et al, 1990). Although these two classes of molecules influence emulsion behavior (eg, emulsion formation, kinetic stability, flow properties, etc), the magnitude of their effects is different. Indeed, due to their differences in composition and conformation, their influence based on physical interactions for both of them, whether intermolecular or intramolecular, and mediated by the chemical nature of the environment on either side of the interface, is different (Fisher and Parker, 1988). By lowering the interfacial tension between the phases, emulsifiers generally reduce the Laplace pressure and pressure gradients required to disperse one phase into the other (Walstra, 1983). Small molecule surfactants, however, lead to the formation of smaller droplets, by lowering the interfacial tension much more strongly than proteins alone. This is associated with a higher Gibbs elasticity of the film between pairs of approaching droplets during emulsification (Dickinson et al, 1989b). Similarly, by accumulating at interfaces, emulsifiers surround fat globules and form a stabilizing layer on their surfaces which protects them against subsequent coalescence. The structure and properties of that layer, which remarkably influence the properties of emulsions (eg, stability and rheology), is widely determined by the nature of the emulsifiers used. On the one hand, protein layers appear rather viscoelastic and tend to dampen down surface fluctuations and to inhibit the mechanism responsible for film rupture; on the other hand, small molecule surfactants which induce low interfacial tension gradients and low shear surface viscosities seem less efficient at preventing layer rup-

ture (Walstra, 1987; Dickinson, 1992). As a first approximation, stability with respect to coalescence tends to increase with the increase of surface viscosity. The relationship between surface viscosity and stability is not clear, however. Random coil-type proteins (eg, caseins) form layers with little cohesiveness (low viscosity) compared to globular proteins (eg, whey proteins) which retain a major portion of their tertiary structure and form layers of higher viscosity (Boyd et al, 1973; Graham and Phillips, 1980; Castle et al, 1987). Nevertheless, it is well known that the former protein type produces more stable emulsions than the latter (Leman and Kinsella, 1989; Lorient et al, 1991). Furthermore, small molecule surfactants, at concentrations well above monolayer coverage, may also provide some additional stabilization by forming liquid crystals at the interface (Friberg et al, 1976; Krog, 1990) or in the continuous phase between oil droplets (Barry, 1975).

All these trends, generally observed in model systems containing either one source of emulsifier (protein or low molecular mass surfactants) or a mixture of two pure surfactants, are further complicated in real systems, such as dairy emulsions. In the latter systems, milk proteins (casein and whey proteins) and oil-soluble low molecular mass surfactants (monoglycerides, phospholipids, free fatty acids, etc) are invariably present in mixture. Two other classes of phenomena must be added to this new situation. First, the difference in magnitude of their own effects leads to competition for space at the interface between i) the various milk protein components (Dickinson et al, 1989a, 1993; Britten and Giroux, 1991; Dalgleish et al, 1991; Dickinson and Gelin, 1992); ii) the various low molecular mass surfactants, deliberately added or inevitably present in oil fraction (Gaonkar and Borwankar, 1991); and finally iii) between these surfactants and the proteins (Dickinson et al, 1990; Courthaudon et al, 1991a, b, c; Dickinson and Tanai,

1992a; Tomas et al, 1994b). In the latter case, strong and thickly adsorbed layers produced by interactions between proteins and surfactants have also been reported (Wüstneck et al, 1984; Doxastakis and Sherman, 1986; Wüstneck and Müller, 1986). Second, milk proteins can also be partially or totally displaced from the interface by low molecular surfactants, as has been theoretically predicted (Cohen Stuart et al, 1984) and experimentally observed (Oortwijn and Walstra, 1979; De Feijter et al, 1987; Dickinson et al, 1989c; Heertje et al, 1990; Courthaudon et al, 1991b, c; Krog, 1991). In practice, however, a clear distinction between competitive adsorption and displacement is sometimes difficult. Indeed, the ease as well as the extent of protein displacement by other surface active molecules are narrowly linked to various factors such as the molecular mass (Walstra and Oortwijn, 1982; Brynda et al, 1986; Zsom, 1986), the hydrophobicity of proteins (Nakai, 1983), the structure stability of proteins (Arai and Norde, 1990), the order of exposure to the interface (Dickinson et al, 1989a), the age of the adsorbed protein layer (Dickinson and Matsumura, 1991), the extent of any thermal treatment (Das and Kinsella, 1990; Dickinson and Tanai, 1992b), the protein glycosylation (Closs et al, 1990) and more.

In part I of this series (Robin et al, 1992), we showed the influence of some composition (butter oil, protein and surfactant concentrations) and process variables (pressure and temperature) on the microfluidized fat globule size distribution. From these results, it can be concluded that some competitive adsorption between caseins and monoglycerides occurs. The study of the effects of the same composition and process factors on the surface concentration of adsorbed proteins of microfluidized milkfat globules was carried out with respect to the possible competitive adsorption between proteins and monoglycerides. The amount of adsorbed proteins on milkfat

globules in freshly made emulsions was consequently evaluated.

MATERIALS AND METHODS

Emulsion ingredients

Butter oil (99.1% anhydrous) with a density $\rho_{BO}^{20^\circ\text{C}} = 0.920$ (Liang and Shi, personal communication) was purchased from Ault Foods Limited (Mitchell, ON, Canada) and was stored at -18°C . Distilled monoglycerides (Excel T-95) were obtained from Atkemix (Brantford, ON, Canada). They had an estimated molecular mass, as determined by gas chromatography, of 360 Da, an hydrophile-lipophile balance of 4.5 and the following composition in mass: 94% monoglycerides, 4% diglycerides, 1% glycerol and 1% fatty acids. Sodium caseinates (89.3% protein, 50 mg Ca/100 g) with an approximated average molecular mass of 20 000 Da (Cheftel et al, 1985) were purchased from ICN Nutritional Biochemicals (Mississauga, ON, Canada).

Experimental design and emulsion preparation

As previously reported (Robin et al, 1992), a central composite rotatable design made up of $k = 5$ factors with five levels, and one repetition was developed. The five factors that were varied in the preparation of the emulsions are the following: two process factors - emulsification pressure (P), and temperature (T), and three formulation factors - protein concentration ($[Prof]$) butter oil content ($[BO]$) and monoglyceride content ($[GMS]$). It was anticipated that the influence of composition and process variables (x) on the protein surface concentration, expressed in terms of adsorbed protein fraction (F_{ads}) and protein load (Γ), could be deduced from a second order polynomial model:

$$y = \beta_0 + \sum_{i=1}^5 \beta_i x_i + \sum_{i,j}^{5,5} \beta_{ij} x_i^2 + \sum_{i,j \neq i}^{5,5} \beta_{ij} x_i x_j + \epsilon \quad [1]$$

where y is the dependent variable (F_{ads} or Γ), β_0 , β_i and β_{ij} are coefficients determined by the least squares method, and ϵ is the random component error.

