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PAR

**Christine GRANGER – GUILMIN**

POUR OBTENIR LE GRADE DE

**DOCTEUR**

SPECIALITE : SCIENCES DES ALIMENTS ET NUTRITION

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# INFLUENCE DE LA FORMULATION SUR LA FORMATION ET LA STABILISATION DES INTERFACES HUILE – EAU DANS LES CREMES GLACEES

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*Après avis de :*

D. GOFF, Professeur, Université de Guelph (Canada) Rapporteurs

M. OLLIVON, Directeur de Recherche, CNRS, Châtenay-Malabry

*Devant la commission d'examen formée de :*

J.-L. AZANZA, Professeur, Université Bordeaux 1, Talence Président et Rapporteur

P. BAREY, Degussa Texturant Systems, Baupte

M. CANSELL, Maître de Conférences, Université Bordeaux 1, Talence

J.-L. COURTHAUDON, Professeur, INSERM, Angers

D. GOFF, Professeur, Université de Guelph, Canada

F. LEAL-CALDERON, Professeur, Université Bordeaux 1, Talence

M. OLLIVON, Directeur de Recherches, CNRS, Châtenay-Malabry

# THESIS

Presented to

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BY

**Christine GRANGER - GUILMIN**

SPECIALITY: FOOD SCIENCE AND NUTRITION

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# INFLUENCE OF THE FORMULATION ON THE FORMATION AND THE STABILIZATION OF OIL – WATER INTERFACES IN ICE CREAM AND RELATED PRODUCTS

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Defended on October 28th

*After approval of:*

D. GOFF, Professor, University of Guelph (Canada) Reviewers

M. OLLIVON, Research Director, CNRS, Châtenay-Malabry

*Examination commission composed of:*

J.-L. AZANZA, Professor, Bordeaux 1 University, Talence Président and Reporter

P. BAREY, Application Line Manager, Degussa Texturant Systems, Baupte

M. CANSELL, Assistant Professor, Bordeaux 1 University, Talence

J.-L. COURTHAUDON, Professor, INSERM, Angers

D. GOFF, Professor, Université de Guelph, Canada

F. LEAL-CALDERON, Professor, Bordeaux 1 University, Talence

M. OLLIVON, Research Director, CNRS, Châtenay-Malabry

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# I. SYMBOLS AND UNITS

| | |
|---|---|
| $\gamma$ | Interfacial tension |
| $\dot{\gamma}$ | Angular velocity |
| $\Gamma$ | Apparent protein coverage |
| $d > 2\,\mu m$ | Particles with diameters greater than 2 μm |
| $d_{4,3}$ | Volume weighted average diameter |
| $\eta$ | Viscosity |
| $f$ | Corrective factor |
| G’ | Elastic modulus |
| G’’ | Viscous modulus |
| $\lambda$ | Wavelength |
| m | Mass |
| $\rho$ | Volume mass |
| $r_{int}$ | Internal radius |
| $\tau$ | Shear stress |
| $T_{10}$ | Time necessary to melt 10 g of product |
| $T_{30}$ | Time necessary to melt 30 g of product |
| tanδ | Damping factor |
| V | Volume |

# II. ABBREVIATIONS

| | |
|---|---|
| AFM | Atomic force microscopy |
| BSA | Bovine serum albumin |
| CA | Correspondence analysis |
| CCD | Charged-coupled device |
| DSC | Differential scanning calorimetry |
| FAME | Fatty acid methyl ester |
| FDP | Functional dairy protein |

| | |
|---|---|
| GC | Gas chromatography |
| GPA | Generalized procrustes analysis |
| HAC | Hierarchical ascendant clustering |
| HLB | Hydrophilic lipophilic balance |
| LSD | Least significant difference |
| MDG | Mono- and diglyceride mixture |
| NMR | Nuclear magnetic resonance |
| PAGE | Denaturing polyacrylamide gel electrophoresis |
| PCA | Principal component analysis |
| PLS | Partial least squares |
| SDS | Sodium dodecyl sulfate |
| SFC | Solid fat content |
| SMP | Skim milk powder |
| SNOM | Scanning near-field optical microscopy |
| Trp | Tryptophan residue |

# CONTEXTE DU PROJET

Au cours des dernières années, le développement des connaissances dans des domaines tels que la chimie et le génie des procédés, d'une part, et la maîtrise des procédés de transformation, d'autre part, ont permis aux industries agroalimentaires de produire des **produits alimentaires** diversifiés et de bonne qualité. Ces produits sont en général des matériaux complexes tant au niveau de leur composition que de leurs propriétés structurales. La composition du produit alimentaire ne donne souvent que des informations limitées sur les propriétés physiques, le comportement rhéologique, la texture ou les caractéristiques sensorielles. Ainsi, la compréhension des propriétés de texture des produits alimentaires requiert la connaissance d'éléments structuraux tels que les réseaux de polymères, les cristaux de glace ou de matière grasse et les assemblages de protéines.

Les produits alimentaires sont pour une large part des **systèmes multiphasiques**. Parmi eux, les émulsions alimentaires tiennent une place importante. Il s'agit de systèmes contenant deux liquides immiscibles dont l'un est dispersé sous la forme de petites gouttelettes (phase dispersée) dans une autre phase (phase continue). Les produits foisonnés constituent un autre groupe de systèmes dispersés alimentaires constitués de bulles d'air distribuées dans un réseau liquide visqueux ou une matrice solide. Dans ce cas, si l'air n'est pas comptabilisé dans la composition, il tient une place cruciale dans la mise en place des propriétés structurales du produit alimentaire. Dans les industries agroalimentaires, la formation et la stabilisation des diverses interfaces présentes dans les systèmes colloïdaux (air / eau, huile / eau, huile / air) nécessitent l'utilisation de molécules tensioactives. Bien que peu importants quantitativement dans la formulation des produits alimentaires, les tensioactifs sont essentiels dans la mise en place et le maintien de la structure des produits. Deux catégories de tensioactifs sont classiquement utilisées dans la formulation des systèmes dispersés alimentaires, des molécules de haut poids moléculaire comme les protéines et des émulsifiants de faible poids moléculaire. La présence simultanée dans les formulations de ces deux types de composés amphiphiles peut favoriser des phénomènes d'association ou de compétition aux interfaces selon leurs propriétés et leur rapport pondéral, de sorte que le produit fini présente des caractéristiques inattendues ou un comportement particulier au cours du procédé de fabrication.

La crème glacée est une dispersion alimentaire particulièrement complexe car elle correspond à un système quadriphasique. Des bulles d'air sont maintenues en suspension par la matière grasse émulsifiée et par un réseau de cristaux de glace, le tout étant dispersé dans une phase liquide cryo-concentrée (dite continue) contenant des substances dissoutes comme des sucres, des protéines et des hydrocolloïdes. Ces différentes structures sont créées et mises en place au cours des différentes étapes du procédé de fabrication des crèmes glacées qui vont faire

évoluer le système d'une simple émulsion huile-dans-eau vers une mousse partiellement solide. La maîtrise de la création de ces différentes structures est fondée, non seulement, sur l'application contrôlée de paramètres du process (cisaillement, température, foisonnement) mais encore sur l'utilisation des ingrédients appropriés. La compréhension des différents mécanismes interfaciaux mis en jeu et des propriétés des ingrédients, en tenant compte de leurs interactions, est également nécessaire pour optimiser la fabrication et la stabilisation du produit fini. A terme, l'acquisition de ces connaissances devrait aider à prédire l'influence d'un changement de formulation sur les propriétés du produit fini.

C'est dans le contexte d'une meilleure compréhension de la sélection des ingrédients sur la formation et la stabilisation de la crème glacée au cours de sa fabrication que s'inscrit ce travail de thèse. L'objectif principal de cette thèse est donc de mieux comprendre les mécanismes d'action des émulsifiants et des protéines qui participent à la formation, à la création et à la stabilisation des interfaces dans une crème glacée. Une attention particulière est portée à la nature de la matière grasse entrant dans la fabrication de l'émulsion huile-dans-eau et à son influence sur les propriétés texturales, rhéologiques, et sensorielles des produits finis.

L'**étude bibliographique** présente, d'une part, au niveau moléculaire, l'adsorption et les interactions des tensioactifs (protéines et émulsifiants) ainsi que leurs interactions avec les autres ingrédients au cours du procédé de fabrication, et, d'autre part, décrit, au niveau supra-moléculaire l'influence des ingrédients sur l'organisation microstructurale des crèmes glacées. Les méthodologies employées au cours de cette étude sont détaillées dans la partie **Matériel et Méthodes**. La partie **Résultats et Discussion** comporte trois chapitres distincts. Le premier chapitre concerne la caractérisation et la compréhension des mécanismes mis en jeu aux interfaces huile / eau au sein d'une émulsion. Le deuxième chapitre porte sur la caractérisation physico-chimique du mix de crème glacée. Le troisième chapitre est centré sur l'étude des crèmes glacées en termes de caractérisation des interactions entre les divers constituants aux interfaces, de microstructures et d'analyse sensorielle. Chaque chapitre de la partie Résultats et Discussion s'appuie sur un ou deux articles publiés, précédés d'une introduction qui permet de résumer les principaux résultats obtenus et de les compléter par des résultats non publiés.

# GENERAL CONTEXT

For several years now, the increasing knowledge in fields like chemistry and process engineering, on the one hand, and transformation processes, on the other hand, allowed food grade industries to produce various **food products** with high quality. These products are usually complex materials when considering their composition and their structural properties. Food composition often provides limited data on the physical properties, the rheological behavior or the sensory texture. Indeed, the comprehension of the texture properties of foods requires the control of structural elements such as polymer networks, ice and fat crystals or protein assembling.

Manufactured food products are, to a large extent, **multiphase systems**. A significant proportion of these products are in an emulsified state, so that they contain two immiscible liquids with one of the liquids being dispersed as small droplets (dispersed phase) in the other (continuous phase). Aerated foods are another group of multicomponent systems consisting of air bubbles distributed in a visco-elastic liquid or a solid matrix. In this case, even if air is not mentioned in the food composition, the shelf life, texture and appearance of these foods are strongly influenced by the size, the concentration and the distribution of the bubbles they contain. In the food industry, the formation and stabilization of the various interfaces present in the colloidal systems (air / water, oil / water, oil / air) require the use of surface active compounds. Although surface active compounds are quantitatively minor ingredients in the formulation, they are of widespread technological importance. Indeed, they actively participate to the structure of the food products. Thus, in food dispersions, the formation of various interfaces intensifies the problems related to stabilization, texture and product shelf life. Two main groups of surface active compounds are classically used in food formulation, *i.e.*, large molecules, such as proteins, or small molecules, like low molecular weight emulsifiers. The simultaneous presence in the formulation of these two types of amphiphilic components can lead to association or competition mechanisms at the interfaces according to their properties and their weight ratio. This results in specific functions in the processing and properties of the final product.

Ice cream is a particularly complex food dispersion that can be described as a quadriphasic system. Both, emulsified fat and ice crystal network suspend air bubbles. The whole is dispersed into a cryo-concentrated liquid phase (continuous) containing dissolved substances such as sugars, proteins and hydrocolloids. These various structures appear during the various steps of ice cream processing which evolves from a simple oil-in-water emulsion to a partially solid foam. The formation of these structures is based not only on the controlled application of process parameters (shearing, temperature, and overrun) but also on the use of suitable ingredients. More fundamental information on the properties of the ingredients as well as their

multiple interactions is necessary to optimize the manufacture and the stabilization of the final product. This should favor the comprehension of the dynamics of the physicochemical phenomena that take place during the different stages of ice cream manufacture and especially of the various interfacial mechanisms involved in ice cream processing. In the end, it should help to predict the impact of changes of ingredients within the formulation on the final ice cream properties.

This work takes place in the context of a better understanding of the ingredient selection on the formation and stabilization of ice cream during the different stages of ice cream manufacture, from the formation of the oil-in-water emulsion, to the mix ageing, and to the freezing / whipping steps leading to the final product. This work focuses on the mechanisms of action of low molecular weight emulsifiers and proteins which contribute to the formation of the oil / water interface in ice cream. A peculiar attention is also paid to the nature of the fat used in the initial oil-in-water emulsion and its influence on the textural, rheological, and sensory properties of the final product.

The **bibliography** section presents, on the one hand, the adsorption phenomena of surface-active molecules (proteins and low molecular weight emulsifiers) and their interactions at the interfaces and, on the other hand, described the influence of the ingredients on the microstructural organization of ice cream. The methodologies employed during this study are detailed in the **Materials & Methods** section. The **Results & Discussion** section comprises three distinct chapters. The first one is related to the characterization and the comprehension of the mechanisms involved at the oil / water interfaces within an emulsion. The second chapter deals with the physicochemical characterization of the ice cream mix. The third chapter is centered on the study of the ice creams in terms of characterization of the interactions between the various components, the description of microstructures and sensory analysis. Each Results and Discussion chapter is based on one or two published papers introduced by a summary of the main results including unpublished results if any.

# A. Bibliography

Food dispersions are multicomponent systems containing various ingredients (classically sugars, proteins and fats) associated with other organic components (hydrocolloids, low-molecular weight emulsifiers) and inorganic components (salts) present in small amounts in the formulation. Besides their own properties, all these compounds may interact with each other in different complex ways depending on pH, temperature, and processing history, all of which intensify the problems of the manufacturer attempting to control stability, shelf life, or product texture (Carrera Sánchez, *et al.*, 2005). In addition, in the case of food dispersions in the form of emulsions and foams, the formation of interfaces (oil / water or air / water, for example) and their stabilization require the use of surfactants that show surface activity by themselves or in association with other components (polysaccharides). Manufacturers employ two types of surfactants in food (Dickinson and Woskett, 1989), namely low-molecular weight emulsifiers (mainly mono- and diglycerides) and macromolecules (proteins and peculiar hydrocolloids). Due to their amphiphilic nature, both proteins and low-molecular weight emulsifiers show the potential for association, adsorption, and reorientation at fluid interfaces. However, these properties strongly depend on the nature of the components (Rodríguez Patino and Rodríguez Niño, 1999; Davies, *et al.*, 2001; Rodríguez Patino, *et al.*, 2001b; Dalgleish, *et al.*, 2002) and the protein-emulsifier ratio(Courthaudon and Dickinson, 1991; Chen, *et al.*, 1993; Chen and Dickinson, 1995; Horne, *et al.*, 1998; Sliwinski, *et al.*, 2003).

This chapter will focus on the interfacial behavior of peculiar surfactants, namely, milk proteins and non-ionic lipophilic emulsifiers, since they are commonly used in ice cream formulation. Emphasis will be made on the surfactant adsorption / desorption mechanisms at the different interfaces during the main steps of ice cream processing, *i.e.*, homogenization and ageing of an emulsion premix, followed by aeration and freezing of this premix. Interface composition, protein and emulsifier structure and the competition phenomenon at the interfaces of emulsion and foam will be considered and discussed in terms of formation and stability of ice cream structure.

# I. ADSORPTION AND COMPETITIVE MECHANISM OF SURFACE ACTIVE MOLECULES AT THE OIL / WATER INTERFACE

A large number of studies have been carried out to examine the emulsifying behavior of proteins and low molecular weight emulsifiers at the oil / water interface. The objective of this part is, first, to point out the individual protein and emulsifier functionality as a function of their chemical nature. Then, the case of mixtures of protein and emulsifier is considered in order to enlighten the competitive mechanism that may occur at the interface between these two species. Besides the molecular phenomena, the consequences of this competition on the emulsion stability are also described.

## I.1. Adsorption of surfactants at the oil / water interface

### *I.1.1. Adsorbed protein layer*

The formation of **adsorbed protein layers** is a complex process dictated by a number of factors, including mass transport to the surface (diffusion through a partially formed adsorbed layer), attachment at the surface, adsorbed layer rearrangements such as reorientation of the adsorbing protein and conformational changes (Malmsten, 1998; Carrera Sánchez, *et al.*, 2005). Each stage is postulated to require an activation energy which, once overcome, results in a successive lowering of interfacial energy. Proteins in solution are usually described by two extreme structures: (i) disordered flexible random coil polymers and (ii) globular tightly packed molecules. In the case of milk proteins, caseins have a disordered flexible structure whereas whey proteins have a compact globular structure (Dickinson, 2001). Depending on the protein concentration, the adsorption of proteins at the interface can cause changes in the conformational state and even gradual unfolding (Figure 1). In the case of disordered flexible proteins, at low concentration, the molecules can be represented as a succession of trains of amino acid residues along the interface and loops and tails protruding into each phase. As protein concentration increases, the surface layer becomes compressed. The looped conformation of the protein creates enough electrostatic repulsion and steric hindrance to protect the droplets against recoalescence.