A total of 52 emulsions, each of 600 g, were produced and analyzed. They were prepared by melting butter oil at 50°C and mixing it with sodium caseinate aqueous solutions freshly made in bidistilled water at room temperature. The mix-

ture contained 5.2, 8, 10, 12 or 14.8 wt% of butter oil and 0.5, 0.9, 1.5, 2.5 or 3.9 wt% sodium caseinate based on protein content. The mixtures were then heated to the emulsification temperature (35, 55, 70, 85 or 100 °C), and the mono-glycerides (0.08, 0.2, 0.4, 0.6 or 0.88 wt%) were added. Following premixing at 2 000 rpm for 2 min at the emulsification temperature, the dispersions were emulsified by microfluidization (model 110, Microfluidics Corporation, Newton, MA, USA), in a two-pass process. The first pass was carried out at 7.8, 20.7, 41.4, 62.1 or 76.3 MPa and the second at 4.8 MPa. Further details on the experimental design as well as on the emulsification method may be found in Robin et al (1992).

Evaluation of the relative adsorbed protein fraction and the protein load of the emulsified fat globules

Methods for determining the protein load (Γ), ie, the amount of protein adsorbed per unit area of interface, are generally based on an evaluation of the specific surface area (A_{sp}) of dispersed droplets and the amount of adsorbed proteins. This second parameter can be obtained by determining the protein content in the creamed layer, the serum layer or in both (Mulder and Walstra, 1974; Ogden et al, 1976; Tornberg, 1978; Oortwijn and Walstra, 1979; Blanchot, 1992) after centrifugation of the native emulsion. Recently, Hunt and Dalgleish (1994) described a direct method whereby the amount of adsorbed protein in emulsions is quantified by SDS-PAGE followed by photometric scanning of the stained protein bands.

Evaluation of the specific surface area in emulsion

In the absence of an ideal method to determine the dispersed droplet size distribution parameters of an emulsion, a previously reported procedure based on photon correlation spectroscopy after the protein aggregates were dissociated by an appropriate buffer (Urea, EDTA, β -mercaptoethanol, pH 7) was used to evaluate the volume-weighted average diameter (d_{vs}) and the relative standard deviation around the average (cv) (Robin and Paquin, 1991; Robin et al, 1992).

From the d_{vs} of the fat globule size distribution, the specific surface area (A_{sp}) can be deduced. Assuming that fat globules are perfect spheres,

A_{sp} can be obtained from the ratio of the total area (A_t) divided by the mass of an array of fat globules (M_t), according to:

$$A_{sp} = \frac{A_t}{M_t} = \frac{\pi d_{vs}^2}{\rho n \frac{\pi d_{vs}^3}{6}} = \frac{6}{\rho d_{vs}} \quad [2]$$

where n is the total number of fat globules. A_{sp} is in $m^2 g^{-1}$ if d_{vs} is in μm , and ρ is in $g cm^{-3}$.

Determination of the amount of adsorbed protein

Three separate samples (about 40 mL) from each of ten freshly made emulsions produced under identical conditions to the centre points of the design (ie, $[Prof] = 1.5$ wt%, $[BO] = 10$ wt%, $[GMS] = 0.4$ wt%, $P = 42.4$ MPa and $T = 70$ °C) were subjected to various centrifugation levels. These levels were 10 000, 12 500, 15 000, 17 500, 20 000 or 22 500 rpm for 30 min, at 5 °C (model L8-70M, Beckman Instruments Inc, Palo Alto, CA, USA). The rotor (type 30, $5 \leq r \leq 10.5$ cm, Beckman Instruments Inc) was previously refrigerated at 5 °C for 12 h. After centrifugation, centrifuge tubes (model 3410-2539, Nalgene® Ultratubes, Fisher Scientific, Montreal, PQ, Canada) contained a thin layer of floating material, a middle layer comprising the bulk of volume and a small pellet of sediment. After the middle layer and the sediment were carefully recovered by a syringe and a spatula, respectively, the sedimenting pellet was resuspended in the middle phase with a mortar. Samples of the resulting dispersion (denoted 1), as well as native emulsions (denoted 0) were then analysed for fat and nitrogen content. Fat content was evaluated by infrared analysis (model 133B, Milkoscan, Foss Electric, Hillerød, Denmark) according to a procedure derived from previous results (Remillard et al, 1993) and nitrogen was determined by the general Kjeldahl procedure (International Dairy Federation, 1986). From these analyses, the relative adsorbed protein fraction (F_{ads} , %) was simply deduced from the following expression:

$$F_{ads} = 100 \left(1 - \frac{[Prof]_1 (1 - \Phi_0)}{[Prof]_0} \right) \quad [3]$$

where $[Prof]_0$ is the protein content of the native emulsion (g/g), $[Prof]_1$ is the protein content in the subnatant (g/g), and Φ_0 is the fat content in the native emulsion. The measurement of F_{ads} is relative since it ignores about $1.5 \pm 0.5\%$ of the original fat which is not removed from the lower phase after centrifugation. Furthermore, if the average size of fat globules changes with any of

the processing variables, this will affect the amount of fat in the subnatant and hence will alter the F_{ads} values. In other words, the conditions established for centrifugation and valid for one formulation may not be totally adequate for another. Nevertheless, and aware of this limitation, a centrifugation treatment of 26 700 g for 30 min was applied to the entire experimental design. Beyond these conditions, even though the recovery of fat was not significantly improved, the adsorbed protein fraction slightly decreased. This slight decrease in F_{ads} could be due to the liberation of protein particles possibly entrapped in the oil matrix of the upper phase, and/or the removal of protein particles from the fat globule surface layer. It was under these centrifugation conditions (26 700 g for 30 min at 5 °C) that the relative adsorbed protein fraction (F_{ads} , eq [3]) and the protein load (Γ , mg m⁻²) were evaluated.

The latter was deduced from protein mass balances on the native emulsion and the resuspended phase obtained after centrifugation. The protein mass balance on the native emulsion (denoted 0) was given by:

$$[Prof]_0 = A_{sp_0} \Phi_0 \Gamma + (1 - \Phi_0) [Prof]_2 \quad [4]$$

where A_{sp_0} is the specific surface area (m² mg⁻¹) of dispersed fat in the emulsion and $[Prof]_2$ is the protein content (g/g) of the plasma (denoted 2). As a small fat fraction remains in the subnatant after centrifugation (fig 1), a protein mass balance on the subnatant (denoted 1) must also be used:

$$[Prof]_1 = A_{sp_1} \Phi_1 \Gamma + (1 - \Phi_1) [Prof]_2 \quad [5]$$

where A_{sp_1} is the specific surface area (m² mg⁻¹) of dispersed fat in the subnatant and Φ_1 is its mass fat fraction. Γ is obtained by substituting the analytical expression of $[Prof]_2$ (eq [4]), into eq [5], according to:

$$\Gamma = \frac{[Prof]_0 (1 - \Phi_1) - [Prof]_1 (1 - \Phi_0)}{A_{sp_0} \Phi_0 (1 - \Phi_1) - A_{sp_1} \Phi_1 (1 - \Phi_0)} \quad [6]$$

Electron microscopy

Six emulsions containing the same butter oil concentration (10 wt%) and a variable GMS:protein molecular ratio (0, 1.9, 5.6, 11.2, 55.9 and ∞) obtained by varying the monoglyceride concentration (from 0 to 1.5 wt%) and keeping constant the protein concentration (1.5 wt% except for emulsion 6 where $[Prof] = 0$) were micro-

fluidized under the same emulsification conditions (at 42.4 MPa and 70 °C). About 7 days after production, emulsion samples containing 0.02 wt% of sodium azide (Fisher Scientific, Quebec, QC, Canada) were fixed by mixing equal volume of emulsion and 3% low melting-temperature agarose (Bio-Rad Laboratories, Richmond, CA, USA). After gelation on a porcelain plate, ≈1 mm³ cubes were excised and fixed in a 1% glutaraldehyde solution (JB EM Service, Pointe Claire, Dorval, QC, Canada) at 6 °C for 24 h. The samples were then washed with distilled water and postfixed for 24 h at 6 °C in a 2% osmium tetroxide solution in a 0.05 mol L⁻¹ veronal-acetate buffer, pH 6.85, containing 0.1 mol L⁻¹ (Polysciences, Inc, Warrington, PA, USA) in order to retain liquids (Allan-Wojtas and Kalab, 1984). The samples were then dehydrated in a graded ethanol series, embedded in a Spurr's low viscosity medium (JB EM Services), and sectioned. Sections, ≈90 nm thick, were stained with uranyl acetate and lead citrate solutions (Reynolds, 1963) and examined in a Philips EM 300 electron microscope operated at 60 kV. Micrographs were taken on 35 mm film.

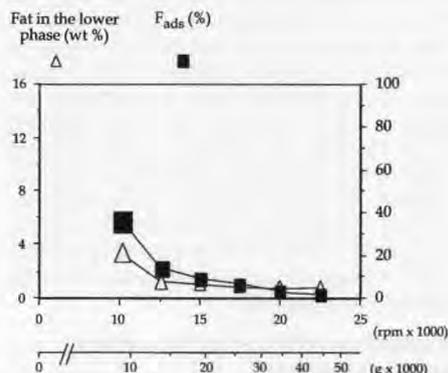


Fig 1. Influence of the centrifugal force expressed in rpm and g (in the middle of the tube) on the fat content of the resuspended phase (fat, %) and on the relative adsorbed protein fraction (F_{ads} , %) after 30 min of centrifugation. The size of the symbols used roughly indicates the reproducibility of measurements.

Influence de la force centrifuge exprimée en rpm et en g (dans le milieu du tube) sur la teneur en matière grasse de la phase inférieure (fat, %) et sur la fraction de protéines adsorbées (F_{ads} , %) après 30 minutes de centrifugation. La taille des symboles utilisés indique grossièrement la reproductibilité des mesures.

Statistical analysis

An analysis of variance and regression calculations on protein surface concentration parameters (F_{ads} and Γ) were carried out using a linear model procedure (SAS, 1990). The nullity of the regression coefficients was tested using a Student test. An adjustment test was used to confirm that the second-order model allowed an adequate description (to $\pm 1\%$) of the variations of the experimental results. Further details on the statistical analysis of this experimental design may be found elsewhere (Robin et al, 1992).

RESULTS

The influence of formulation and process variables on the protein surface concentration

Two analyses of variance were carried out on the values of protein surface concentrations (F_{ads} and Γ) using comparisons between the principal treatments and between the interactions. From these analyses and an F test, which differentiated the treatments and interactions having a significant effect on F_{ads} and Γ , two regression equations were deduced. Finally, an adjustment test was used to evaluate the fitting of experimental results to a second-order polynomial model. Further details may be found in the first study (Robin et al, 1992).

Relative adsorbed protein fraction (F_{ads} , %) in emulsion

Table I shows the analysis of variance of the relative adsorbed protein fraction (F_{ads}) as a function of process and formulation variables. According to the adjustment test, for $P \leq 0.01$ ($F < 4.77$), a second-order polynomial model adequately described the variance of the adsorbed protein fraction ($\sigma_{F_{ads}}$). As expected, composition factors had major effects on F_{ads} since caseinates (sum of linear and quadratic effects), butter oil and monoglyceride concentrations explained 51.0, 26.0 and 12.3% of $\sigma_{F_{ads}}$, respectively. The emulsification pres-

sure and temperature represented 1.2 and 2.2% of $\sigma_{F_{ads}}$, respectively. Finally, among all the possible interaction effects, only the quadratic effect of protein concentration ($[Prof]^2$) significantly influenced $\sigma_{F_{ads}}$. The regression equation calculated from these significant effects ($P \leq 0.05$) explained 92.7% of $\sigma_{F_{ads}}$. All the coefficients were highly significant ($P \leq 0.001$).

Figures 2A and B display the response surfaces obtained from the second-order model for process and formulation variables in the range of the study. In Figure 2A the values of F_{ads} (%), for a fixed temperature (50 °C) and for fixed caseinates (1.5 wt%) and monoglyceride (0.4 wt%) concentrations, are expressed as a function of the butter oil concentration ($[BO]$, wt%) and the emulsification pressure (P , MPa). Since the adsorbed protein fraction (F_{ads}) is directly proportional to the total fat surface area, any increase in emulsification pressure and/or butter oil concentration favours caseinate adsorption and increases F_{ads} values (fig 2A). Nevertheless, this last statement is only true if the fat is mainly found in the upper layer. In emulsions containing 0.4 wt% of monoglycerides, adsorbed protein fractions (F_{ads}) ranged from ≈ 1 to $\approx 13\%$ (fig 2A), which is drastically lower than those found in emulsions stabilized only by proteins. Indeed, in the absence of monoglycerides ($[Prof] = 1.5$ wt%), $F_{ads} = 87\%$ for an emulsion produced under identical conditions to the centre points (ie, $[BO] = 10$ wt%, $P = 42$ MPa and $T = 70$ °C) (fig 3A). This result is in good agreement with those of other authors. For example, Oortwijn and Walstra (1979) and Courthaudon et al (1991b) found, under different homogenization conditions, that all of the protein emulsifier was adsorbed at the oil droplet surface in emulsions. Their emulsions were respectively made with either skimmed milk or whey, or β -casein (0.4 wt%, 20 wt% *n*-hexadecane, pH 7.0). Finally, electron micrographies 1A and B (photo 1), corresponding to the emulsion