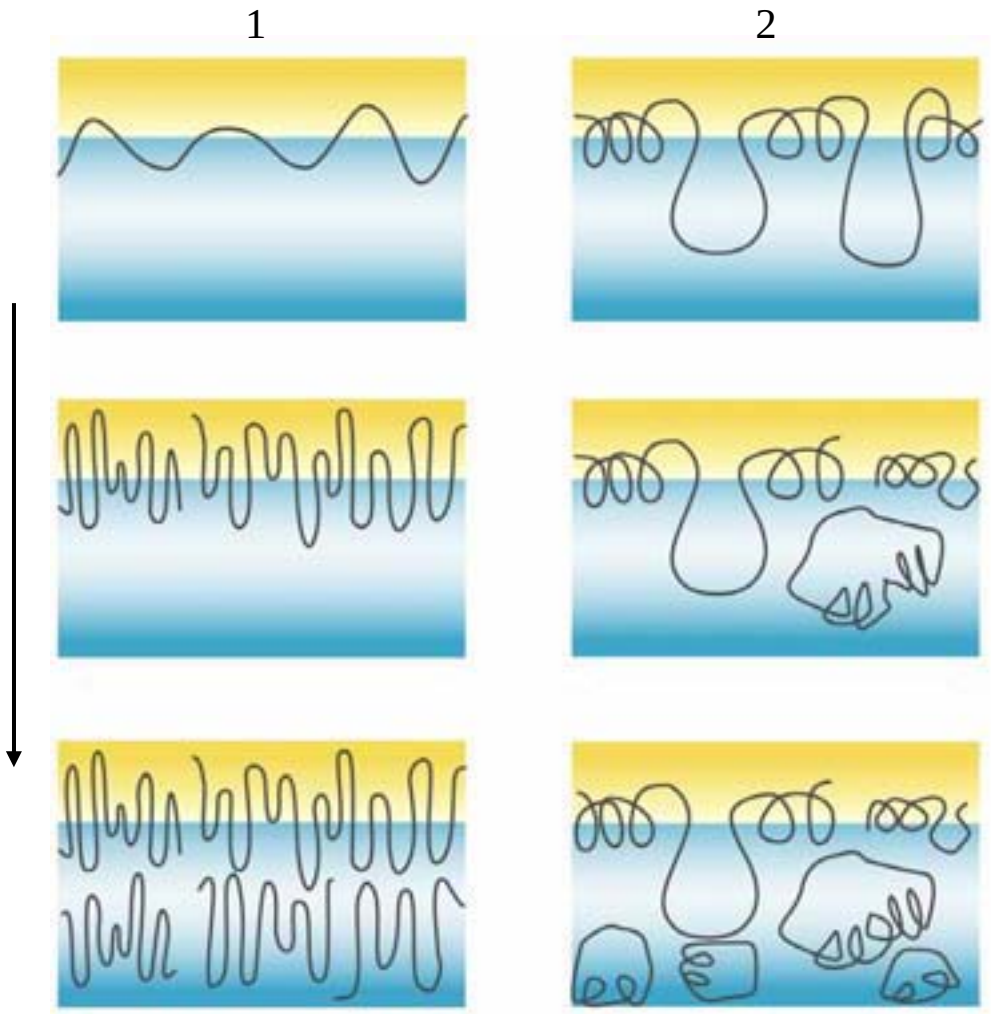


**Figure 1: Schematic representation of protein structure at a fluid interface.**

The interface is shown in cross section, the aqueous inter-lamellar phase is represented in blue and the oil phase is represented in yellow: (1) flexible, random-coil proteins; (2) globular, highly structured proteins. The arrow denotes increasing protein concentration.

The disordered flexible proteins are more closely packed and more loops and tails are formed. In the case of globular proteins, a dense two-dimensional assembly of highly interacting particles is observed (Dickinson, 1997). The lateral interactions between the proteins are due to hydrogen bonding, electrostatic and hydrophobic interactions (Bos, *et al.*, 1997). Covalent bonding between proteins may also occur (Bos, *et al.*, 1997). As a consequence, the diffusion of molecules in the adsorbed layer is inhibited. The adsorbed layer in protein-stabilized thin films is stiff and often shows visco-elastic or even solid-like properties. This enables the fluid interface to resist tangential stresses from the adjoining flowing liquids (Dickinson, 1999). At high protein concentration, multilayer formation can occur and serves to further mechanically strengthen the adsorbed layer. Depending on the tertiary and quaternary structures of the protein, various degrees of molecular unfolding may be observed leading to a range of different interfacial structures (Darling and Birkett, 1987). In parallel, the proteins can also be classified as “hard” and “soft” depending on the extent of the unfolding of the macromolecule upon adsorbing (Malmsten, 1998; Dickinson, 1999). The soft proteins, like caseins, experience a greater loss of secondary and tertiary structure upon adsorption than the hard ones, like whey proteins, whose adsorption is accompanied by minimal or no structural changes.

Proteins are large complex amphiphilic molecules containing combination of ionic, polar and non-polar regions. Thus, they act as polymeric **surface active molecules** with multiple encroaching sites at the interface that, together with the unfolding process, stabilize the interfacial layer kinetically. For random-coil proteins such as caseins, molecular unfolding is related to a loss of internal hydrophobic interactions as surface-active residues gradually orientate themselves at the interface. In contrast, the unfolding of globular proteins may involve a denaturation phenomenon including a loss of the ternary structure and the rupture of hydrogen bonds (Darling and Birkett, 1987). In addition, partial unfolding of the monomer allows exposure of the free sulfhydryl group, which leads to slow polymerization of the protein in the adsorbed layer via the interchange between the sulfhydryl and disulfide groups (Dickinson, 1997).

The thickness of the adsorption layer can be expressed as the **surface excess** which is governed by the surfactant concentration and the specific interfacial area. Table 1 presents values of the surface excess obtained for protein systems in emulsions. Generally speaking, a surface excess of about 1 mg $m^{-2}$ corresponds to a monolayer of unfolded peptide chains. It is formed when the adsorbed molecules have space to unfold at the oil / water interface. Globular proteins adsorbed without much change in conformation give surface excess values close to 3 mg $m^{-2}$. For

**Table 1: Surface excess of proteins at the interface of oil-in-water emulsions obtained with protein systems.**

| Protein type | Concentration (wt.%) | pH | Oil type | Surface excess of proteins (mg $m^{-2}$) | Reference |
|---|---|---|---|---|---|
| β-casein | 0.001[a] | 6.7 | Sunflower oil | 3.0 | (Bos and van Vliet, 2001) |
| | 0.01[a] | 7.1 | | 4.2 | |
| | 0.4 | 7.0 | n-Tetradecane | 1.8 | (Courthaudon and Dickinson, 1991) |
| | 0.4 | 7.0 | Soya oil | 1.0 | |
| | 0.4 | 7.0 | n-hexadecane oil | 3.0 | (Chen, *et al.*, 1993) |
| | 0.4 | 7.0 | Triglyceride oil | 1.6 | (Dickinson, 1997) |
| Na-caseinate | 0.03[a] | 6.7 | Sunflower oil | 3.3 | (Bos and van Vliet, 2001) |
| | 2.4 | 6.7 | Soybean oil | 3.4 | (Sliwinski, *et al.*, 2003) |
| β-lactoglobulin | 0.4 | 7.0 | Hydrocarbon oil | 1.4 | (Chen, *et al.*, 1993) |
| α-lactalbumin | 0.45 | 7.0 | n-Tetradecane | 1.5 | (Dickinson, 1997) |
| BSA | 0.01[a] | 6.7 | Sunflower oil. | 2.4 | (Bos and van Vliet, 2001) |
| | 0.001[a] | 7.1 | | 2.0 | |
| WP concentrate partially denaturated | 1.0 | 6.7 | Sunflower oil | 10.5 | (Sünder, *et al.*, 2001) |
| WP isolate | 1.0 | 6.7 | Sunflower oil | 6.3 | (Sünder, *et al.*, 2001) |
| | 2.4 | 6.7 | Soybean oil | 4.0 | (Sliwinski, *et al.*, 2003) |

[a] Concentrations are calculated from values in g $l^{-1}$ with the density of the aqueous phase taken equal to 1.

BSA: Bovine Serum Albumin, WP: whey protein.

values higher than 5 mg $m^{-2}$, molecular aggregates are generally adsorbed (Walstra, 1987). However, various factors influence the surface excess value:

- the **protein type** (molecular weight, conformation). For example, for a similar amount of protein (0.4 wt.%), the surface excess values vary from 3.0 to 1.4 mg $m^{-2}$, for β-casein and β-lactoglobulin, respectively, in hydrocarbon oil-in-water emulsion. The influence of the protein conformation on the surface excess is particularly well illustrated with protein subjected to denaturation by a heat treatment (Sünder, *et al.*, 2001; Rodríguez Patino, *et al.*, 2001b). This may account for an increase in surface activity because the conformational changes of the molecules during heating may induce further unfolding, reorganization, and aggregation of the molecules to bring more hydrophobic segments from the interior of the molecule to the oil / water interface (Rodríguez Patino, *et al.*, 2001b);
- the **protein concentration** (below the saturation surface coverage);
- the **pH** and **ionic strength** of the solution (that in turns affect the protein conformation). The surface excess tends to be higher at a solution pH close to the isoelectric point of the protein (Bos and van Vliet, 2001);
- the **oil type** used for the emulsion preparation. The surface excess values of β-casein vary between 1.0 and 3.0 mg $m^{-2}$, for soya oil and hydrocarbon oil-based emulsions, respectively.

In common dairy ingredients, such as sodium caseinate or whey protein concentrate, there is potential for **competitive adsorption** between the various proteins due, at least partly, to the variation of the surface activities of the individual milk proteins (Dickinson, 1997). Table 2 presents some examples of interfacial protein ratio at the oil-in-water emulsions for different initial protein mixtures. Caseins are often preferentially adsorbed at the interface compared to whey proteins. In emulsions prepared with skim milk powder, caseins are in the highly aggregated form of micelles. Preferential adsorption of the casein fraction is generally reported for homogenized dairy emulsions (Dickinson, 1997). Moreover, β-casein is able to displace adsorbed $\alpha_{s1}$-casein since the former is slightly more hydrophobic and surface-active than the latter (Dickinson, 1997). However, making an emulsion with one pure milk protein and adding a second pure protein afterwards does not mean that the most surface-active protein will actually finish by dominating the interface (Dalgleish, 1997; Dickinson, 1999). Indeed, the competitive adsorption is affected by factors other than just relative affinity for the surface, *e.g.*, the

**Table 2: Interfacial protein composition at the interface of oil (soybean oil)-in-water emulsions obtained with protein mixtures.**

| Protein mixture type | Initial protein ratio | Concentration (wt.%) | pH | Interfacial protein ratio | Reference |
|---|---|---|---|---|---|
| Caseinate / β-lg | 80 / 20 | 2.50 | 7.0 | 97 / 3 | (Dalgleish, *et al.*, 2002) |
| Caseinate / WP | 73 /27 | 2.75 | | 97 / 3 | |
| Caseinate / WP | 50 / 50 | 2.40 | 6.7 | 88 / 12 | (Sliwinski, *et al.*, 2003) |
| | 40 / 60 | | | 87 / 13 | |
| | 90/10 | | | 50 / 50 | |

β-lg: β-lactoglobulin; WP: whey protein.

macromolecular mobility and flexibility at the interface (Dickinson, 1997), the irreversibility of the adsorption event in the case of globular proteins (Dickinson, 1999). As a rule of thumb, the adsorbed layer will be dominated by the protein that presents itself first to the interface (Dickinson, 1999).

### *I.1.2. Adsorbed low molecular weight emulsifier layer*

In this part, we will only focus on oil-soluble, non-ionic low molecular weight emulsifiers and especially on mono- and diglyceride mixtures since they are commonly used in ice cream formulation to improve fat network formation and the air phase stabilization.

**Lipophilic emulsifiers** (monoglycerides, propylene glycol esters), at low concentrations, form a partition between the oil phase and the interface (Krog, 1977; Kanelo, *et al.*, 1999; Awad and Sato, 2002). The adsorption of monoglyceride at the oil / water interface can be described by three consecutive or concurrent steps: (i) diffusion of whole surfactant molecules to attach the interface; (ii) spreading or unfolding of already adsorbed molecules; and (iii) molecular rearrangements of the adsorbed molecules. The two latter mechanisms involve transport of molecular elements or surfactant segments on the surface and account for the rapid diffusion or migration of emulsifiers at the interface (Gibbs – Marangoni mechanism).

The adsorption of low molecular weight emulsifier to fluid interfaces modifies the interfacial layer surface properties although the modifications depend on the intrinsic characteristics (lipophilic / hydrophilic properties, polymorphic behavior) of the molecule, its concentration, and the temperature (Table 3). It is generally admitted that emulsifiers form a mobile, fluid-adsorbed layer at temperature above their transition temperature (Rodríguez Patino, *et al.*, 2001a). Moreover, the adsorption of low molecular weight emulsifier to the interface leads to a reduction of the surface tension (Wong, 1989; Carrera Sánchez, *et al.*, 2005), monoglycerides being more surface active than diglycerides (Wong, 1989). Concerning the **polymorphic behavior**, non-ionic low molecular weight emulsifiers can be classified into two main groups:

- **emulsifiers exhibiting a lyotropic mesomorphism**, like monoglycerides. In the anhydrous state, monoglycerides can present two polymorphic forms, namely $\alpha$ and $\beta$, which differ in the way the molecules pack in the crystal structure. Depending on the crystallization conditions, the $\alpha$-metastable crystalline structure or the $\beta$-state are

**Table 3: Interfacial tension values of low molecular weight emulsifier at oil (sunflower oil) / water interface.**

| Emulsifier type | Concentration in the oil phase (%) | Interfacial tension values (mN/m) | | Reference |
|---|---|---|---|---|
| | | 40°C[a] | 5°C[b] | |
| Propylene glycol monostearate | 2.0 | 23 | 3 | (Barfod, *et al.*, 1989) |
| Glycerol monostearate | 0.03 | 28 | 23 | (Krog and Larsson, 1992) |
| | 0.1 | 23 | 1 | (Barfod, *et al.*, 1991) |
| | 0.1 | 27 | 6 | (Krog and Larsson, 1992) |
| | 0.2 | 17 | 2 | (Krog and Larsson, 1992) |
| Glycerol monomyristate | 0.2 | 18 | 2 | (Krog and Larsson, 1992) |
| Glycerol monopalmitate | 0.2 | 17 | 2 | (Krog and Larsson, 1992) |
| Glycerol monobehenate | 0.2 | 18 | 4 | (Krog and Larsson, 1992) |

[a] Interfacial tension above the melting point;

[b] Interfacial tension below the melting point.

formed (Small, 1986). In water, monoglycerides through their interaction with water often exhibit a complex liquid-crystalline phase behavior with the formation of different types of mesophases such as lamellar, cubic or reversed hexagonal structures, depending on the concentration of the emulsifier and the temperature (Krog, 1977; Small, 1986; Larsson, 1994b; Bergenståhl, 1997). Under certain conditions, the β-crystal form of monoglyceride interacting with water can lead to the α-lamellar liquid crystal form. Upon cooling, this form can transform to a so-called α-gel phase (Krog, 1977). The change from the lamellar form to the α-gel phase results in a decrease in surface area of the emulsifier of about 30 %, and the subsequent formation of the β-crystal structure releases a large amount of water (Euston, 1997). This phase behavior is accompanied by a large decrease in the interfacial tension below the melting point (Barfod, *et al.*, 1989; Barfod, *et al.*, 1991) (Table 3). Several hypotheses have been proposed to explain this phenomenon: (i) a more effective packing or an increased density of polar groups of the emulsifier near the crystallization point; (ii) the crystallization of a monomolecular layer of emulsifier adsorbed at the interface; (iii) micelle formation near the interface;

- **non-polymorphic emulsifiers**, so-called α-tending emulsifiers, such as propylene glycol monostearate, acetylated monoglycerides, or lactylated monoglycerides. α-Tending emulsifiers can only form an hydrated gel phase structure (α-crystalline form) below the melting temperature of the hydrocarbon chains and are practically insoluble in water (Westerbeek, 1991).

Although the competitive adsorption of binary mixtures of protein is a well-documented phenomenon (§ A.I.1.1), binary mixtures of oil-soluble low molecular weight emulsifiers are poorly investigated. However, it seems that a competitive adsorption may also occur for this type of surfactants. The final composition of a mixture of low molecular weight surfactants at the interface would be affected by: (i) the binding capacity for the surface of each molecule; (ii) the bulk concentration of the surfactants; (iii) the molecule-molecule interactions, and (iv) the size ratio of each species (Pugnaloni, *et al.*, 2004).

## I.2. Composition of the interfacial protein / emulsifier film at oil / water interface

Food emulsions are generally prepared using mixtures of both proteins in soluble and dispersed form and low molecular weight emulsifiers. These various surfactants contribute to both short-term (at the time of formation) and long-term (for product shelf-life) stability (Darling and Birkett, 1987). Differences in the properties of oil-in-water emulsions arise largely from the differences in structure and composition of the absorbed protein and / or low molecular weight emulsifier layers at the surface of the fat globules.

During emulsion formation, the surfactant molecules become rapidly adsorbed at the surface of the newly formed oil droplets leading to competition and / or interactions between the different molecular species. The basic principles of competitive adsorption and displacement of proteins by emulsifiers are well understood for water-soluble (Tween, etc.) low molecular weight emulsifiers (Pugnaloni, *et al.*, 2004; Wilde, *et al.*, 2004). They can be summarized as follows: at low emulsifier concentration, small pools of surfactant develop in the defects of the protein monolayer. With increasing surfactant bulk concentration, these surfactant domains grow, compressing the protein film. Beyond a critical surface pressure, the protein film starts buckling into the bulk phase and consequently increasing the film thickness. As surfactant molecules continue adsorbing, the surfactant domains start to coalesce and parts of the protein film detach from the interface. Finally, a continuous surfactant phase develops at the interface, leaving just a few disconnected protein areas adsorbed, which eventually also detach from the interface. This mechanism has led to the description of the competitive displacement process as “orogenic displacement”.

Relatively little research has been performed on oil-soluble emulsifiers although they are commonly used in food application. In this chapter, we will only present the competitive adsorption and displacement of proteins by oil-soluble low molecular weight emulsifiers.

### *I.2.1. Adsorbed layer from mixtures of proteins and emulsifiers*

Different mechanisms may account for the composition of the interfacial layer when both proteins and low molecular weight emulsifiers are present:

- **a control** of the adsorbed layer either **by the protein or by the emulsifier**. This mechanism corresponds to the fact that one type of surfactant is more surface-active than the other. In a solution containing both protein and emulsifier, emulsifier is likely

to prevail at the interface, after equilibration, if both species are present at high enough bulk concentrations. This is due to the fact that emulsifiers are much smaller in size than proteins, so they can reduce the interfacial tension more efficiently by adsorbing a much larger number of molecules within the same surface area (Bos and van Vliet, 2001; Pugnaloni, *et al.*, 2004);

- **a competitive adsorption between proteins and low molecular weight emulsifiers** at the interface. The adsorbed layer characteristics are found intermediately between those of pure protein and emulsifier films. The adsorption of the protein is affected by the binding of the emulsifier to the protein itself and/or the fluid interface (Dalgleish, 1997; Bos, *et al.*, 1997; Dickinson, 1999; Dickinson, 2001; Pugnaloni, *et al.*, 2004). The competition mechanism results in the disruption of the interfacial protein-protein interactions by the emulsifier (Bos and van Vliet, 2001) and, thus, in the decrease of the equilibrium surface concentration of protein (Darling and Birkett, 1987);
- a **cooperative adsorption** at the interface. Protein and emulsifier can interact in solution to form complexes that have different properties from those of pure protein. In particular, protein / emulsifier complexes can themselves be surface active and compete for the interface with the emulsifier and protein molecules (Bos and van Vliet, 2001). Non-ionic emulsifiers may bind strongly to proteins, but this is a highly specific effect involving a small number of hydrophobic sites per protein molecule (Bos and van Vliet, 2001). At low emulsifier concentration, the presence of oil-soluble emulsifier at the oil / water interface would tend to drag more protein molecules to the interface due to the interactions between the emulsifier and protein molecules.

Table 4 presents the mechanisms proposed for some **oil-in-water emulsions** prepared with mixed solutions of proteins and oil-soluble emulsifiers.

The structures and composition of the interfacial mixed protein - low molecular weight emulsifier layer encountered depend on numerous parameters:

- the **type of protein** (disordered or globular, bond-forming or weakly interacting) and its **concentration** (whether or not the interface is saturated by the protein);
- the **type and concentration of the emulsifier**. At high concentration, emulsifiers usually lead to a lower equilibrium surface tension than proteins (Euston, *et al.*, 2001);
- the **protein / emulsifier ratio**. Milk proteins exhibit a greater thermodynamic affinity for the adsorbed state and saturate fluid interfaces at much lower concentrations than

**Table 4: Behavior of protein-emulsifier films adsorbed at the oil / water interface (ambient temperature).**

| Protein mixture | | Emulsifier | | Adsorption mechanism | | Reference |
|---|---|---|---|---|---|---|
| Type | Concentration (wt.%) | Type | Concentration (wt.%) | **Short time** [a] | **Long time** [b] | |
| WPI | $1.10^{-2}$ | Monopalmitin | $1.10^{-4}$ | A | A | (Rodríguez Patino, *et al.*, 2001a)[c] |
| | | | $1.10^{-3}$ | A | B | |
| | | Monoolein | $1.10^{-4}$ | A | B | |
| | | | $1.10^{-2}$ | C | C | |
| | $1.10^{-4}$ | Monopalmitin | $1.10^{-4}$ | C | C | |
| | | | $1.10^{-3}$ | D | D | |
| | | Monoolein | $1.10^{-4}$ | C | C | |
| | | | $1.10^{-2}$ | C | C | |
| Sodium caseinate | $1.10^{-2}$ | Monooleyl-glycerol | $1.10^{-1}$ | D | | (Heertje, *et al.*, 1996)[d] |
| | | | 0.36 | D | | |
| | $8.10^{-2}$ | | $1.10^{-1}$ | C | | |
| | | | 0.36 | D | | |

[a]: Time lower than approximately 10 h.

[b]: Time higher than approximately 15 h.

[c]: Trisun oil / water interface.

[d]: Sunflower seed oil / water interface.

A: Adsorbed layer controlled by the adsorption of protein.

B: Cooperative adsorption in the mixed solution.

C: Competitive adsorption in the mixed solution.

D: Adsorbed layer dominated by the adsorption of the emulsifier.

WPI: whey protein isolate.

do small molecule emulsifiers like monoglycerides (Dickinson, 1999; Euston, *et al.*, 2001) (Dickinson, 1999; Euston, *et al.*, 2001). Thus, at the same low (molar) bulk protein concentration, proteins induce a greater lowering of the equilibrium interfacial tension than small molecule amphiphiles (Dickinson, 1999). However, a converse situation occurs at high bulk concentration, where the small molecule emulsifier gives a lower tension and a more densely packed layer (Dickinson, 1999). Thus, high concentrations of emulsifier are required to displace proteins from emulsion droplets (Rodríguez Patino, *et al.*, 2001a; Wilde, *et al.*, 2004);

- the **surface properties**, *i.e.*, the nature of the fluid interface. However, the influence of the fat phase on protein-lipid mixed films at the oil / water interface at positive temperature is poorly documented;
- the **adsorption time**. The characteristic adsorption time (in diffusion limited conditions) of proteins is much longer than the characteristic adsorption time for small amphiphiles, due to the larger molecular mass of the proteins (Kilara and Keeney, 1989);
- the **temperature**. The influence of this parameter is detailed in the next paragraph (§ A.I.2.2.) since it is crucial in the ice cream process.

In **ice cream mixes**, where protein concentration is high, it is generally admitted that, at temperature above the crystallization temperature of the emulsifier, proteins and emulsifiers both occupy the fat globule surface, with the lipophilic emulsifier having a relatively small effect on the protein surface coverage (Buchheim, 1991; Dickinson, 1996b; Euston, 1997; Pelan, *et al.*, 1997). For example, interfacial tension measurements achieved on propylene glycol monostearate-based whippable emulsions, show that both protein and emulsifier are located at the oil / water interface above 20°C (Barfod, *et al.*, 1989). Thus, the oil-soluble emulsifiers are not able or inefficient in displacing proteins from the oil / water interface. Besides the argument of the difference in surface activity, this could mean that the adsorbed protein layers are, to a large extent, positioned close to the interface in the water phase, rather than equally distributed at both sides of the interface or at the oil side interface (Bos and van Vliet, 2001). In other words, oil soluble emulsifiers adsorb to the interface from the oil phase and wet the oil phase preferentially. Consequently, only a small part of the molecule may enter the aqueous phase and have the potential to interact with protein molecules (Euston, *et al.*, 2001). However, emulsifiers present at too low concentration to cause protein displacement can affect the adsorbed protein layer by modifying inter-protein interactions (Euston, *et al.*, 2001).

### *I.2.2. Effect of temperature decrease on the interfacial protein / emulsifier film composition of oil-in-water emulsion*

Surface activity of oil-soluble non-ionic emulsifiers is strongly temperature-dependent (Barfod, *et al.*, 1989; Gelin, *et al.*, 1995). For example, the interfacial tension of a sunflower oil containing monostearin is drastically reduced on cooling between 30 and 0°C (from 7 to 95 % depending on the emulsifier concentration) (Krog and Larsson, 1992). The temperature dependence of emulsifier surface activity can be explained in terms of their **phase behavior** in an aqueous solution (§ A.I.1.2.). Depending on the amphiphile concentration, both monoglycerides and α-tending emulsifiers gel at low temperature but the stability of the α-gel phase of monoglycerides is relatively poor in contrast to that of α-tending emulsifiers that are stable below the crystallization temperature of the hydrocarbon chain. In the case of α-tending emulsifier, this stability may account for the hydration of the polar head group. It has been hypothesized that the phase transition of the adsorbed monoglycerides from a water-containing lamellar liquid-crystalline phase into the α-gel phase and, then, into the stable β-crystalline state, is responsible, at least partly, for the protein desorption from the fat globule interface (Berger, 1976) (Figure 2). This crystallization includes: (i) a **reorientation** of the polar groups towards the interface just before the start of crystallization following the adsorption of the emulsifier from the bulk of the oil phase. The increased hydrophobicity of the oil / water interface weakens the hydrophobic lipid-protein binding initiating protein desorption from the interface (Barfod, *et al.*, 1989). Because of the high water-binding capacity of emulsifier at low temperature, water penetrates into the emulsifier multi-layers in the fat phase, accelerating protein desorption; and (ii) the **formation of crystals** at the interface. These crystals were identified for monoglycerides (Krog and Larsson, 1992; Larsson, 1994a), diglycerol esters (Holstborg, *et al.*, 1999), and their mixtures with proteins. They are called “surface active crystals” since they expose the methyl end group towards the oil phase and the polar head towards water.

Another parameter that may favor protein desorption is the change in the **physical state of the oil** that begins to crystallize when temperature is decreased. Indeed, in cooled mix prepared without emulsifier, the protein load at the fat globule surface is slightly reduced (Barfod, *et al.*, 1991; Krog, 1998). This reduction is due partly to the crystallization of the fat globules. Because emulsifiers are known to influence fat crystallization in emulsion (Barfod, *et al.*, 1991; Euston, 1997; Abd El-Rahman, *et al.*, 1997), they can participate indirectly to protein

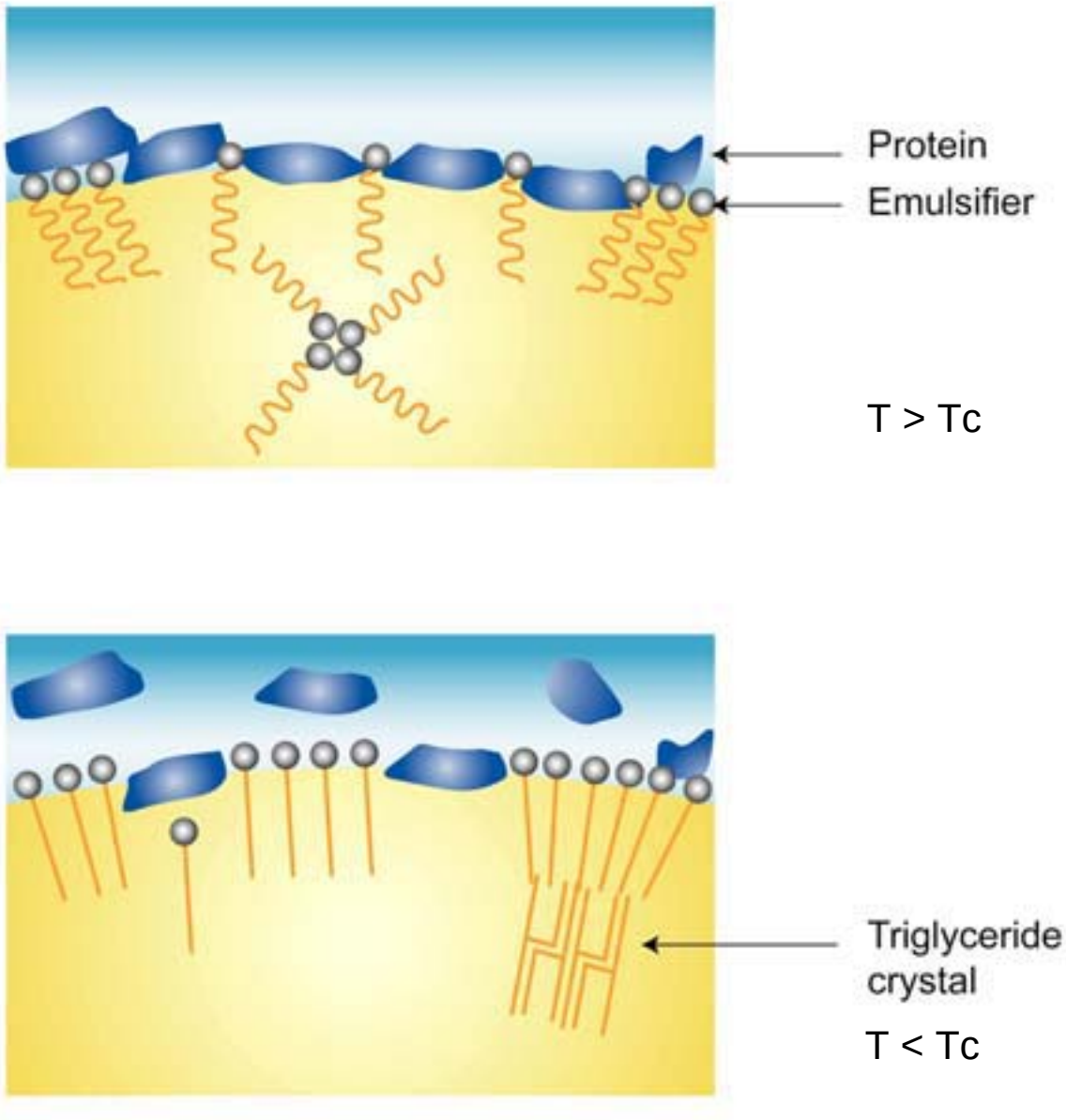


**Figure 2: Schematic diagram showing the possible effect of temperature on the protein layer at the interface of oil-in-water emulsion.**

Part of oil droplet is shown in cross section, the aqueous interlamellar phase is represented in blue and the oil phase is represented in yellow. $T_C$: crystallization temperature of the emulsifier.

desorption. Fat crystallization appears to be correlated to the interfacial effects of monoglycerides at low temperature (Barfod, *et al.*, 1991). Monoglycerides are known to be initiators of fat crystallization (Barfod, *et al.*, 1991; Davies, *et al.*, 2001). Such a template effect of monoglycerides should greatly influence the triglyceride surface crystallization kinetic and, possibly, polymorph. Emulsifiers containing saturated hydrocarbon chains are better at initiating fat crystallization than those with unsaturated hydrocarbon chains (Barfod, *et al.*, 1991; Davies, *et al.*, 2001). If there is a match in saturation and number of carbon atoms in the fatty acid chains, it is assumed that monoglyceride will more readily incorporate into growing triglyceride crystals. If there is a slight mismatch in the number of carbon atoms or if there is a kink in the carbon chain due to a double bound, the monoglyceride will fit less well with the oil triglyceride.