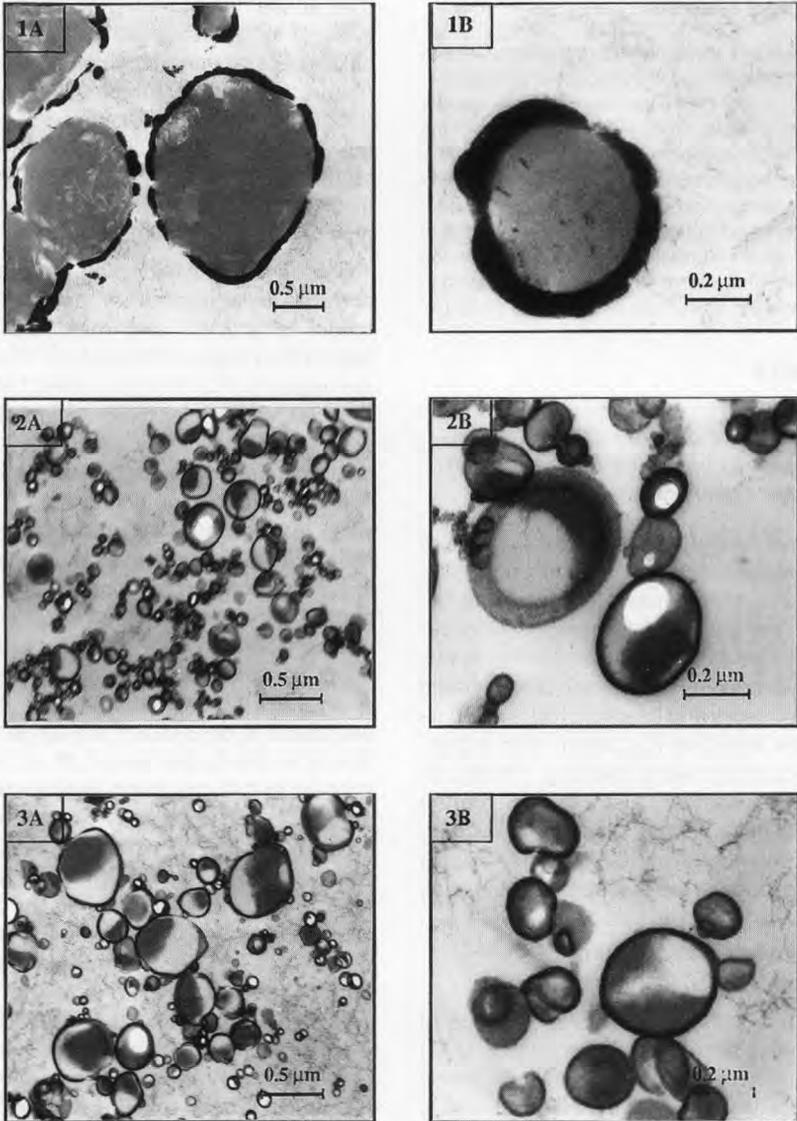


Photo 1. Electron micrographs of various microfluidized emulsions. The GMS:protein molecular (R) was increased by varying $[GMS]$ while the other conditions remained constant ($[Prot] = 1.5$ wt%, $[BO] = 10$ wt%, $P = 50$ MPa and $T = 70$ °C). If not specified, magnifications were 9 030 x and 24 580 x for the A and B series, respectively. (1) $R = 0$, A x 7 190; (2) $R = 1.9$ and (3) $R = 5.6$.

Photographies de microscopie électronique pour diverses émulsions microfluidisées. Le ratio moléculaire GMS:protéine (R) a été augmenté en faisant varier $[GMS]$, les autres conditions expérimentales restant inchangées $[Prot] = 1,5\%$, $[BO] = 10\%$, $P = 50$ MPa, $T = 70$ °C). En l'absence de spécification contraire, les grossissements utilisés étaient respectivement de 9 030 x et de 24 580 x pour les séries A et B. (1) $R = 0$, A x 7 190; (2) $R = 1,9$, et (3) $R = 5,6$.

free of monoglycerides, show milkfat globules with very thick protein membranes which illustrates a high sodium caseinate adsorption on the globule surface. In these electron micrographs, the adsorbed layers of proteins were about 45 to 60 nm thick.

The adsorbed caseinate fraction decreased with increasing emulsification temperature. This decrease was about 3.9%/10 °C between 35 and 100 °C (table I). A similar tendency was also reported by Walstra (1975) and Oortwijn and Walstra (1979) with skimmed milk.

The influence of emulsifier type and concentration on the adsorbed protein frac-

tion (F_{ads}) is presented in figure 2B where F_{ads} is plotted against the surfactant ($[Prof]$ and $[GMS]$) concentrations for a fixed butter oil concentration (10 wt%) and for fixed emulsification pressure (40 MPa) and temperature (50 °C). As expected, when sodium caseinates were present, any increase in $[GMS]$ induced a proportional decrease in F_{ads} . On the contrary, when monoglycerides were present, an increase in $[Prof]$ only induced a slight increase in the amount of adsorbed proteins, which eventually rapidly leveled off. These results suggest that because monoglycerides are more surface active, they first accumulate

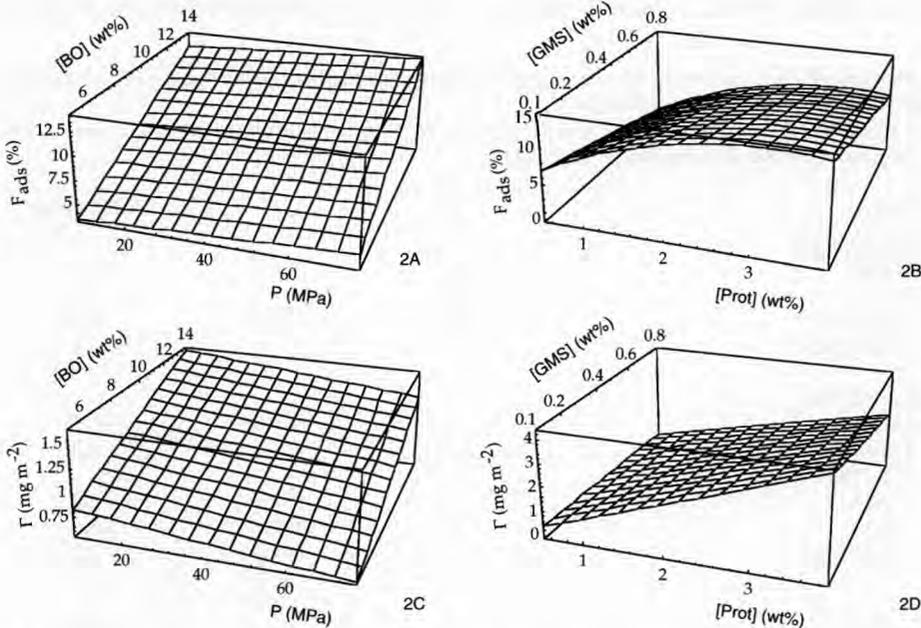


Fig 2. Response surfaces relating the values of adsorbed protein fraction (F_{ads}) and protein load (Γ) as a function of the butter oil concentration ($[BO]$) and the emulsification pressure (P) (A and B, respectively), and as a function of emulsifier type and concentration ($[Prot]$ and $[GMS]$) (C and D, respectively). The other variables were fixed ($T = 70$ °C, $[Prot] = 1.5$ wt%, $[GMS] = 0.4$ wt% and/or $P = 50$ MPa, $[BO] = 10$ wt%).

Surfaces de réponse reliant les valeurs de F_{ads} et Γ à la concentration en huile de beurre ($[BO]$) et la pression d'émulsification (P) (A et B, respectivement), et à la nature et la concentration des émulsifiants ($[Prot]$ et $[GMS]$) (C et D, respectivement). Les autres variables étaient fixées ($T = 70$ °C, $[Prot] = 1,5$ %, $[GMS] = 0,4$ %, et/ou $P = 50$ MPa, $[BO] = 10$ %).

at the interface, leaving little free space to proteins. Protein adsorption is then rapidly limited. This hypothesis is partly confirmed by the electron micrographs [photos 1 (2A to 3B) and 2] since distinctly finer milkfat globule membranes (about 15 nm) were observed at GMS:protein molecular ratio (R) as low as 1.9. A regression analysis was finally carried out on the F_{ads} values as a function of R , $[BO]$, P and T . The obtained relationship, between F_{ads} and R , appears to fit an exponentially decreasing function of R (fig 3A). Although this form of curve seems to describe a rather simple mechanism of competitive adsorption, it should be considered as a rough approximation since this relationship only explains 77% of the total variance of F_{ads} , compared to more

than 90% when the five process and formulation variables are used.