Several studies on oil-in-water emulsions (Barfod, *et al.*, 1989; Dickinson and Tanai, 1992; Davies, *et al.*, 2001) and ice cream mix (Barfod, *et al.*, 1991) indicate a displacement of protein from the oil / water interface by emulsifiers at temperatures between 4 and 10°C. Tables 5 and 6 present the adsorbed protein amount at the fat globule surface found in oil-in-water emulsions and ice cream mixes, respectively, when temperature is reduced to typically around 5°C. It appears that protein desorption is function of numerous factors:

- the **ageing period** (Barfod, *et al.*, 1991; Gelin, *et al.*, 1994; Gelin, *et al.*, 1996; Abd El-Rahman, *et al.*, 1997; Krog, 1998). Most of the studies show that the amount of adsorbed protein at the fat globule surface in ice cream mix decreases during the ageing period. However, opposite results are also found (Abd El-Rahman, *et al.*, 1997). Interestingly, the effect of monoglycerides on protein load is reversible (Krog, 1998). If a mix is reheated after being kept at low temperature for a given period, the protein load will regain the value it had before it was cooled;
- the **emulsifier** type (unsaturation degree and fatty acid chain length) and concentration (Pelan, *et al.*, 1997; Davies, *et al.*, 2000; Bolliger, *et al.*, 2000a; Davies, *et al.*, 2001). Variations in the effect of saturated and unsaturated monoglycerides regarding protein desorption have been reported. Some works are in disagreement (Davies, *et al.*, 2001) or were not able to confirm (Pelan, *et al.*, 1997) the reported findings (Goff and Jordan, 1989; Barfod, *et al.*, 1991) which suggest that unsaturated monoglycerides are more effective at displacing milk proteins than saturated monoglycerides. Concerning the chain length, palmitic acid based monoglyceride leads to higher milk protein desorption than stearic acid based monoglyceride (Davies, *et al.*, 2001);

**Table 5: Values of apparent protein surface coverage (Γ) for some proteins and protein mixtures in oil-in-water emulsions.**

| Protein mixture | | Emulsifier | | | Oil type | Ageing[a] time (h) | Γ ($mg.m^{-2}$) | Reference |
|---|---|---|---|---|---|---|---|---|
| Type | Conc. (wt.%) | Type | % MG | Conc. (wt.%) | | | | |
| Na-caseinate | 1 | GMO | 100 | 0 | Groundnut oil + tristearin | 1 | 1.08 | (Davies, *et al.*, 2000) |
| | | | | 0.8 | | | 0.45 | |
| | | | | 1.3 | | | 0.20 | |
| Na-caseinate | 1 | GMS | 100 | 0.8 | Groundnut oil + tristearin | 1 | 0.17 | (Davies, *et al.*, 2001) |
| | | GMP | 100 | 0.8 | | 1 | 0.05 | |

[a] ageing corresponds to an incubation of the samples around 5°C.

Conc.: concentration; GMO: glycerol monoolein; GMS: glycerol monostearate; GMP: glycerol monopalmitate.

**Table 6: Values of apparent protein surface coverage (Γ) for some proteins and protein mixtures in ice cream mixes.**

| Protein mixture | | Emulsifier | | | Oil type | Ageing[a] time (h) | Γ (mg.m$^{-2}$) | Reference |
|---|---|---|---|---|---|---|---|---|
| Type | Conc. (wt.%) | Type | % MG | Conc. (wt.%) | | | | |
| SMP | 13 | GMP | 50 | 0.1 | Butter oil | 2 | 14.5 | (Pelan, *et al.*, 1997) |
| | | | | 0.3 | | | 10.1 | |
| | | | | 0.5 | | | 8.1 | |
| | | Saturated | 100 | 0.12 | | | 12.9 | |
| | | | | 0.3 | | | 7.4 | |
| | | | | 0.5 | | | 5.8 | |
| | | Partially unsaturated (70 %) | 100 | 0.12 | | | 10.1 | |
| | | | | 0.3 | | | 7.0 | |
| | | | | 0.5 | | | 7.4 | |
| | 11.5 | / | 100 | 0 | Hardened coconut fat | 2 | 14.2 | (Barfod, *et al.*, 1991) |
| | | GMS | | 0.2 | | | 12.5 | |
| | | GMO | | 0.2 | | | 7.4 | |
| | 11.5 | / | 100 | 0 | Hardened coconut fat | 24 | 11.0 | (Barfod, *et al.*, 1991) |
| | | GMS | | 0.2 | | | 7.0 | |
| | | GMO | | 0.2 | | | 4.7 | |
| | 10 | Saturated MDG | 40 | 0 | Anhydrous milk fat | 24 | 11.4 | (Bolliger, *et al.*, 2000a) |
| | | | | 0.075 | | | 8.8 | |
| | | | | 0.15 | | | 9.0 | |
| | 10 | Saturated MDG | 60 | 0.3 | Hydrogenated palm kernel oil | 24 | 10.1 | (Sourdet, *et al.*, 2002) |
| Milk protein | 4 | GMS | 100 | 0.2 | Coconut fat | 0 | 10.5 | (Krog, 1998) |
| | | | | | | 1 | 7.7 | |
| | | | | | | 2 | 7.0 | |
| | | | | | | 24 | 3.0 | |
| SMP/WPI | 6.7 | Saturated MDG | 60 | 0.3 | Hydrogenated palm kernel oil | 24 | 10.2 | (Sourdet, *et al.*, 2002) |
| WPI | 3.5 | | | | | | 4.4 | |

[a] ageing corresponds to an incubation of the samples around 5°C.

Conc.: concentration; MDG: mono- and diglyceride mixture, SMP: skim milk powder; WPI: whey protein isolate, GMO: glycerol monoolein; GMS: glycerol monostearate; GMP: glycerol monopalmitate.

the protein type (Sourdet, *et al.*, 2003), concentration (Sourdet, *et al.*, 2003), and conformation (Relkin, *et al.*, 2003b). The proportion of proteins adsorbed at the fat globule surface, at the end of the ageing period, seems to be more important with decreasing casein-to-whey protein ratio (Sourdet, *et al.*, 2002; Sourdet, *et al.*, 2003). Variations in the conformation of proteins, such as the increase of surface hydrophobicity by heat treatment for whey proteins, lead, in some cases, to higher proportion of adsorbed proteins (Sourdet, *et al.*, 2003; Relkin, *et al.*, 2003b);

- the **oil type**. For example, after the ageing step, low melting milk fat fraction leads to higher adsorbed proteins than cream, anhydrous milk fat, and very high melting fraction (Abd El-Rahman, *et al.*, 1997).

Although there is substantial evidence that protein desorption occurs during the ageing process and that it is affected by numerous physicochemical parameters, the results presented in the various studies are hardly comparable. This accounts for differences in formulation (purity of the raw materials, proportions of the various ingredients), the ageing conditions (time and temperature), and analytical methods (extraction and protein dosage).

## I.3. Consequences of the adsorption / desorption phenomena on ice cream mix characteristics

Ice cream process begins with a homogenization step. The mix passes through one or two homogenizing valves, creating fat globules, the size of which ranges from 0.5 to 2.0 μm (Berger, 1976). Homogenization results in a large increase in the area of the oil / water interface by adsorption of surface-active components from the oil and aqueous phases. At this stage, adsorption of surfactant material occurs in order to rapidly reduce the interfacial tension (Segall and Goff, 1999). Indeed, it is crucial to avoid coalescence of the dispersed droplets. Although low molecular weight emulsifiers diffuse more rapidly to the newly created interface than milk proteins, proteins serve as the principal emulsifying agents in stabilizing the dispersed oil droplets (Doxastakis, 1989). The role of proteins is to form a mechanically strong interfacial film on the surface of the oil droplets. Protein coating on the fat droplets is relatively thick due to the excess protein available in the system. Indeed, the concentration in a standard mix is more than that required to stabilize the fat droplets in an emulsion. As a result, the droplets are stable and the system is stabilized against immediate coalescence (Krog, 1977).

Different protein blends in ice cream mixes cause the emulsion to behave differently to stabilization (Dickinson, 1997). For example, micellar caseins produce a much larger quantity of adsorbed material per surface area than globular whey proteins (Goff, 1997). The presence of caseins in the micellar form, at the surface of oil droplets, creates a steric stabilizing layer that maintains the repulsion between fat globules and protects the droplets against destabilization (Berger and White, 1971). In contrast, due to their compact globular structures, whey proteins form close-packed protein monolayers, described as dense-dimensional assemblies of highly interacting deformable particles (Dickinson, 2001). This molecular arrangement may prevent a sufficient protein surface loading. In addition, the use of whey proteins partially denatured by heat treatment, may favor the association of polymers at the oil / water interface through weak interactions and the formation of condensed aggregates due to disulphide groups between protein molecules (bridging flocculation) (Segall and Goff, 1999). The extent of bridging flocculation depends on the size, conformation and chemistry of the absorbing species (Darling and Birkett, 1987; Dickinson, 2001).

In the formation of ice cream, the emulsifiers (typically, mono- and diglycerides), are added to break the adsorbed layer of protein during the ageing step at low temperature (Goff, 1997). The competitive adsorption and / or displacement between emulsifiers and proteins at fluid / fluid interfaces should occur during / or after this step. Because protein molecules are considerably larger than the emulsifier molecules, the decrease in the adsorbed protein amount at the fat globule surface causes the actual membrane to become weaker (Goff, 1997). However, a certain level of protein depletion must be exceeded in order to significantly weaken the fat globule membrane system (Bolliger, *et al.*, 2000a). The adsorption of the protein is affected by the binding of the surfactant to both the protein and the fluid interface. Thereby, the balance of protein-protein, protein-emulsifier and emulsifier-emulsifier interactions, both at the interface and in the bulk solution is modified (Dalgleish, 1997; Dickinson, 2001). A mixed protein / emulsifier film induces a higher molecular mobility in the adsorbed layer (Krog, 1998). The disruption of the protein-protein interactions by the emulsifier gives a drop in the interfacial shear viscosity and interfacial shear modulus to values characteristics of low molecular weight emulsifiers (Dalgleish, 1997; Bos and van Vliet, 2001; Rodríguez Patino, *et al.*, 2001a). The surfactant-induced disruption of protein layers can promote or enhance destabilization in ice cream emulsions (Dickinson, 1997). It is worth noting that the destabilization due to protein desorption may not be readily revealed at a supra-molecular level. Indeed, the ageing of ice cream mix does not cause any change in droplet size distribution (Gelin, *et al.*, 1994). The

absence of fat globule coalescence is observed for different casein-to-whey protein ratios (Barfod, *et al.*, 1991; Gelin, *et al.*, 1994; Bolliger, *et al.*, 2000a; Sourdet, *et al.*, 2002; Sourdet, *et al.*, 2003). Saturated mono- diglycerides and casein-based ice cream mixes resist aggregation due to a specific adsorption of casein molecules (Sourdet, *et al.*, 2002).

On the whole, low molecular weight emulsifiers do not play a major role in ice cream mix stabilization just after homogenization at high temperature, due to an excess of protein in the mix formulation. The stabilization of the fat globules against coalescence is achieved thanks to the presence of sufficient protein to cover fully the droplet surface. The resulted emulsions normally retain good stability towards coalescence during quiescent storage, even in the presence of competitively adsorbing emulsifiers (Dickinson, 2001). However, as a function of the protein type, *i.e.*, disordered or globular, some flocculation may occur. In contrast, low molecular weight emulsifiers play a major role after homogenization during the ageing step in causing a subsequent protein desorption. Nevertheless, the fat globule size characteristics are hardly affected by the changes in the interfacial composition.

## II. PARTIAL COALESCENCE PHENOMENON OF FAT GLOBULES

In ice cream formulation, oil-soluble emulsifiers (typically mono- and diglycerides) are added to favor the emulsion destabilization during the freezing step. Emulsifier crystallization is accompanied by a drop in the interfacial tension. As a result, protein is displaced from the fat surface. Because emulsifier breaks the adsorbed layer of protein, it diminishes the effective film thickness (Darling and Birkett, 1987) and the membrane becomes more susceptible to subsequent destabilization (Goff, 1997). The emulsifier is unable to maintain the fat in an emulsified state on its own, probably due to the decreased steric stabilization (Barfod, *et al.*, 1989). In particular, the fat globules with adsorbed emulsifier membranes become much less able to remain individual. Coalescence refers to a decrease in the number and an increase in the size of individual globules. This phenomenon is controlled by the thinning and rupture of liquid films between two colliding globules. Then, the fat generally flows together, causing the loss of identity of the original particles and creating a larger one. Simultaneously to fat droplet coalescence occurring due to the protein replacement by the low molecular weight emulsifier at the interface, spontaneous fat

crystallization occurs. This last phenomenon prevents the total fusion of the colliding particles and leads to the so-called partial coalescence phenomenon.

## II.1. General description of the partial coalescence phenomenon

Numerous papers dealing with partial coalescence of fat globules are available in literature (Walstra, 1987; Boode and Walstra, 1993; Goff, 1997; Rousseau, 2000). This phenomenon is of peculiar importance in the food industry, and particularly in dairy products, where many triglyceride emulsions are manufactured and stored at temperatures at which fat is in a crystallized state. The mechanism called partial coalescence occurs with fat globules containing a solid network of fat crystals. It results in the formation of fat irregularly shaped clumps. The crystal network in the globules prevents their complete coalescence into bigger ones. It is worth noting that partially coalesced droplets are stable as long as there is a solid fat network to support the shape but when the solid fat is melted, the partially coalesced droplets collapse and merge to form a larger droplet through true coalescence (Vanapalli and Coupland, 2001; Palanuwech and Coupland, 2003).

The partial coalescence phenomenon is often described as a three stage process (Berger, 1976; Boode and Walstra, 1993; Rousseau, 2000; Vanapalli and Coupland, 2001): (i) **droplet contact** favored by the presence of fat crystals that protrude through the interfacial membrane. This implies that crystals are actually present at the interface and that they are correctly orientated towards the surface of the droplet; (ii) **interdroplet piercing.** Crystals are believed to occasionally puncture the thin film separating two colliding droplets on approach of other droplets through application of shear forces. This induces fat / fat contact. A prerequisite for piercing to occur is that the distance between globules is small enough; (iii) **oil mixing** that corresponds to the flow out of residual liquid oil to wet the crystal linkage. This tightens the link between the two droplets. As soon as a crystal touches the oil phase of another globule, partial coalescence is inevitable because the crystal is better wetted by oil than by water. However, in spite of the oil flow, the mechanical strength of the internal crystal network limits the merging of the oil and is sufficient to maintain the droplet shape. This implies that there is a sufficient amount of solid fat.

As a consequence, the partial coalescence phenomenon and the extent of fat globule instability are greatly influenced by numerous factors such as the **amount of crystallized**

**matter**, the **size and shape of the fat crystals**, the **orientation of the crystals** at the interface, the **surfactant type and concentration** (Goff and Jordan, 1989; Barfod, *et al.*, 1991; Pelan, *et al.*, 1997; Bolliger, *et al.*, 2000b; Palanuwech and Coupland, 2003), **droplet diameter** (Boode and Walstra, 1993; Thomsen and Holstborg, 1998), and **shear rate** (Boode and Walstra, 1993; Rousseau, 2000). A recent study (Thanasukarn, *et al.*, 2004) also points out that the formation of **ice crystals** could enhance partial coalescence. The ice crystal role is based on differences in oil behavior upon cooling observed when the temperature is reduced to -10°C (only oil crystallization) and -20°C (oil and water phase crystallization). Water crystallization could adversely affect emulsion stability through a variety of mechanisms, including freeze-concentration, penetration of membrane by ice crystals, cold-denaturation of proteins and emulsifier dehydration.

## II.2. Influence of fat

Fat crystals in the oil droplets play an important role in the destabilization of the emulsion. The ability of triglyceride crystals to protrude from the droplet surface and pierce the thin film between globules depends on the solid fat content, the particle microstructure and the surface tension.

The **solid fat content** is a factor in the emulsion stability (Boode and Walstra, 1993; Abd El-Rahman, *et al.*, 1997; Davies, *et al.*, 2001). Crystals can flocculate into a large continuous network enhancing the likelihood of partial coalescence occurring when the solid fat content approximately ranges between 10 and 50 % (Walstra, 1987). Below this value, there are not sufficient crystals present to form a continuous network and therefore the system may maintain stability or normal coalescence may occur. When the majority of fat is solid, the droplets will not coalesce (Boode and Walstra, 1993; Davies, *et al.*, 2000).

The **microstructure** implies **fat polymorphism and crystal morphology** (Rousseau, 2000; Davies, *et al.*, 2001). The change in microstructure influences the droplet coverage and fat crystal contact angle. The rigid crystals are more able to overcome the bending imposed by the surface (Coupland, 2002). It is generally admitted that triglycerides can present three polymorphic forms, $\alpha$, $\beta$' and $\beta$ polymorphs, differing in their stability. $\alpha$ Crystals contain fatty acids in a more disordered arrangement. The resulting fat crystals are softer, more able to deform and they follow the contours of the fat droplet more easily. Consequently, they are less likely to protrude from the surface. The $\beta$-crystalline structure is a true solid, with the fatty acid molecules

arranged in ordered arrays. The β crystals exhibit a greater mechanical strength and are able to distort the shape of the fat droplets. This leads to their bursting out of the droplet surface into the aqueous phase (Euston, 1997; Coupland, 2002).

The **surface tension** is developed at the interface between crystal and oil, crystal and water, and oil and water. The surface tension determines the wettability of the crystals at the interface, *i.e.*, the location of the fat crystals towards the interface. If the fat crystals are completely wetted by the oil phase, they become fully dispersed in the oil phase and no effect on emulsion coalescence is observed (Rousseau, 2000).

## II.3. Influence of tensioactive molecules at the interface

Because the composition of the fat globule interface plays an important role in partial coalescence, the influence of surface-active molecules such as protein and low molecular weight emulsifier has received considerable research attention.

### *II.3.1. Influence of protein*

Protein may influence the partial coalescence mechanism at two levels: (i) protein amount involved in the coverage of the fat droplet and (ii) thickness of the protein layer at the interface. Some studies show that lower value of **protein surface load** is associated with higher degree of fat droplet coalescence (Bolliger, *et al.*, 2000a; Relkin, *et al.*, 2003b). Penetration of the droplet surface by fat crystals inside droplets becomes easier when less protein is present at the interface. Moreover, surfactants that form **thicker interfacial membranes** are more effective at preventing partial coalescence (Goff, 1997; Palanuwech and Coupland, 2003; Thanasukarn, *et al.*, 2004). Emulsions stabilized by caseins are less susceptible to partial coalescence than emulsions stabilized by whey protein. As already mentioned (§ A.I.1.1.), caseins are largely random coil proteins which form a thick interfacial layer (approximately 8 nm). This thick layer can prevent fat crystals from protruding from the droplet surface. In contrast, whey protein isolate forms a relatively thin interfacial layer (approximately 2 nm) that may favor partial coalescence. Even when whey protein isolate stabilized-emulsion is denatured, presumably leading to interfacial polymerization and increased interfacial visco-elasticity, the thin layer remains vulnerable to partial coalescence (Palanuwech and Coupland, 2003).

### *II.3.2. Influence of low molecular weight emulsifier*

Emulsifiers (such as mono- and diglycerides, polysorbates) play a critical role in the promotion of partial coalescence in partially crystalline emulsions. It is suggested that the low molecular weight emulsifier changes the physical properties and behavior of the fat globule in ice cream due to protein desorption and the impact on fat crystallization.

We have already mentioned (§ A.I.2.2.) that, upon cooling, emulsifier displaces part of the protein from the surface of the homogenized fat globules. With **protein displacement**, the membrane becomes thinner because protein molecules, particularly caseins, are considerably larger than the emulsifier. Thus, emulsifiers constitute a thinner oil-water interfacial layer that can be easily penetrated by fat crystals (Barfod, *et al.*, 1991; Abd El-Rahman, *et al.*, 1997; Davies, *et al.*, 2001; Palanuwech and Coupland, 2003; Thanasukarn, *et al.*, 2004). In addition, emulsifiers, by way of their surface activity, alter the surface tension, and this may result in the crystals being able to penetrate further into the aqueous phase. Finally, mixed interfacial films exhibit reduced visco-elastic properties compared to a pure protein film. This would make it easier for the triglyceride crystals within the droplets to penetrate the oil-water interface and to connect to other droplets (Davies, *et al.*, 2000). The direct relationship between protein, at the fat globule surface, resulting from displacement by emulsifiers, and partial coalescence depends on the nature of the protein and emulsifier. For example, in agreement with unsaturated mono-diglycerides being more effective in displacing protein from the fat globule interface than saturated mono- diglycerides, the addition of unsaturated mono- diglycerides induced greater partial coalescence than the saturated ones (Barfod, *et al.*, 1991; Pelan, *et al.*, 1997; Barfod, 2001).

An emulsifier may promote partial coalescence by contributing to **fat crystallization** but few studies demonstrate the direct correlation between partial coalescence and fat crystallization (Krog and Larsson, 1992; Davies, *et al.*, 2000). The emulsifier could play a role at two levels; (i) by its adsorption onto the triglyceride crystals. Consequently, the mechanical strength of the crystal network may be affected, bringing crystals close to the interface and so increasing the likelihood of penetration of the droplet surface by crystals (Davies, *et al.*, 2000); and (ii) by increasing the amount of triglyceride crystals formed so that they are directed towards the oil-water interface which is a favorable condition for partial coalescence to occur (Davies, *et al.*, 2000). Indeed, crystallization is promoted by the presence of the emulsifier at the fat globule

interface as revealed by an increase in solid fat content in a stabilized emulsified system (Krog and Larsson, 1992). Recent studies clearly show that some emulsifiers play the role of a catalytic template for the heterogeneous nucleation of the oil when the hydrophobic tails of the emulsifier solidify early on cooling. This was actually observed in the case of the addition of diacylglycerols with behenic, stearic and palmitic acid moieties (Awad, *et al.*, 2001). These emulsifiers modify the crystallization behavior of n-hexadecane in oil-in-water emulsions. In particular, the nucleation process is accelerated by the emulsifier concentration. Similarly, sucrose oligoesters having palmitic and stearic moieties accelerate the rate and extent of the crystallization of palm kernel oil in an emulsion system but retarded the crystal growth of palm kernel oil (Awad and Sato, 2002). These results were generalized to polyglycerol fatty acid esters used in emulsified systems based on various vegetable fats (Sakamoto, *et al.*, 2004).

## II.4. Influence of the process parameters

A very important feature of partial coalescence is the role of **shear** (Goff, 1997). The velocity gradient in liquid emulsions containing partially crystallized fat globules increased the rate of partial coalescence by a factor $10^6$ (van Boekel and Walstra, 1981a; van Boekel and Walstra, 1981b). The shear rate mainly influences the initial coalescence efficiency. A minimum shear rate is necessary to obtain a film thickness that is smaller than the protrusion distance of a crystal into the aqueous phase (Boode and Walstra, 1993). Shear leads to greater tendency towards partial coalescence by increasing the rate of collisions between droplets (Palanuwech and Coupland, 2003) and by removing mixed films from the fat surface since they have a looser adherence to the surface than a pure protein film (Krog, 1998). Shear-induced destabilization occurs at a certain critical shear stress, dependent on the emulsifier concentration and the concentration of triglyceride crystals (Davies, *et al.*, 2000). The **homogenization** pressure and selective homogenization processes also affect fat agglomeration (Koxholt, *et al.*, 2001). Indeed, the partial coalescence phenomenon and the extent of fat globule instability is influenced by the droplet diameter (Boode and Walstra, 1993; Thomsen and Holstborg, 1998). The coalescence efficiency increases with the globule size.

In the case of ice cream manufacture, the sequential process of partial coalescence during freezing is well investigated (Goff and Jordan, 1989; Goff, *et al.*, 1999; Bolliger, *et al.*, 2000b; Koxholt, *et al.*, 2001; Relkin, *et al.*, 2003b). The incorporation of air alone or the shearing action alone, independently of freezing, are not sufficient to cause the same degree of destabilization as

when the ice crystallization occurs concomitantly (Goff, *et al.*, 1995). The freezing process causes an increase in concentration of the mix components, such as proteins and mineral salts, in the unfrozen water phase. Besides their role in the penetration of fat membrane due to their physical shape already mentioned, ice crystals contribute to the shearing action on the fat globules through a freeze-concentration mechanism that also leads to enhanced destabilization (Thanasukarn, *et al.*, 2004). When emulsions freeze, the lipid droplets become progressively concentrated into the freeze-concentrate phase thereby coming into close contact with one another in the unfrozen aqueous channels between the ice crystals. The concentration of the lipid droplets in these narrow channels could promote aggregation, flocculation and/or coalescence during the freeze-thaw process (Thanasukarn, *et al.*, 2004). Moreover, low temperature extrusion process (-15°C), batch freezing and high back pressure continuous freezing lead to more fat destabilization in ice cream product in comparison with conventional continuous freezing (Goff, *et al.*, 1999; Bolliger, *et al.*, 2000b).

# III. AIR BUBBLE FORMATION AND STABILIZATION

## III.1. Surfactant adsorption at the air / water interface

**Proteins** are able to adsorb at the air / water interface. The mechanisms involved in this adsorption are very similar to those described in the case of water / oil interface (A.I.1.1.) including multiple anchoring sites and an unfolding process. Briefly, the adsorption can be described by a three-step mechanism: (i) the protein moves from the bulk phase to the sub-surface (a layer immediately adjacent to the interface) by diffusion and / or convection; (ii) the protein adsorbs and unfolds at the interface, and (iii) the previously adsorbed amino-acid segments slowly rearrange at the interface (Damodaran, 1997; Rodríguez Patino and Rodríguez Niño, 1999). Thus, the protein interfacial behaviour is dependent on the structure and rigidity of the molecule. All of the individual caseins, except κ-casein, show a strong tendency to adsorb the air / water interface (Carrera Sánchez and Rodríguez Patino, 2005). Since caseins are flexible and disordered macromolecules, their adsorption results in the formation of a film with a very low visco-elasticity (Rouimi, *et al.*, 2005). This contrasts with the high rigid film formed by the whey

proteins (especially β-lactoglobulin) that can be attributed to the high packing density and strong intermolecular interactions (Rouimi, *et al.*, 2005).

During the protein adsorption, the surface tension of air / fluid interfaces decreases. The surface tension measured for protein depends on numerous factors:

- **the protein concentration**. At low concentration, the protein spreads at the interface forming a monolayer. The protein may retain elements of the native structure, with incomplete unfolding at the interface. As protein concentration increases, part of the protein can be displaced towards the bulk phase and / or unfolding may occur. At a given surface concentration, the film properties change significantly. The protein collapses and amino-acid segments are extended into the aqueous solution (Rodríguez Patino and Rodríguez Niño, 1999; Carrera Sánchez and Rodríguez Patino, 2004);
- **the protein molecular size and structure**. Disordered, small and flexible proteins like β-casein reduce the surface tension earlier and faster than ordered, rigid and larger proteins (Bos, *et al.*, 2003);
- **the aqueous phase pH**. For example, the β-casein film structure was more condensed at pH 5. This behavior could be attributed to a reduction in the repulsive interactions between negative amino-acid residues at the isoelectric pH (Rodríguez Patino and Rodríguez Niño, 1999);
- **the temperature**. Depending on authors, the equilibrium surface pressure for β-casein is affected (Rodríguez Patino and Rodríguez Niño, 1999) or not (Carrera Sánchez, *et al.*, 2005) by temperature. For whey protein isolate, equilibrium surface pressure increases with temperature (Carrera Sánchez, *et al.*, 2005).

It is worth noting that, as already mentioned in the case of water / oil interface, protein competitive adsorption exists at the air / water interface. A competitive adsorption is shown between β-casein and $\alpha_{s1}$-casein (Carrera Sánchez and Rodríguez Patino, 2005). Moreover, in the case of a protein mixture, some phase separations may be observed in the mixed film due to protein incompatibility. This is the case for bovine serum albumin / β-casein / water ternary system at the air / water interface. The mixed monolayer film shows distinct phase-separated bovine serum albumin-rich regions and β-casein-rich regions coexisting with inhomogeneous mixed regions (Sengupta and Damodaran, 2000).

**Low molecular weight emulsifiers** adsorb strongly to air / water interface giving close molecular packing at the interface to produce low surface tensions. Mono- and diglycerides

spread on water can exist in different monolayer states, such as liquid-expanded, liquid-condensed, solid, or collapse phase. Only the gaseous phase is not observed (Rodríguez Patino and Rodríguez Niño, 1999). A decrease in temperature produces transitions towards more condensed structures or monolayer collapse (Rodríguez Patino and Rodríguez Niño, 1999). In contrast, some studies show that the equilibrium surface pressure of monoglycerides is not affected by temperature (Carrera Sánchez, *et al.*, 2005).

The interfacial characteristics of **mixed protein and low molecular weight emulsifier films** at air / water interfaces depend at least on the interfacial composition and on the protein / emulsifier ratio (Carrera Sánchez, *et al.*, 2005). For an equimolar ratio of whey protein isolate and monopalmitin, and at surface pressures lower than that for whey protein isolate collapse, protein and monoglyceride domains are present at the interface in the mixed film, but with few interactions between them. In the region near and after the protein collapse, it is suggested that monopalmitin is able to displace protein residues from the interface towards a sub-layer beneath the emulsifier monolayer. Near to the collapse of the mixed films, monopalmitin predominates at the interface and imposes its structural and topographical characteristics to the mixed film (Carrera Sánchez and Rodríguez Patino, 2004). Similar results are obtained for the whey protein isolate and monoolein system (Carrera Sánchez and Rodríguez Patino, 2004). These results suggest that whey protein isolate and monopalmitin or monoolein are practically immiscible with regions of emulsifier or protein alternating at the air / water interface, at the highest surface pressures (Carrera Sánchez and Rodríguez Patino, 2004). It is worth noting that this notion of immiscibility stresses the importance of the experimental conditions. Thus, differences in whey protein isolate displacement by monoglycerides (monopalmitin or monoolein) are observed in the absence or in the presence of shear (Carrera Sánchez and Rodríguez Patino, 2004). Shear may induce heterogeneity in the microstructure of the mixed monolayer and could favor the displacement of the protein by the monoglyceride (Carrera Sánchez and Rodríguez Patino, 2004).

Protein displacement by surfactant is heterogeneous and not a simple exchange mechanism (Leser and Michel, 1999). A mechanism describing the protein displacement induced by the emulsifier addition has been proposed (Gunning, *et al.*, 1999). The so called “orogenic” mechanism is divided into three stages:

(i) The emulsifier adsorbs and penetrates into the polar lipid – protein mixed film either within defects in the protein network or at the polar lipid locations. The emulsifier forms separate adsorbed domains. In the case of water-insoluble emulsifiers, a displacement front of emulsifier domains is produced. Further adsorption of emulsifier into the emulsifier domains reduces the

local surface tension. Due to emulsifier adsorption, the protein surface area decreases but the film thickness does not increase. The increased surface pressure difference between the surfactant and protein domains leads to compression of the protein network without displacement of the proteins from the surface, reflecting probably, a denser packing of the proteins within the network;

(ii) When the protein film can no longer be compressed, a buckling of the monolayer is observed. Reordering of the molecules occurs that may include a dissociation of at least some of the proteins from the surface but not from the film itself. In response to the decreasing surface coverage, the protein film gets thicker;

(iii) At sufficiently high surface pressures, the protein network begins to fail allowing individual molecules or small protein aggregates to desorb from the interface.

The final failure depends on the type of protein structure, the extent of unfolding upon protein adsorption, and the type and strength of intermolecular interactions at the surface, related to the miscibility, topography and molecular structure of the proteins and emulsifiers. The more elastic whey protein isolate film is more resistant to displacement than the less elastic β-casein films (Carrera Sánchez, *et al.*, 2005). Caseinate film spread at the air / water interface can be displaced by monoglyceride (monopalmitin, monoolein and monolaurin) (Carrera Sánchez, *et al.*, 2005). Monoolein has a lower capacity than monopalmitin for protein displacement (Carrera Sánchez, *et al.*, 2005).

## III.2. Foam formation and stabilization

We have previously described the process of surfactant adsorption to the air / water interface on a flat surface. However, in food industry, the air interface is created by a whipping process. This dynamic process introduces new variables in the constitution of the interface. Moreover, in the case of ice cream process, a freezing step also modifies the air / water interface characteristics.

### *III.2.1. Foamed systems*

In a **protein** solution, foam formation occurs when air is mechanically incorporated. Air bubbles are thought to be first surrounded by a proteinaceous membrane similar to that discussed in an emulsion system (Brooker, 1993; Goff, 1997; Goff, *et al.*, 1999). The major

thermodynamic driving force of protein to adsorb and concentrate at fluid / fluid interfaces is relative to the removal of non-polar amino acid sequences from the unfavorable environment of the bulk aqueous solution. Moreover, protein plays an important role in the stabilization of air bubbles since during the formation of the foam system, the adsorbed protein prevents the recoalescence of the initially formed bubbles. This is achieved by reinforcing the repulsive forces between the air cells, forming a steric barrier and increasing viscosity in the aqueous solutions in the lamella (Wong, 1989). During the protein adsorption, the surface tension of the interface decreases. This allows the optimization of the input energy involved in the foaming process (Thakur, *et al.*, 2005) and, finally, small bubble sizes are obtained which is an important factor for the stability of the dispersed system.