Protein load (Γ , mg m⁻²) of the microfluidized fat globules

Table II shows the analysis of variance of the protein load (Γ) as a function of compositional and process variables. According to the adjustment test, a second-order polynomial model adequately described the variance of the protein load (σ_{Γ}) within the range of the study. As previously mentioned, the linear effects of the principal factors were highly significant (more than 95%). The protein concentration, as expected from eq [6], had a major effect on Γ since it explained more than 87% of σ_{Γ} . The butter oil and monoglyceride concentrations

Table I. Analysis of variance and second-order model of the adsorbed protein fraction (F_{ads} , %) versus formulation and process parameters.

Analyse de variance et modèle de second ordre de la fraction protéique adsorbée (F_{ads} , %) en fonction des paramètres de composition et de procédé.

Source	df ¹	F ²	% Explained
<i>Before pooling</i>			
Model	20	35.7 ³	96.7
Error	31		3.3
Lack of fit	22	2.7 ³	2.9
Pure error	9		0.4
<i>After pooling</i>			
Model	6	147.1 ³	92.7
Error	45		7.3
Significant effects			
[Prot]	1	454.5 ³	46.3
[BO]	1	239.2 ³	26.0
[GMS]	1	114.2 ³	12.3
P	1	11.1 ³	1.2
T	1	20.1 ³	2.2
[Prot] ²	1	43.8 ³	4.7

$$F_{ads} = -3.78 + 6.47 [Prof] + 1.05 [BO] - 7.58 [GMS] + 0.02 P - 0.04 T - 1.02 [Prof]^2$$

Validity intervals

$$\begin{array}{ll} 0.5 \leq [Prof] \leq 3.9 \text{ wt \%} & 5.2 \leq [BO] \leq 14.9 \text{ wt \%} \\ 0.08 \leq [GMS] \leq 0.8 \text{ wt \%} & 7.8 \leq P \leq 76.3 \text{ MPa} \\ \text{or } 3.5 \leq R \leq 77.8 & 35 \leq T \leq 100 \text{ } ^\circ\text{C} \end{array}$$

¹ df : degree of freedom; ² F : Fisher ratio; ³ $P < 0.001$.

explained 3.6 and 5.5% of σ_{Γ} , respectively. The influence of process factors, although having minor effects on σ_{Γ} , was nevertheless significant. The emulsification pressure and temperature respectively accounted for 1.1 and 1.6% of σ_{Γ} . The last significant effect, according to the analysis of variance, was the $[Prof] \times [GMS]$ interaction. It explained 1.1% of σ_{Γ} . Other effects were not significant ($P > 0.05$); the error term represented approximately 9.4% of σ_{Γ} . The regression equation (table II) calculated from these significant effects ($P < 0.05$) explained 90.6% of σ_{Γ} . All the coefficients were highly significant ($P \leq 0.001$).

Figure 2C is the response surface obtained from a second-order model relating the

values of Γ (mg m^{-2}) as a function of butter oil concentration ($[BO]$, wt%) and of emulsification pressure (P , MPa), for a fixed temperature (50 °C) and for fixed protein (1.5 wt%) and monoglyceride concentrations (0.4 wt%). As with F_{ads} values, an increase in butter oil concentration, by increasing the average diameter of fat globules (Robin et al, 1992), lead to a reduction in specific surface area (A_{sp}), which enhances Γ . On the contrary, an increase in emulsification pressure induced a considerable increase in A_{sp} and led to a slight but significant decrease of Γ . The increase in A_{sp} was about 74% for a pressure increase from 10 to 60 MPa ($[Prof] = 1.5$ wt%, $[BO] = 10$ wt%, $[GMS] = 0.4$ wt% and

Table II. Analysis of variance and second-order model of the protein load (Γ , mg m^{-2}) versus formulation and process parameters.

Analyse de variance et modèle de second ordre de la charge protéique (Γ , mg m^{-2}) en fonction des variables de formulation et de procédés.

Source	df ¹	F ²	% Explained
<i>Before pooling</i>			
Model	20	88.6 ³	98.0
Error	31		2.0
Lack of fit	22	4.7 ³	1.5
Pure error	9		0.5
<i>After pooling</i>			
Model	6	112.6 ³	90.6
Error	45		9.4
Significant effects			
$[Prof]$	1	586.0 ³	87.7
$[BO]$	1	25.9 ³	3.6
$[GMS]$	1	39.4 ³	5.5
P	1	5.6 ³	1.1
T	1	11.7 ³	1.6
$[Prof] \times [GMS]$	1	8.1 ³	1.1

$$\Gamma = -0.35 + 1.19 [Prof] + 0.09 [BO] - 0.29 [GMS] - 0.004 P - 0.008 T - 0.60 [Prof] \times [GMS]$$

Validity intervals

$$0.5 \leq [Prof] \leq 3.9 \text{ wt \%} \quad 5.2 \leq [BO] \leq 14.9 \text{ wt \%}$$

$$0.08 \leq [GMS] \leq 0.8 \text{ wt \%} \quad 7.8 \leq P \leq 76.3 \text{ MPa}$$

$$\text{or } 3.5 \leq R \leq 77.8 \quad 35 \leq T \leq 100 \text{ }^{\circ}\text{C}$$

¹ df : degree of freedom; ² F : Fisher ratio; ³ $P < 0.001$.

$T = 70\text{ }^{\circ}\text{C}$). A similar pressure effect on Γ values was also reported on protein-stabilized emulsions by Tornberg (1978).

Although the temperature effect on the amount of adsorbed proteins was similar to that affecting F_{ads} values, its magnitude was higher: the decrease of Γ as a function of T was about $5.8\%/10\text{ }^{\circ}\text{C}$ between 35 and $100\text{ }^{\circ}\text{C}$. A decrease in Γ from 10.7 to

6.0 mg m^{-2} due to a temperature increase from 40 to $60\text{ }^{\circ}\text{C}$ (about $22\%/10\text{ }^{\circ}\text{C}$) was also reported for recombined milk (Oortwijn and Walstra, 1979).