The tendency of proteins to adsorb onto the fluid interfaces determines the quality of their good foaming properties. More precisely, the foamability of a protein solution is determined by the rate at which the protein can reduce the interfacial tension as new interfacial area is being continuously created during whipping. Close relationships exist between foaming and the rate of diffusion of caseinate to the air / water interface (Carrera Sánchez and Rodríguez Patino, 2005). The rigidity of the protein is an important factor because protein with high molecular weight and rigid structure does not adsorb and unfold sufficiently fast enough at the interface to produce much foam (Damodaran, 1997). All caseins (β-casein, $\alpha_{s1}$-casein, κ-casein and $\alpha_{s2}$-casein) exhibit foaming properties (Zhang and Goff, 2004; Carrera Sánchez and Rodríguez Patino, 2005). Adsorption of β-casein into foam phases occurs if partial dissociation of the casein micelles is achieved (Zhang and Goff, 2004). β-casein is considered to be the most surface-active protein of the caseins, due to its high mean residue hydrophobicity and its unordered structure. Studies on foaming properties of β-casein, bovine serum albumin, and lysozyme showed that relative foamability of these proteins followed the order β-casein > bovine serum albumin > lysozyme, which is the same order in which they undergo conformational change and affect the rate of decay of surface tension in model systems (Damodaran, 1997). In foams made of incorporating caseinate, the individual caseins seem to be adsorbed at fluid interfaces in proportion to their incorporation in solution. However, a distinction exists between caseinate and mixtures of purified individual caseins, since the latter show competitive adsorption between β-casein and $\alpha_{s1}$-casein (Carrera Sánchez and Rodríguez Patino, 2005). Competitive milk protein adsorption to air / water interfaces in aqueous foam is also demonstrated when foams are made by whipping protein solutions containing skim milk powder and whey protein isolate (Zhang and Goff, 2004).

The lower enrichment ratio of whey proteins in the foam phase can be explained by their rigid structures and relative low surface hydrophobicity (Zhang and Goff, 2004).

**Low molecular weight emulsifier** is able to stabilize dispersed air bubbles. However, in contrast to protein that stabilizes bubbles by forming a rigid layer of interlinked molecules at the interface, emulsifier forms a densely packed but much less rigid monomolecular layer, which is stabilized by dynamic processes (*i.e.* Gibbs-Marangoni effect), whereby if local thinning occurs, emulsifier rapidly diffuses into the depleted area, sweeping liquid in with it, to re-thicken the thinned region. Foam formed in presence of small molecule surfactants is well known for its instability due to the lack of the desired rheological properties compared to that of protein foam.

When **proteins and emulsifiers** are **mixed**, they may lead to an antagonistic effect on foam formation due to the "orogenic" mechanism already discussed for the flat air / water interface. Systems of protein and emulsifier can be unstable, as the surfactant dilutes the proteins and prevents their interlinking, while the proteins interfere with the rapid surface diffusion of the emulsifier (Bos, *et al.*, 1997). As a consequence, collapse of protein foam in the presence of emulsifier may result from the interrupted interaction between proteins and the consequent loss of desired rheological properties (Rodríguez Patino and Rodríguez Niño, 1999; Zhang and Goff, 2004).

### *III.2.2. Foamed and frozen systems*

Some food products, like ice cream, are aerated and frozen. On an industrial scale, aeration, cooling and freezing are generally carried out simultaneously under steady state conditions in scraped surface heat exchangers with high dasher rotational speed and attached surface-scraping knives. Such exchangers are usually called freezers. As freezing begins, a reduction in maximum air cell size is observed due to the increased shear stress applied to disrupt the air cells (Chang and Hartel, 2002a).

In ice cream, the gas phase volume is generally found around 50 % (Walstra and Jonkman, 1998) but can be as low as to 10–15 % (Goff, 2002). During freezing, several processes occur simultaneously. Air bubbles are beaten in and broken up into smaller ones. Coalescence of bubbles also occurs until an approximately steady state is reached (Walstra and Jonkman, 1998). As a result, air is distributed in the form of numerous small air bubbles. In conventional frozen ice cream, the air bubble size often ranges from 20 to 50 μm (Turan, *et al.*, 1999; Bolliger, *et al.*, 2000b; Goff, 2002; Sofjan and Hartel, 2004) but air cell size and

distribution are dependent on process parameters: low-temperature extruder (Bolliger, *et al.*, 2000b; Thakur, *et al.*, 2005), level of emulsification (Bolliger, *et al.*, 2000b), temperature (Chang and Hartel, 2002a; Caillet, *et al.*, 2003), overrun (Chang and Hartel, 2002a; Sofjan and Hartel, 2004). The smallest air bubble size distributions could result from longer residence times in the scraped surface freezer (Goff, 2002). Minimization of air cell size distributions have also been claimed to be a benefit at low temperature extrusion (Bolliger, *et al.*, 2000b; Thakur, *et al.*, 2005).

Aeration and freezing involve numerous physical changes in the structure and the texture of frozen dairy foams that determine the formation of a stable foamed product: the adsorption of proteins on the gas interface; protein desorption from fat globule surface by low molecular weight emulsifiers; partial coalescence of fat droplets; ice crystallization (Goff, 1997). During the aeration and freezing process, **protein** acts as foaming agent in forming and stabilizing the foam phase (Walstra and Jonkman, 1998; Goff, *et al.*, 1999; Goff, 2002; Zhang and Goff, 2005). **Low molecular weight emulsifiers** are added to achieve different purposes: (i) they can break down and displace the adsorbed layer of protein at the air interface (Carrera Sánchez, *et al.*, 2005; Zhang and Goff, 2005), (ii) they allow the adsorption of fat to the surface of the air bubble (Buchheim, 1991; Pelan, *et al.*, 1997; Thomsen and Holstborg, 1998; Goff, *et al.*, 1999; Zhang and Goff, 2004; Carrera Sánchez, *et al.*, 2005; Zhang and Goff, 2005); (iii) they increase the stability of the air bubbles (Turan, *et al.*, 1999; Barfod, 2001; Zhang and Goff, 2005).

The **effects of formulation** (protein, fat, emulsifier types) on development and stability of air cells have been widely investigated (Pelan, *et al.*, 1997; Chang and Hartel, 2002c; Zhang and Goff, 2004; Zhang and Goff, 2005). The different roles of the low molecular weight emulsifier in protein desorption, fat partial coalescence and foam stabilization is well pointed out in ice creams prepared without emulsifiers. These ice creams are characterized by low concentration of fat droplets at the air cell interface (Buchheim, 1991; Goff, *et al.*, 1999) and by the presence of large air cells, some of which have coalesced (Pelan, *et al.*, 1997). The coalescence indicates poor air stability, which is probably related to the lack of fat droplets at the air interface. Saturated monoglyceride reduces the air cell size more than unsaturated monoglyceride. A possible explanation is that unsaturated monoglyceride causes too much fat globule destabilization and therefore ultimately air cell coalescence, whilst saturated monoglyceride promotes adsorption of fat droplets at the air cell interface but does not lead to extensive fat aggregation (Pelan, *et al.*, 1997). However, another study that underlined the impact of the emulsifier type on bubble size (Thakur, *et al.*, 2005), showed that, for similar protein and fat types, unsaturated mono-diglycerides always permit the development of smaller bubbles compared to saturated

emulsifiers. In a recent study on ice cream (Zhang and Goff, 2005), the compositions of the air interface were quantified for different protein mixtures and emulsifiers used in the formulation. When whey protein is used as the only source of protein, fat globules do not adsorb to the air interface of ice cream to the extent that they do when skim milk powder is the protein source. When ice cream is made from skim milk powder and glycerol monostearate, casein micelles, $\beta$-casein and $\beta$-lactoglobulin are all detected at the air interface. Glycerol monooleate strongly displaces caseins from the fat interface, introduces more partially coalesced fat adsorbed to the air interface and seems to displace more proteins from the air interface (Zhang and Goff, 2005). Some studies show that the fat level and emulsifier content have no effect on the air cell (Chang and Hartel, 2002c).

The foam structure is also stabilized by **partial coalescence of the fat droplets** (Walstra, 1987; Brooker, 1993; Goff, 1997; Euston, 1997; Thomsen and Holstborg, 1998; Goff, *et al.*, 1999; Leser and Michel, 1999; Koxholt, *et al.*, 2001; Goff, 2002; Chang and Hartel, 2002b; Chang and Hartel, 2002c). Increased levels of fat destabilization are associated with an increased concentration of discrete fat globules at the air interface and increased coalescence and clustering of fat globules both at the air interface and within the serum phase (Goff, *et al.*, 1999). Partial coalescence of the fat emulsion causes both adsorption of fat at the air interface and formation of fat globule clusters that stabilize the lamellae between air bubbles. When the fat globules and agglomerates reach sizes in the range of the width of the foam lamellae, they block them and impede the drainage of the serum. Additionally, they form loose bridges between air bubbles and prop them up against each other which leads to a mechanically stable, mousse-like foam matrix (Koxholt, *et al.*, 2001). Air bubbles in stable ice creams are not only stabilized by partially destabilized fat agglomerates. There are also areas of the air bubbles that are covered with small and intact fat globules (Walstra and Jonkman, 1998) and other areas where no fat can be found. The fat-free regions must be stabilized by proteins and emulsifiers (Koxholt, *et al.*, 2001). Moreover, the coalescing fat interacts with the proteins at the air bubble membrane (Goff, 1997; Koxholt, *et al.*, 2001). There does not seem to be significant fat spreading or continuous fat layers at the air interface, even with the highest levels of fat destabilization (Goff, *et al.*, 1999).

### III.3. Foam evolution

Aqueous foams are thermodynamically unstable and can be subjected to three main physical mechanisms of destabilization: disproportionation (Ostwald ripening), coalescence and

drainage (Dickinson, 1996a; Rodríguez Patino, *et al.*, 1997; Chang and Hartel, 2002c). These mechanisms are to some extent interdependent.

**Disproportionation** occurs due to differences in Laplace pressure between two air bubbles of different sizes, *i.e.*, different curvatures of the surface. The Laplace pressure difference induces a concentration gradient in the liquid layer surrounding the bubbles, which causes different concentrations of gas between the smaller to the larger bubble. Consequently, smaller bubbles disappear at the expense of large bubbles, and the foam becomes coarser with time. In ice cream, disproportionation results in a bimodal distribution with both small and large air cells being present. In the early stages of disproportionation, a net decrease in mean size may be observed. As more air cells disappear, however, a gradual increase in the mean size is observed over time.

**Coalescence** of air cells in close proximity leads to a loss of two small bubbles and the formation of a single, larger bubble. Thus, foam that undergoes coalescence also becomes coarser over time. This instability leads to formation of a bridge between two bubbles, which eventually leads to the two bubbles growing together into a single larger bubble. Coalescence may be promoted by the presence of hydrophobic solid particles (fat crystals for example) that connect two air bubbles at an obtuse angle, causing the film to break.

**Drainage** is the liquid flow from the foam as a result of gravity and capillary forces, at the same time that buoyancy forces cause a rise in the air cell. The larger the air cell, the faster it rises. Drainage by itself does not change the air cell distribution; rather it changes the film thickness between the air cells and promotes coalescence. The drainage forces are governed by the interfacial tension of the air cell, the viscosity of the fluid matrix and the height of the foam system.

In ice cream products, the three different instability processes may take place either during ice cream production, *i.e.*, freezing and hardening, or during storage of the final product. It is worth noting that when coalescence occurs during storage, the loss of discrete nature of the gas bubbles and channeling may occur, leading to a continuous network of coalesced bubbles. This results in a product collapse or shrinkage. A better understanding of the stability of the air phase has been obtained by studies based on examining the response of frozen ice cream to fluctuation pressure (Turan, *et al.*, 1998; Turan, *et al.*, 1999) or temperature (Chang and Hartel, 2002c; Sofjan and Hartel, 2004). During short periods of storage, the mechanisms that increase the air cell size distribution are dependent on the storage temperature. Disproportionation and

coalescence occur at the same time when temperatures are below −10°C. However, only coalescence occurred at −20°C (Sofjan and Hartel, 2004). For extended storage time, the main mechanism of size increase is coalescence, as significant interconnection and channeling between air cells are observed (Turan, *et al.*, 1998; Turan, *et al.*, 1999; Chang and Hartel, 2002c; Sofjan and Hartel, 2004).

Whether these different instability processes take place, and at what rate, depends on the physical properties of both the continuous liquid phase and the gas phase. It is possible to increase foam stabilization: (i) by decreasing air bubble size (increase in surfactant surface coverage) to limit disproportionation; (ii) by forming a thick film on the surface of the air cells via adsorption of solid particles to gas cells. This would reduce drainage and disproportionation. The internal semi-continuous fat network created in the frozen product by entrapping air within the coalesced fat, offers resistance to collapse during meltdown (Goff, 1997); (iii) by increasing the viscosity of the serum phase (decrease of the diffusion rate of gas between bubbles, inhibition of the mobility of air cells and decrease of the ripening due to drainage) using stabilizer (Stanley, *et al.*, 1996; Leser and Michel, 1999; Bolliger, *et al.*, 2000b; Chang and Hartel, 2002a; Chang and Hartel, 2002b; Chang and Hartel, 2002c); (iv) by decreasing temperature; (v) by increasing the density of ice crystals. Generation and growth of ice crystals serve to stabilize air cells (Sofjan and Hartel, 2004); (vi) by decreasing the storage temperature (product hardening); (vii) by acting on the formulation. For example, disproportionation of air cells is inhibited by addition of emulsifiers (Chang and Hartel, 2002c).

**In conclusion**, the creation and the setting of the fat network and foam structure in ice cream result from different interconnected processes due to a combination of air, applied shear stress, freeze concentration of the premix and ice formation. These processes include emulsifier adsorption at the oil / water or air / water interfaces, protein desorption from the different interfaces, partial coalescence of fat globules, significant concentration of discrete fat globules and clusters at the air interface, clustered fat extending away from the air interface into the serum phase, cluster fat and ice crystals in the serum phase independent of the air interface.

The different networks determine the properties of ice creams such as the shelf-life and textural and organoleptic characteristics. In particular, the melting resistance of ice cream is related to the breakdown of the foam structure in the product (Pelan, *et al.*, 1997; Sofjan and Hartel, 2004) and to the amount of partially coalesced fat product (Pelan, *et al.*, 1997; Sofjan and Hartel, 2004; Zhang and Goff, 2005). Moreover, the creation of a matrix of fat throughout the product results in the beneficial properties of a smooth-eating texture in the frozen dessert (Goff,

1997; Thomsen and Holstborg, 1998). The amount of air incorporated and the distribution of sizes of the air cells in ice cream provides a smooth texture (Thomsen and Holstborg, 1998; Goff, 2002). The cooling sensation perceived by the consumers when eating ice creams is largely conditioned by the distribution, the size and the morphology of the ice crystals. In particular, organoleptic evaluation of ice cream has shown that small air cells and ice crystals are associated with increased creaminess and reduced iciness (Turan, *et al.*, 1999).

# IV. SCIENTIFIC GOAL

The bibliography section clearly demonstrates that the formation of ice cream structure results from complex physicochemical phenomena during the different stages of ice cream manufacture, from the oil-in-water formation, to the maturation of the mix and, then, to the freezing and whipping steps. Although the impact of the nature of the ingredients used in ice cream formulation is well established, comparison between the different studies of the literature is often difficult. Indeed, variations in purity, sources, and proportions of the different ingredients, added to different experimental set-ups and/or conditions often prevent the accurate comparison of the results. Moreover, most studies focus on the role of the proteins and low molecular weight emulsifiers on the ice cream structure formation and textural resulting properties. Only few works deal with the influence of the fat sources used in the preparation of ice cream emulsion, although numerous studies agree with the effects of fat destabilization on ice cream quality.

The present work was based on the characterization of the behavior of surface-active molecules which take part in the formation and the stabilization of the oil /water interface in ice cream. The aim of this study was to understand and control the mechanisms of action of the emulsifiers during the different steps of ice cream manufacture. Indeed, even if it is clearly established that emulsifiers are of technological importance in ice cream structure, a better comprehension of the interactions between the different ingredients present in the formulation will facilitate the design of ice creams with required characteristics. Thus, we focused on two mono- and diglyceride mixtures usually used in ice cream application and comprising different unsaturation degree. Because the low molecular weight emulsifiers are, in general, combined with other stabilizing molecules such as proteins, two protein sources were also investigated. The

protein mixtures differed by the whey proteins / caseins ratio and by their interfacial properties. We hypothesized that this ingredient selection would allow the development of different emulsifier - protein interactions. Although commercial ice cream includes hydrocolloids of various sources, in this study, the impact of hydrocolloids was minimized by using a constant blend of guar gum - locust bean gum (50/50 v/v) known to poorly interact with proteins. Besides the selection of the surfactant molecules, four sources of fat were chosen to highlight the emulsifier - fat and protein - fat interactions. These fats varied in the composition in fatty acids, *i.e.*, unsaturation degree and chain length. The study took into account the various steps in ice cream process and supported the whole ice cream structure. Thus, three systems were studied, *i.e.*, oil-in-water emulsion, ice cream mix and ice cream, to investigate the behavior of surface-active molecules (protein and emulsifier), and their interactions, between each other and with fat.

# B. MATERIALS AND METHODS

# I. EMULSION AND ICE CREAM PREPARATION

## I.1. Products

Different fats, originated from vegetable sources, *i.e.*, hydrogenated and refined coconut oils (SIO), refined palm oil (SIO), high oleic sunflower oil (Lesieur) and animal source *i.e.*, anhydrous milk fat (France Beurre) were used. Physicochemical characteristics of the fats are presented in Table 7. Milk solids-non-fat was either skim milk powder (SMP) provided by Coopérative d'Isigny Sainte-Mère or functional dairy protein (FDP) (Bel Industries). The functionalization consisted in a partial protein denaturation by a heat treatment performed by the supplier. The chemical composition of the two protein mixtures is presented in Table 8. Emulsifiers, *i.e.*, saturated and partially unsaturated MDG (60% alpha monoglycerides) were given by Degussa Food Ingredients. Physicochemical characteristics of the low molecular weight emulsifiers are presented in Table 9. Sucrose and corn syrup solids (40 DE, 80 Brix solid content) were purchased from Eurosucre and Cerestar, respectively. Stabilizers (guar gum and locust bean gum) were donated by Degussa Food Ingredients.

## I.2. Emulsion preparation

The emulsions were prepared using a two-step homogenizer (SA 1200 AB, Manton Gaulin) operating at a pressure of 17.5 MPa in the first stage, 3.0 MPa in the second stage, at 90°C. Initially, two premixes, one containing the fat and the emulsifier, the other the aqueous protein mixture solution, were prepared and preheated separately at 70°C. Immediately, they were mixed, brought under continuous stirring to 90°C and maintained at this temperature for 5 s. After homogenization, the emulsions were first cooled down to 20°C then to 4°C and aged for 24 h at 4°C whilst stirring.

Each oil-in-water emulsion was based on one type of fat, one type of emulsifier and one type of protein mixture chosen among the various ingredients presented in Table 10. The proportions used are also indicated in Table 10. It is worth noting that similar proportions of protein mixture (SMP or FDP) were used although these mixtures did not contain the same

**Table 7: Physicochemical characteristics of the fats used.**

| | Hydrogenated coconut oil | Refined coconut oil | Refined palm oil | Anhydrous milk fat | Sunflower oil |
|---|---|---|---|---|---|
| Solid fat content (%) | | | | | |
| 5°C | 91.6[a] | 86.4[a] | 54.5[a] | 71[b] | |
| 10°C | 89.1 | 78.8 | 52.6 | 60 | |
| 15°C | 78.0 | 64.5 | 45.9 | 40 | |
| 20°C | 51.3 | 36.3 | 30.6 | 13 | |
| Wt.% of total fatty acids[c] | | | | | |
| 4:0 | - | - | - | 2 | - |
| 6:0 | 6 | - | - | 2 | - |
| 8:0 | 6 | 6 | 0 | 1 | 0 |
| 10:0 | 45 | 6 | 0 | 3 | 0 |
| 12:0 | 19 | 45 | 0 | 3 | 0 |
| 14:0 | 10 | 19 | 1 | 11 | 0 |
| 16:0 | 13 | 11 | 44 | 32 | 5 |
| 18:0 | 0 | 3 | 5 | 12 | 4 |
| 18:1 | 0 | 8 | 38 | 22 | 43 |
| 18:2 | | 0 | 10 | 2 | 46 |

[a] Determined by pulsed nuclear magnetic resonance of proton (§ B.IV.); [b] Data given by France Beurre; [c] Determined by gas chromatography (§ B.II.); -: not determined.

**Table 8: Chemical characteristics of the two protein mixtures used.**

| Composition (wt.%) | SMP | FDP |
|---|---|---|
| Proteins | 35[a] | 20[a] |
| *Caseins* | *28*[a] | *8*[a] |
| *Whey proteins* | *7*[a] | *12*[a] |
| Moisture | 4.0[b] | 3.5[c] |
| Fat | 1.5[b] | 1.5[c] |
| Lactose | 52.0[b] | 62.0[c] |
| Ashes | 8.1[b] | 8.5[c] |
| Calcium | 1.30[b] | 1.01[c] |
| Potassium | 1.55[b] | 2.31[c] |
| Sodium | 0.60[b] | 0.56[c] |
| Phosphorus | 1.05[b] | 0.79[c] |
| Chlorides | 1.10[b] | 1.47[c] |

[a] Determined by denaturing polyacrylamide gel electrophoresis and densitometry; [b] Data given by Coopérative d'Isigny Sainte-Mère.; [c] Data given by Bel Industries.

**Table 9: Physicochemical characteristics of the mono- and diglyceride mixtures (MDG).**

| | Saturated MDG | Partially unsaturated MDG |
|---|---|---|
| Solid fat content (%)[a] | | |
| 15°C | 96.0 | 91.9 |
| 25°C | 95.7 | 88.0 |
| 35°C | 95.5 | 75.9 |
| 45°C | 94.1 | 43.9 |
| 55°C | 72.9 | 8.2 |
| Wt% of total fatty acids (%)[b] | | |
| 16:0 | 56 | 49 |
| 18:0 | 42 | 26 |
| 18:1 | 0 | 20 |
| 18:2 | 0 | 3 |

[a] Determined by pulsed nuclear magnetic resonance of proton (§ B.IV.); [b] Determined by gas chromatography (§ B.II.).

**Table 10: Compounds and proportions used to formulate the different oil-in-water emulsions and ice cream mixes.**

| System | | Compound | Proportion (wt.%)[a] | Type |
|---|---|---|---|---|
| **Ice cream mix** | **Oil-in-water emulsion** | Fat * | 8 | Hydrogenated coconut oil<br>Refined coconut oil<br>Refined palm oil<br>Anhydrous milk fat |
| | | Emulsifier* | 0.3 | Saturated MDG<br>Partially unsaturated MDG |
| | | Protein mixture* | 10 | SMP<br>FDP |
| | | Sugar | 12 | Sucrose |
| | | | 6 | Corn syrup solids |
| | | Hydrocolloids | 0.2 | Guar gum, locust bean gum |

* Each oil-in-water or mix formulation contained only one type of fat, one type of emulsifier and one type of protein mixture; [a] Proportions are completed to 100% with water. MDG: mono- and diglyceride mixture; SMP: Skim milk powder; FDP: Functional dairy protein.

protein amount (Table 8). It was not possible to hold the amount of proteins constant because in the case of mixes based on FDP, they exhibited high viscosity that made them difficult to handle.

### I.3. Ice cream preparation

The different ice cream mixes were prepared based on compounds and proportions presented in Table 10. The manufacturing process of the ice cream involved the steps described in Figure 3. The fat, the corn syrup solids and a fraction of water were introduced into a tank with a double layer (20 N0, Guédu), whilst stirring, at 40°C for 10 min. The protein mixture, sucrose, hydrocolloids and emulsifiers were dry blended, mixed with water separately for 10 min and immediately blended at 65°C with the melted fat phase, for 15 min (pre-heating step). Homogenization was performed with a double piston, two stages homogenizer (1st stage: 17.5 MPa, 2nd stage: 3.0 MPa) (Lab 60, APV France) on batches of ice cream mix of 20 L. The mix was pasteurized (APV Junior, APV France) (85°C, 30 s), and immediately cooled at 4°C. Ageing took place at 4°C for 16 h, whilst slow stirring in a water bath. A sample of the mix was taken at 4°C before and after the ageing period for mix analysis. At the end of the ageing period, the vanilla flavor (LAB 15359, Degussa Food Ingredients) was dispersed into the mix. The freezing step was carried out using a continuous freezer (WCB ice cream CS 100, Waukesha Cherry Burrell). Before introducing the mix into the freezer, the flushing of the apparatus with sweetened solution (24 wt.% sucrose, 6 wt.% dextrose, 0.7 wt.% hydrocolloids adjusted to 100 wt.% with water) made it possible to reach the operating conditions of temperature, air pressure and number of revolutions of the blades. The air (nitrogen, 20 mPa) was injected into the mix at the entrance of the freezer. The temperature of the evaporating gas in the freezer double layer was about -40°C. The outlet temperature was close to -5°C. The final product expansion was expressed by the ratio:

$$100 \times \left( \frac{mass\ of\ \ a\ volume\ of\ \ mix - mass\ of\ \ the\ same\ volume\ of\ \ ice\ cream}{mass\ of\ \ the\ same\ volume\ of\ \ ice\ cream} \right)$$

The overrun was pre-fixed at 100% m/m. At the exit of the freezer, the semi-solid product was packaged. Hardening was carried out by a hardening tunnel (Fouchard) with cold air circulation (-40°C, 30 min). Ice cream products were stored in a freezer at -20°C before analysis.

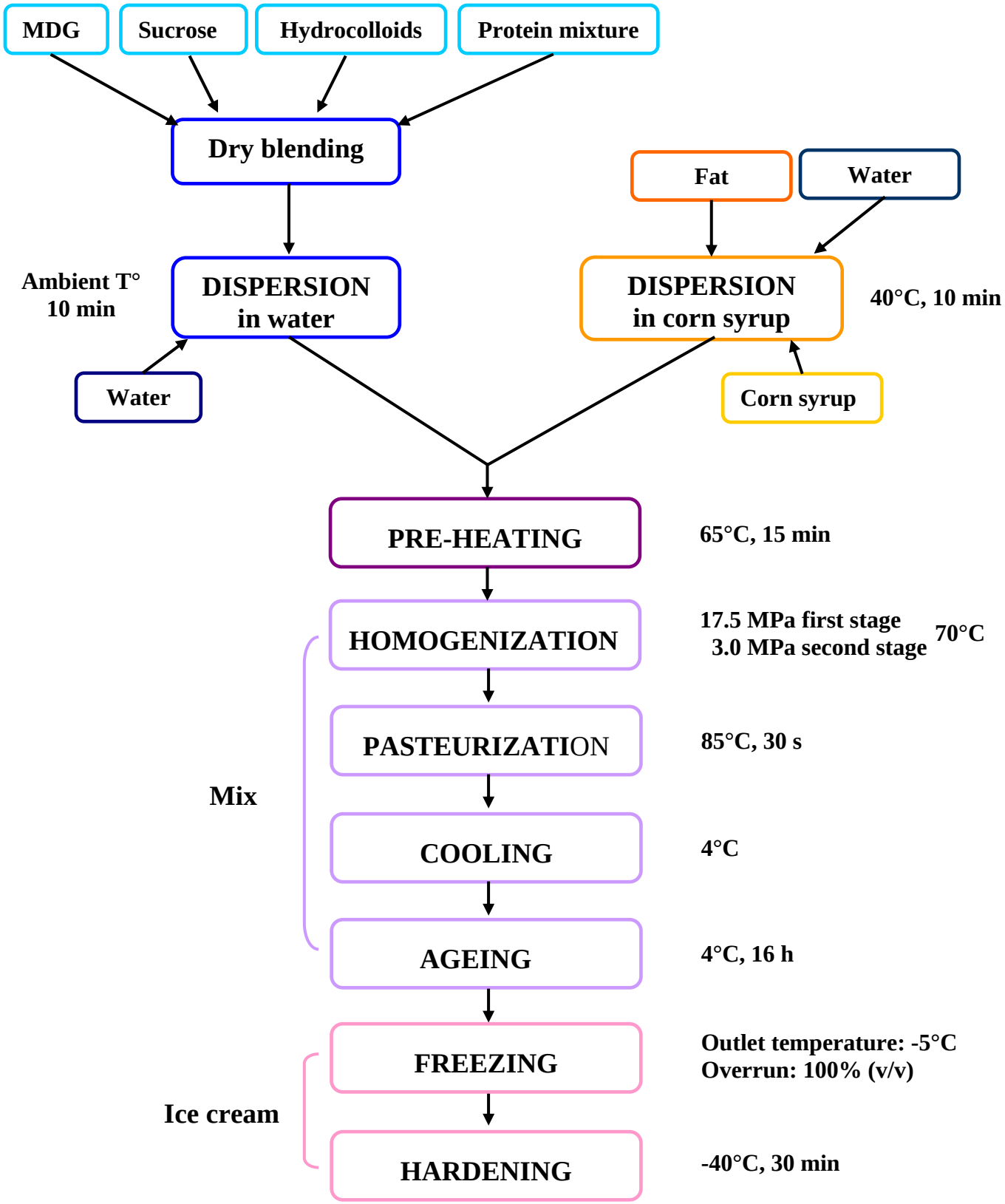


**Figure 3: Diagram of ice cream fabrication**

# II. LIPID ANALYSIS

The Vigneron technique was used to prepare fatty acid methyl esters (FAME) from **oils and fats** (Vigneron, *et al.*, 1973). Forty mg of lipids were mixed with 3 ml of hexane and 0.2 ml of a methanolic sodium hydroxide solution (2N). The mixture obtained was vigorously mixed for 10 s, then heated in a water bath at 50°C for 30 s. This step (agitation/heating) was repeated twice. Then, 0.2 ml of hydrochloric methanol (2N) was added to acidify the solution. The hexane phase was taken after centrifugation and washed with 1 ml of distilled water. The FAME in hexane solution was analyzed directly by gas chromatography (GC).

The Morrison technique (Morrisson and Smith, 1964) was used for **emulsifier** fatty acid analysis. It is worth noting that before FAME preparation, MDG was purified according to the Folch method (Folch, *et al.*, 1957) using a chloroform/methanol mixture (2/1; v/v) at a rate of 20 volumes per volume of material. After the two aqueous washings of the lipid phase, several additional washings with methanol were performed to limit crystallization of the emulsifiers. The preparation of FAME from the MDG mixture was carried out at 90°C in the presence of a mixture (1.5 ml) composed in equal parts (0.5 ml) of hexane, methanol and a solution of 10 % boron trifluoride in methanol. The trans-methylation required 60 min. The FAME was extracted, after addition of 1 ml of distilled water, by 2 ml of hexane, repeated three times. The connected hexane phases were concentrated and after washing with 1 ml of distilled water were stored at -20°C before GC analysis.

The FAME composition was established by GC under the following conditions: chromatograph Hewlett Packard (HP 5890) equipped with a capillary column BPX (length = 60 m, internal diameter = 0.25 mm). The temperatures of the injector and the detector were both equal to 250°C; the pressure of the carrier gas (hydrogen) was 0.1 MPa. The analysis was performed in "Split" injection mode. The temperature programs used for the different fats and oils are presented in Table 11. The chromatograms were obtained and analyzed using an integrator (Hewlett Packard). The identification of FAME was obtained by a comparison of the retention times with those of standards (Sigma) analyzed using the same chromatographic conditions.

# III. INTERFACIAL TENSION MEASUREMENTS

The interfacial tension at the oil/water interface was measured by the weighed drop method (Harkins and Brown, 1919). When the volume of the growing drop exceeds its maximum possible value (*i.e.* if the drop weight exceeds the forces acting vertically upward on the drop), the drop (volume *V* and mass *m*) falls from the needle tip. The forces are found at the level of the triple contact between the needle tip and the two fluids (water and oil), due to the interfacial tension $\gamma$. The assessment of the forces is given by:

$$2\pi r_{\text{int}}\gamma + mg\left(\frac{\varphi_{\text{eau}}}{\varphi_{huile}}\right) = mg$$

where *g* is the gravity acceleration, $r_{int}$ the internal radius of the needle and $\rho$ the volume mass. This leads to the expression of the interfacial tension $\gamma$:

$$\gamma = \frac{mg(\varphi_{\text{eau}} - \varphi_{huile})}{2\pi r_{\text{int}} \times \varphi_{\text{eau}}}$$

This equation is an approximation that does not take into account the lengthening of the drop under the gravity effect. To take this phenomenon into account, a corrective factor *f* is introduced. This factor *f* is dependent on the ratio $\frac{r_{\text{int}}}{V^{\frac{1}{3}}}$.