Figure 2D shows the influence of sodium caseinates and monoglyceride concentrations on the amount of adsorbed proteins on fat globules per unit of area (Γ , mg m^{-2}) for a fixed butter oil concentration ($10\text{ wt}\%$) and for fixed emulsification pressure (40 MPa) and temperature ($50\text{ }^{\circ}\text{C}$). In emulsions containing as low as $0.08\text{ wt}\%$ of monoglycerides, Γ ranged from 0.5 to 4 mg m^{-2} for $0.5 \leq [Prof] \leq 3.9\text{ wt}\%$. In the absence of monoglycerides, the protein load was $2.9 \pm 0.2\text{ mg m}^{-2}$ for an emulsion produced under identical conditions to the centre points (ie, $[Prof] = 1.5\text{ wt}\%$, $[BO] = 10\text{ wt}\%$, $P = 42\text{ MPa}$ and $T = 70\text{ }^{\circ}\text{C}$). This value is consistent with that found for β -casein adsorbed at the planar oil-water interface (Graham and Phillips, 1979) and in various casein or NaCas-stabilized emulsions (Tornberg, 1978; Walstra and Oortwijn, 1982; Courthaudon et al, 1991b). Lower values ($0.8 \leq \Gamma \leq 1.2\text{ mg m}^{-2}$) were also reported in a $10\text{ wt}\%$ soybean emulsion, $0.5\text{ wt}\%$ casein, Tris-HCl buffer (Dickinson et al, 1993). Even though increases in $[GMS]$ lead to decreases in Γ at any given $[Prof]$ (fig 2D), the Γ increase remained proportional to $[Prof]$, which is different from the effect of $[Prof]$ on F_{ads} (fig 2C). Finally and as previously done, a regression analysis was carried out on the Γ values as a function of R , $[BO]$, P and T . From statistical analysis a decreasing exponential relationship between Γ and R was obtained, explaining about 70% of σ_{Γ} (fig 3B). These figures compare well the behaviour of systems made of β -casein, with or without α_{s1} -casein, and zwitterionic surfactants, such as phosphatidylcholine (Courthaudon et al, 1991a) or monoglycerides (Dickinson et al, 1993; Tomas et al, 1994b) at n -alkane or triglycerides interfaces. Although comparisons must be made with caution, exponential decreasing

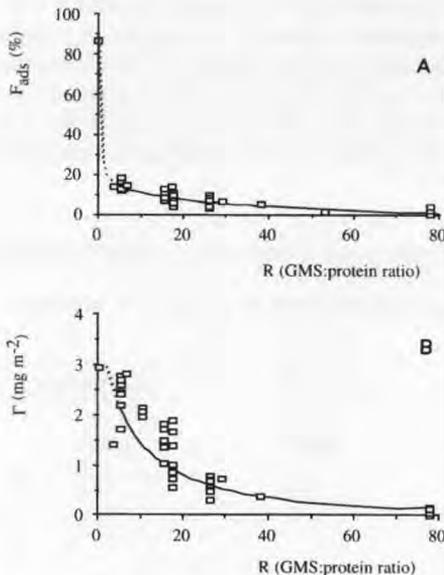


Fig 3. Effect of the GMS:protein molecular ratio (R) on the protein surface concentration expressed in terms of (A) adsorbed protein fraction (F_{ads} , %) and (B) protein load (Γ , mg m^{-2}). (\square) are the 52 experimental points: 24 and 12 points are hidden in (A) and (B), respectively. (—) are the theoretical curves determined by an analysis of regression between F_{ads} or Γ and R , $[BO]$, P , T and their significant interactions.

Effet du ratio moléculaire GMS:protéine (R) sur la concentration de protéines en surface, exprimée soit sous forme de (a) fraction adsorbée (F_{ads} , %), ou (b) charge protéique (Γ , mg m^{-2}). (\square) sont les 52 points expérimentaux dont 24 et 12 sont respectivement masqués dans (A) et (B). (—) sont les courbes théoriques obtenues par une analyse de régression entre F_{ads} ou Γ , et R , $[BO]$, P , T , et leurs interactions significatives.

behaviour, between Γ and R for $4.5 \leq R \leq 100$, was also reported by Dickinson et al (1993) and Tomas et al (1994a, b). However, they do not seem to agree with the results that are found using electron microscopy. Indeed, if the values of Γ are both around 3 mg m^{-2} for $R = 0$ and $R = 1.9$, the estimated thickness from micrographies suggests a difference in load of about a factor 4.

DISCUSSION

The homogenization process induces both a decrease in the average size of fat globules and in the spread of distribution that lead to the creation of a large new fat-serum interface, and the development of a new membrane. In dairy emulsions, this membrane is represented by the material adsorbed at the interface between the fat and serum phases. It contains essentially caseins, in the case of homogenized milk or cream (Darling and Butcher, 1978; Oortwijn and Walstra, 1979; Dalgleish, 1994), or both proteins and low molecular mass surfactants, as suggested by various works of Pr Dickinson's research group. During homogenization, parameters which characterize protein adsorption are mainly influenced by the emulsification pressure and temperature (Walstra and Oortwijn, 1982), the fat concentration (Walstra et al, 1984), the protein nature and concentration (Leman and Kinsella, 1989; Britten and Giroux, 1993) and by the presence of surfactants (Dickinson and Woskett, 1989).

Accuracy and reproducibility of the protein surface concentration measurements

In theory, the centrifugation methods at medium-high speed would be appropriate to evaluate the protein surface concentration if emulsions are stable against coalescence and free of very small fat globules, and if proteins suspended in the aqueous

phase do not sediment. In practice however, it is a bit different. Indeed, the centrifugation of oil-in-water dairy emulsions generally leads to a separation into three layers: a thin layer of floating material, a middle layer comprising the bulk of the volume and a sediment. The physicochemical characteristics of these three layers depend on the centrifugation conditions. Dalgleish and Robson (1985), studying various sedimenting pellets obtained from centrifugal fractionations of homogenized milks, have shown that the fat:protein ratio as well as the milkfat globule diameter increased as the centrifugation force was increased. Similar trends have also been reported by Blanchot (1992) when studying the centrifugation behaviour of the same model emulsions as those used in this study. Some conclusions can be drawn from these results. First, it seems clear that variable proportions of proteins adsorbed or accumulated on fat globules and proteins in solution co-sediment. Second, the protein concentration on fat globules varies with the fat globule diameter; this was confirmed by dissociating proteins with EDTA. These results, which partly reflect structural differences between centrifuged particles, must necessarily have repercussions on the evaluation of the relative F_{ads} . If the average reproducibility of measurements for F_{ads} was satisfactory ($\pm 2.7\%$), it was estimated by Blanchot (1992) that approximately 2–5% (depending on the processing variables) of adsorbed proteins on sedimented fat globules were not taken into account in evaluating F_{ads} .

According to this procedure, the estimation of the protein load (Γ) rests both on the determination of the interfacial area obtained from the average diameter of dispersed particles, and on a protein mass balance. Without lingering on a previous discussion (Robin et al, 1992), the average diameter obtained by laser light scattering is not a simple average. It depends upon the amount of light scattered at 90° by all

the particle sizes in a given sample, that is, upon the range of the particle size distribution. Furthermore, eq [5] which describes the protein mass balance in phase 1 (middle phase and sedimenting pellet), assumes that Γ is the same for all fat globules. In homogenized milks, Oortwijn and Walstra (1979) have clearly shown that Γ was higher for smaller globules. To take this difference into account, they introduced a correction factor into eq [6]. In the present study, as in others (Hunt and Dalgleish, 1994), we have no reason to believe that the adsorbed protein concentration is dependent on size.

The influence of process variables on the protein surface adsorption (F_{ads} and Γ)

The effect of emulsification pressure

High energy densities generated during microfluidization increase both the collision number between proteins and fat globules and the fat globule dispersion. The former enhances the protein adsorption (F_{ads}) even though the latter reduces the amount of adsorbed proteins per unit of area (Γ). A different result, that is, an increase of Γ with P , was reported in milk, however (Walstra et al, 1984). This result may be due both to the milk emulsion itself and to the protein transport toward the fat during emulsification. The latter is roughly proportional to the protein concentration, and $(d_g + d_p)^3$, where d_g and d_p denote fat globule and protein particle diameter, respectively (Walstra and Oortwijn, 1982). From the previous relationship and if proteins are relatively abundant, which is the case in milk, the smallest fat globules have on average a higher surface layer; consequently, Γ may increase with P .

The effect of emulsification temperature

The actual protein load and membrane composition are affected by the emulsification temperature. This variable induced a decrease in the protein adsorption param-

eters ranging from about 4 to 6%/ 10 °C for F_{ads} and Γ , respectively. Two influences might explain this decrease. First, increases in emulsification temperature induced a strengthening of hydrophobic interactions (at least up to 60–65 °C) and a weakening of the molecular structure of water, both of which may have favoured the adsorption of monoglycerides. Second, an increase in the emulsification temperature induced an increase in the spreading rate of proteins at the oil–water interface, and consequently led to a reduction in Γ values, as suggested by Oortwijn and Walstra (1979).

The influence of compositional factors on the protein surface adsorption (F_{ads} and Γ)

The effect of butter oil concentration

Any increase in the butter oil concentration increases the amount of adsorbent material, and the average fat globule diameter. Two hypotheses might explain the increase of the protein adsorption parameters (F_{ads} and Γ) with the butter oil content (fig 2A and C). The first is classical. Any increase in the [BO] enlarges the total surface area (A_t) of the milkfat membrane, ie, the free space at the interface that favours protein adsorption (F_{ads}). Furthermore, by leading to a drop in the specific surface area (A_{sp}), since the average fat globule diameter increases (eq [2]), the increase of [BO] also has a positive and a mathematical effect on Γ (eq [6]). The second, though more, must be mentioned. The increase in the fat globule diameter in the butter oil fraction might not be caused by a less efficient emulsification, but rather by some recoalescence favoured by the high frequency of droplet collisions in the emulsification chamber. In this case, the reduction of the interfacial area which occurs during recoalescence induces a compression of the protein film, which then enhances Γ . This effect was initially reported by Pearce and Kinsella

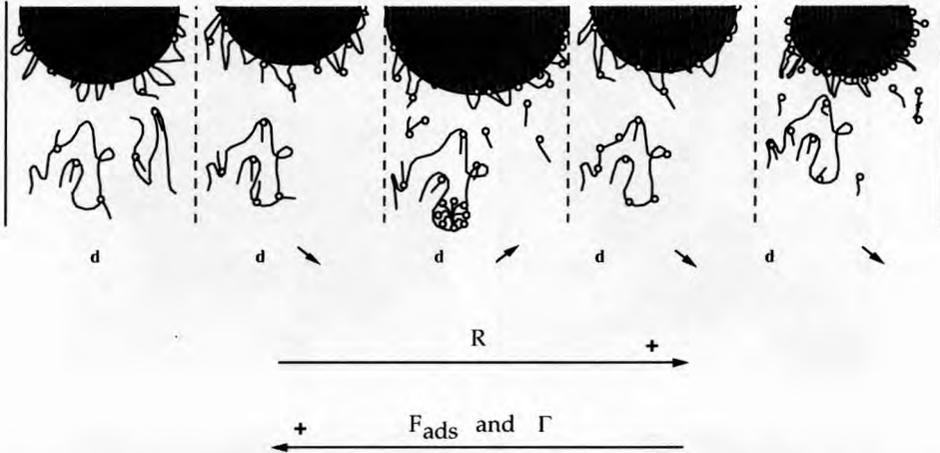


Fig 4. Schematic representation of the protein-monoacylglyceride competitive adsorption effects on fat globules as a function of d (average particle size), R (GMS:protein molecular ratio), F_{ads} (adsorbed protein fraction), and Γ (protein load). Fat is represented by the shaded grey area (inspired from Dickinson and Woskett, 1989; adapted from Robin et al, 1993).

Représentation schématique de l'effet de l'adsorption compétitive protéine-monoacylglycéride sur le globule gras en fonction de d (diamètre particulaire moyen), R (ratio moléculaire GMS:protéine), F_{ads} (fraction de protéines adsorbées), et Γ (charge protéique). La matière grasse est représentée par les zones ombragées en gris (inspiré de Dickinson et Woskett, 1989 ; adapté de Robin et al, 1993).

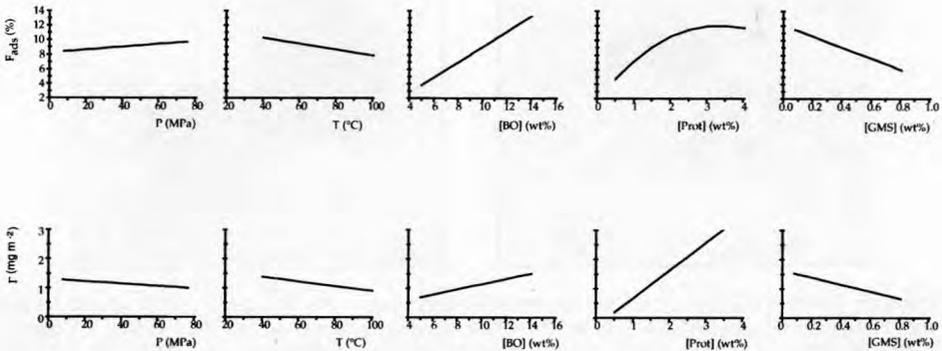


Fig 5. Effects of process and composition variables on the protein adsorption parameters (F_{ads} and Γ). For each graph, values of four variables were either specified or fixed. In the latter case, their values were $P = 50$ MPa, $T = 70$ °C, $[BO] = 10$ wt%, $[Prot] = 1.5$ wt% and $[GMS] = 0.4$ wt%.

Effets des variables de procédés et de composition sur les paramètres de l'adsorption protéique. Pour chaque figure, les valeurs des quatre variables étaient soit spécifiées, soit fixées. Dans ce dernier cas, leur valeur était : $P = 50$ MPa, $T = 70$ °C, $[BO] = 10$ %, $[Prot] = 1,5$ % et $[GMS] = 0,4$ %.

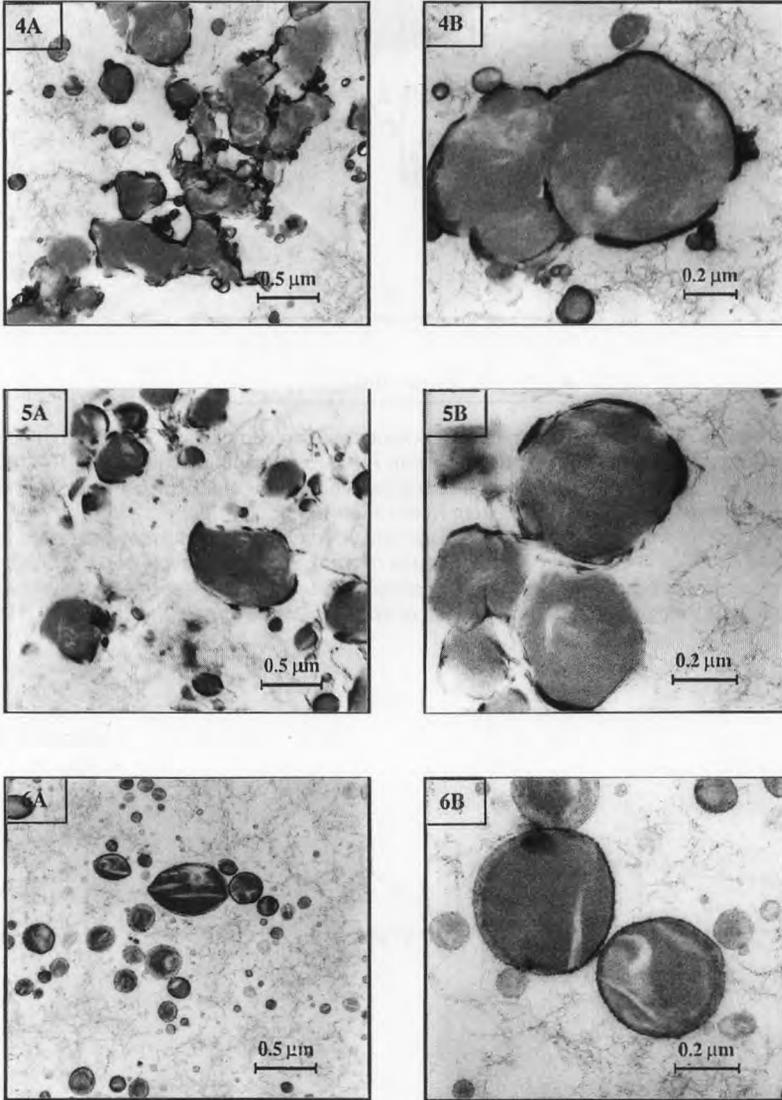


Photo 2. Electron micrographs of various microfluidized emulsions. The GMS:protein molecular (R) was increased by varying $[GMS]$ while the other conditions remained constant ($[Prot] = 1.5$ wt%, $[BO] = 10$ wt%, $P = 50$ Mpa and $T = 70$ °C). If not specified, magnifications were 9 030 x and 24 580 x for the A and B series, respectively. (1) $R = 11.2$, B x 19 200; (2) $R = 55.9$ and (3) $R = \infty$.
Photographies de microscopie électronique pour diverses émulsions microfluidisées. Le ratio moléculaire GMS:protéine (R) a été augmenté en faisant varier $[GMS]$, les autres conditions expérimentales restant inchangées [$Prot$] = 1,5%, $[BO]$ = 10%, P = 50 MPa, T = 70 °C). En l'absence de spécification contraire, les grossissements utilisés étaient respectivement de 9 030 x et de 24 580 x pour les séries A et B. (1) $R = 11,2$, B x 19 200 ; (2) $R = 55,9$, et (3) $R = \infty$.

(1978) while studying peanut oil-in-water emulsions stabilized by whey proteins.

The effect of surfactant concentrations

When fat globules are entirely covered with a layer of completely spread protein molecules (the cross-sectional area per amino acid residue is about 15.3 \AA^2), an approximate calculation shows that the protein load (Γ) of milkfat globules would theoretically be about 1.2 mg m^{-2} . With a single layer of casein submicelles, Γ would be about 5.5 mg m^{-2} and about 41.3 mg m^{-2} with a single layer of whole casein micelles. All the intermediate situations may also occur, and with sodium caseinates-stabilized emulsions a Γ value ranging between 1.2 and 5.5 mg m^{-2} should be expected. In emulsions free of low molecular weight surfactants, the interpretation of Γ values, which may be roughly deduced from the $[Prot]:A_{sp}$ ratio, should take into account the two opposing influences of the protein concentration. First, and as logically expected, by enhancing the probability of interfacial adsorption, any increase in the caseinate concentration increases Γ . Second, by enhancing the dispersion of droplets, the interfacial spreading of caseinates could also decrease the amount of adsorbed proteins per unit of area. In emulsions that contain a mixture of likely competitive surfactants, such as caseinates and monoglycerides, it seems that the influence of the caseinate concentration can be interpreted in terms of competition between i) monoglycerides and proteins to be adsorbed on the fat globule surface, and ii) fat and monoglycerides to bind proteins at high $[GMS]$. From figures 2B, 2D and 3, it can be concluded that any reduction of the GMS :protein molecular ratio favours a better protein adsorption at the fat globule surface, and consequently an increase in F_{ads} and Γ ; more abundant and/or more surface active than proteins, monoglycerides probably settle at the interface quicker than proteins. The protein adsorption should

then be evaluated in terms of the remaining free space at the serum-fat interface. In addition, it was previously observed that the average droplet size of milkfat globules increased with $[GMS]$ at high $[Prot]$ (Robin et al, 1992), which is not invalidated by electron micrographies 3A, B (photo 1) and 4A (photo 2). Indeed, in these emulsions where $[GMS]$ and $[Prot]$ were quite high ($[Prot] = 1.5 \text{ wt\%}$ and $0.3 \leq [GMS] \leq 1.5 \text{ wt\%}$), fat globules were no longer spherical but partly melted due to a possible deficit in membrane material (photo 2, 4B). This may be due to some kind of competition for protein binding between monoglycerides and fat. This competition could arise from the higher number of monoglyceride molecules per residue of caseinate and should be characterized by the formation of surface inactive weak complexes produced by the binding of hydrophobic areas of proteins and monoglycerides. At higher R , fat globules transform back to spheres with distinct and uniform membranes (photo 2, 6A and B). These two hypotheses have been schematically summarized in figure 4 where the influence of R on the average fat globule diameter (d_{vs}) and on the protein adsorption parameters (F_{ads} and Γ) have been illustrated.

CONCLUSION

The studied process and composition variables affected in varying proportions, the parameters of protein surface adsorption (F_{ads} and Γ) in model dairy oil-in-water emulsions which both contained proteins (sodium caseinates) and a low molecular mass surfactant (monoglycerides). The respective influences of these process and composition variables on F_{ads} and Γ have been summarized in figure 5. In order of importance, the sodium caseinate concentration, the monoglyceride concentration, the butter oil concentration, the emulsification pressure and finally the emulsification temperature had the greatest influence on the protein surface concentration parameters (F_{ads} or Γ).

The determination of the amount of adsorbed protein, by centrifugation straight after homogenization and by evaluation of the protein content, showed that sodium caseinates, as a group, seem much less easily adsorbed than GMS molecules when the GMS:protein molecular ratio R is higher than 5.

Finally and although they are preliminary results, the competitive adsorption between proteins and monoglycerides during emulsification appears to follow a rather simple mechanism (some kind of decreasing exponential function linking F_{ads} or Γ to R) for $R \geq 5$. This competitive adsorption mechanism underlies the concept, though not completely satisfying, of the 'remaining' free space at the interface.

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