If $\frac{r_{\text{int}}}{V^{\frac{1}{3}}} < 0.4$, then $f = 1.017 - 1.334 \times \frac{r}{V^{\frac{1}{3}}} + 1.477 \times \left(\frac{r}{V^{\frac{1}{3}}}\right)^2 - 0.471 \times \left(\frac{r}{V^{\frac{1}{3}}}\right)^3$

If $\frac{r_{\text{int}}}{V^{\frac{1}{3}}} > 0.4$, then $f = 0.9 - 0.71 \times \frac{r}{V^{\frac{1}{3}}} + 0.4245 \times \left(\frac{r}{V^{\frac{1}{3}}}\right)^2$

The method proves to be accurate if the diameter of the needle and the conditions of damping are well defined. Moreover, an equilibration period is necessary to leave sufficient time for the emulsifier to adsorb at the interface.

The experimental setup used in this work consisted in a set of syringe / needle of known and constant diameter ($r$ = 0.406 mm), and a micro-metric screw allowing delivery of the syringe's content in a controlled way. Interfacial tension measurements were carried out in a two phase system: a dense phase (distilled water) and a less dense phase (oil). The emulsifier (saturated or partially unsaturated MDG) was incorporated at 0.3 wt.% in 10 g of oil phase as its hydrophilic-lipophilic balance (HLB) was low. The oil phase was either high oleic sunflower oil

**Table 11: Temperature programming of the gas chromatography analysis.**

| Lipid | Step | Initial temperature (°C) | Speed (°C/min) | Final temperature (°C) | Temperature holding time (min) |
|---|---|---|---|---|---|
| **Butter** | 1 | 120 | 1.3 | 200 | 0 |
| | 2 | | 2.5 | 230 | 6 |
| | 3 | | 20.0 | 245 | 15 |
| **Vegetable fats and MDG** | 1 | 150 | 1.3 | 200 | 0 |
| | 2 | | 1.5 | 230 | 6 |
| | 3 | | 20.0 | 245 | 10 |

or a mixture of high oleic sunflower oil and vegetable fat incorporated at a level of 20 wt.%. The protein mixture (SMP) was dissolved in water (10 wt.%). The different aqueous and lipid mixtures were heated for 15 min at 70°C in a water bath. Then, the temperature of the mixtures was brought back to the required temperature. The aqueous phase was placed into the syringe and the oil phase into a weighted tube. Three to five drops were weighed in order to obtain a significant result. The interfacial tension was measured at temperatures ranging from 45 to 4°C. The temperature control of the solutions was carried out with a water bath. Measurements at 4°C were performed in a cold room.

## IV. SOLID FAT CONTENT DETERMINATION

The solid fat content (SFC) of fats was obtained by the measurement of the signal of the nuclear magnetic resonance of the proton ($^1$H-NMR) as a function of temperature. The measurements were carried out by the French Institute for Fats and Oils (ITERG) on a pulse low-resolution NMR spectrometer (Minispec PC20, Bruker Spectrospin) operating at 20 MHz for protons. SFC analysis of the **raw vegetable fats and MDG mixtures** was performed according to the normalized method ISO 8292. SFC was also determined on emulsions after homogenization at 20 and 4°C and after ageing (24 h at 4°C). In **emulsions**, solid + liquid signal amplitude (*s* + *l*) 11 µs after end pulse, and liquid signal amplitude (*l*) 70 µs after end of pulse were used. To compensate for the dead time of the receiver circuit, a corrector factor, *f*, was used to obtain the initial solid signal level (*fs*). SFC in the emulsions was calculated as described in (Barford, *et al.*, 1991):

$$\%\ SFC = \frac{fs \times 100}{fs + (l - 0.8 \times l_{"H_2 0"})}$$

The liquid signal from the "$H_2O$" sample was subtracted from the total signal of the emulsion to obtain a more realistic value of the liquid oil signal. The liquid signal was multiplied by 0.8, since about 8 % of fat was present in the emulsion.

# V. FAT PARTICLE SIZE ANALYSIS

The size and the particle distribution of the fat globules in the emulsions, mixes and ice creams were measured by light scattering. The method is based on the theory of Mie. A laser beam which interacts with a particle is not only diffracted, but also reflected and diffused. The diffracted light was measured on the level of a central detector composed of 31 concentric sectors: the intensity measured on a sector was representative of a class of sizes, particles with lower diameters scattering at higher diffraction angle.

In this type of measurement, the preliminary knowledge of the refractive indexes of the dilution medium and of the dispersed particles is required: the values of 1.456 for the lipid droplets and 1.330 for water were used. The value of adsorption was 0.003. No correction was carried out when the sodium dodecyl sulfate (SDS) was used as dilution medium. Measurements were performed on two different particle-measurement instruments, a Mastersizer 2000 for emulsion and a Mastersizer S for mix and ice cream (Malvern). The volume weighted average diameter of the fat globules ($d_{4,3}$) as well as the particle size distribution were obtained.

## V.1. Measurement of fat globule size in emulsion and mix

Two dilution media were used in order to make the distinction between the "apparent" droplet size (measurements in non-dissociating conditions, tap water, ambient temperature) and the "real" droplet size (after aggregate dispersion in a powerful anionic detergent solution of SDS 1 wt.%). Emulsions were analyzed after homogenization (20°C) and rapid cooling (4°C) and after the ageing step (24 h, 4°C). Mixes were analyzed after homogenization and cooling (4°C) and after the ageing step (16 h, 4°C). The sample volume was about 1 ml. The dilution factor of the sample was approximately 1/1000. For all samples, at least two measurements were carried out.

## V.2. Measurement of fat globule size in ice cream

The method used to measure the droplet size in ice cream consisted in a direct defrosting of a sample in the dilution medium (water or SDS solution 1 wt.%). The application of ultrasounds (frequency = 27 KHz, maximum power = 50 W) made it possible to get rid of the air bubbles in the sample. The scale of ultrasounds was characterized by a quantity of heat applied per volume unit ($Ws.l^{-1} = J.l^{-1}$). This scale was composed of 4 stages: 1 measurement without

ultrasounds, three measurements at 100 % of the maximum power, two measurements at 30 % of the maximum power then, and seven measurements at 50 % of the maximum power. This procedure ensured that the size of the globules or of the aggregates remained intact (Gelin, 1995). For all samples, at least two measurements were carried out.

# VI. VISCOSITY MEASUREMENTS

Viscosity measurements are based on the determination of the torque necessary to the rotation of a cone at the surface of the studied material. The viscosity ($\eta$) is the relationship between the tangential force exerted on the surface of the cone ($\tau$, shear stress) and the angular velocity ($\dot{\gamma}$). It is given by:

$$\eta = \frac{\tau}{\dot{\gamma}}$$

## VI.1. Analysis of emulsion

Viscosity measurements were recorded on a Physica Modular Compact Rheometer 300 (Anton-Paar) with a cone (7.5 cm diameter, 0.992° angle, 0.05 mm gap) and a plate geometry with smooth surfaces. Viscosity measurements were performed on emulsions at 20°C (after homogenization) and at 4°C (after the 24 h-ageing period). Samples were transferred onto the rheometer plate set at the working temperature, and left 5 min in the rheometer before beginning the experiment. Viscosity was recorded at a shear rate of 1 $s^{-1}$ and measured for 3 min. For all samples, at least two measurements were carried out.

## VI.2. Analysis of mix

The apparent viscosity of ice cream mix taken after homogenization and after maturation was measured using a rotary viscosimeter Brookfield (model RVT). Temperatures of measurement were close to 4°C after homogenization and ageing. The samples (500 ml) were carried out in glass beaker. The value was taken after ten revolutions of the cylinder.

# VII. DYNAMIC RHEOLOGICAL CHARACTERIZATION

Dynamic rheological measurements are based on the application of a control stress on the sample. The elastic (G') and viscous (G'') moduli were determined. The damping factor $\tan\delta \left( = \frac{G''}{G'} \right)$ was calculated.

## VII.1. Analysis of aged emulsion

Measurements were carried out using a controlled stress rheometer (Physica MRC 300, Anton-Paar) provided with a plate geometry with smooth surfaces and a cone module of 7.5 cm diameter and 0.992° angle (CP-50-1). The gap between the plate and the module was 0.05 mm. A deformation of 0.5 % was used. The amplitude was chosen on the basis of the determination of the linear visco-elastic regime of an emulsion by a deformation amplitude test carried out at 4°C. Approximately 2 ml of an aged emulsion were placed in the center of the plate. The sample was protected from the evaporation phenomena by placing oil around the geometry. G' and G'' were measured according to a frequency sweep test between 5 and 0.05 Hz, at 4°C. For all samples, at least two measurements were carried out.

## VII.2. Analysis of ice cream

Rheological profiles of ice creams were obtained in the temperature range from -10 to 60°C (0.5°C.min$^{-1}$) with the Physica MCR300 rheometer equipped with streaked parallel plates. The streaked plates (4 cm diameter) avoided any phenomenon of slip. A gap of 1 mm was selected in order to take into account the heterogeneity of the product but also its change of state during measurement. The starting temperature of -10°C was selected as a compromise between a correct implementation of the sample into the apparatus and a limited evolution of the product as ice cream characteristics started to change above -20°C. Ice creams were stored for 2 h at -10°C before going to the rheometer. They were placed on the lower level of the geometry, then, crushed between the two plates. Oil with a low crystallization temperature was used to prevent the evaporation phenomena during the experiment. Moreover, one lid was laid out around the geometry in order to avoid the formation of white frost or condensation onto the geometry. After setting, the sample was left 15 min at -10°C before the beginning of the measurement in order to

allow the stabilization of the product. G', G" and tanδ were measured as a function of temperature at 1 Hz and at a deformation amplitude of 0.05 % between -10 and 5°C and 0.1 % between 5 and 60°C. These amplitudes were chosen on the basis of the linear visco-elastic regime of ice cream by a deformation amplitude sweep test at various temperatures. At 5°C, before the change in deformation amplitude and at 60°C at the end of measurements, a frequency sweep test from 5 to 0.5 Hz at a percentage of deformation of 0.1 % was carried out. At the end of measurements, at 60°C, a deformation amplitude sweep test (0.1 to 100 %) was also performed. For all samples, at least two measurements were carried out.

## *VIII.* ICE CREAM MELTING TEST

Ice cream samples, with a mass of 270 g ± 10 g each, were removed from their packaging and placed on horizontally mesh fixed grids (mesh size: 1 cm × 1 cm), located above balances (Metter PJ 600). The melting test was carried out in a temperature controlled room at 29°C, under constant hygrometry. The mass of the product passing through the screen was uninterrupted and weighed for 2 h. The layout of the melting curves was obtained: mass melted according to time. The average time at the end of which 10 then 30 g of product melted ($T_{10}$ and $T_{30}$) was determined.

## IX. DIFFERENTIAL SCANNING CALORIMETRY

In order to characterize the thermal behavior of fat in the mix and ice cream, differential scanning calorimetry (DSC) was used. This technique is based on maintaining at the same temperature, the sample and a reference (often air) placed in different pans, during a given temperature program. The electric output necessary for the compensation between the different pans corresponds to a calorimetric effect. This latter can be endothermic (fusion phenomenon) or exothermic (crystallization phenomenon).

The DSC experiments were performed in collaboration with A. Schöppe (Degussa Food Ingredients) using a MDSC 2920 (V2.6A) (TA Instruments). Samples of **mix** (≈ 10 mg) aged at 4°C for 16 h were sealed in aluminum pans. The samples were first rapidly heated to 60°C. Heat

flow was recorded as a function of decreasing temperatures from 60 to -20°C at 5°C.min$^{-1}$. Since the size of fat droplets might influence the crystallization process (Palanuwech and Coupland, 2003), for each formulation, the absence of fat globule size evolution as a function of temperature was checked in the conditions of the thermal analysis. Samples of **ice cream** (≈ 80 mg) stored at -20°C were equilibrated at –30°C for 10 min in the DSC instrument. Eighty mg of ice cream corresponded approximately to the complete filling of the pan that allowed a good heat exchange between ice cream and the surface of the cell and avoided the artifact phenomenon caused by the presence of air bubbles. Heat flow was recorded as a function of increasing temperatures from –30 to 65°C at 1°C.min$^{-1}$.

The thermograms allowed the determination of the enthalpy for the various peaks. The temperature of crystallization or of melting corresponded to the temperature at which the peaks were at maximum. However, because this value varied with the mass sample, the onset and offset temperatures were also determined and defined as the beginning of the crystallization and fusion peaks, respectively, by the intersection of the tangent to the peak with the base line. For each mix and ice cream preparation, the experiment was conducted at least in duplicate.

The percentage of the crystallized matter (fat and MDG) in the ice cream and the mix was determined from the enthalpy of the peaks associated with the fat fraction related to the enthalpy associated with the initial fat present in the formulation. This calculation was based on the hypothesis that no other event than fat melting or crystallization occurred in the temperature range considered.

# X. QUANTIFICATION OF PROTEIN PARTITIONING IN EMULSION AND ICE CREAM

## X.1. Spectrophotometric method

The protein partitioning determination was based on the quantification of the amount of proteins present in the aqueous phase, *i.e.*, non-adsorbed at the different interfaces. Measurements concerned **emulsion** and **ice cream** systems. This method required a preliminary separation of the emulsified fat phase and, in the case of ice cream, of the aerated phase from the aqueous phase.

Samples, 25 g of emulsion (fresh and aged) or 20 g of melted ice cream (one night at 4°C), were placed in centrifugation tubes (Nalgene 3139-0050, 50 ml) and were centrifuged at 15 000 g, 30 min, at 20 or 4°C for emulsion and at 4°C for ice cream product (Suprafuge 22 - Heraeus Sepatech). The aqueous phase consisted in the subnatant and the protein solid base. Samples were frozen at -20°C until analysis.

The quantification of the amount of total proteins in the aqueous phase was done using the Bradford method (Bradford, 1976). This method is based on fixing the Coomassie dye to the aromatic and basic amino-acid residues of the proteins (arginine, phenylalanine, and tyrosine). The complex formation modifies the initial brown color of the reagent in blue. The optical density was read with a Lamba Bio-20 spectrophotometer (Perkin Elmer) at 595 nm and was compared with that of a standard bovine serum albumin (BSA) solution (from 50 to 800 $\mu g.ml^{-1}$) to obtain the protein concentration. The samples were diluted at 1/100 in distilled water, then 50 µl were taken and added to 1500 µl of Coomassie reagent in to micro-cells at ambient temperature. The calculation of the protein concentration at the surface of the fat globules is presented in Appendix 1. The final results were expressed as a percentage of proteins adsorbed at the fat globule surface in the case of emulsion and as a percentage of proteins present in the aqueous phase in the case of ice cream.

## X.2. Determination of the protein types in the aqueous phase

A separative technique was used to determine, in a qualitative way, the various protein types present in the aqueous phase. The quantitative determination of each protein fraction was obtained by densitometry on the various separated fractions. This determination was only performed on the aqueous phases recovered after the centrifugation of fresh and aged **emulsions**.

The technique of polyacrylamide gel electrophoresis in a SDS medium (SDS-PAGE) was used for the separation of the protein types present in the aqueous phase on the basis of the molecular mass of the different species. The association of a powerful anion detergent, a reducing agent and an elevated temperature made it possible to break the electrostatic and disulfide connections between the proteins. The reducing agent used (2-mercaptoéthanol) caused a cut of the disulfide bridges by a nucleophilic substitution mechanism taking place in neutral or basic medium. The temperature (100°C, 5 min) allowed accelerating the dissociation reactions.

SDS-PAGE separation of the protein types present was carried out on acrylamide pre-cast gel at 15% of acrylamide (Bio-Rad). The migration buffer used was made of tris-glycine SDS.

The samples from the aqueous phase defrosted beforehand were diluted at the 1/40 for SMP and at 1/15 for FDP in a buffer (Tris 50 mM, NaCl 100 mM, pH 7.5). The samples were then heated (100°C, 5 min). The applied volume of the samples was of 10 µl corresponding to a protein concentration of about 1mg.ml$^{-1}$. A suspension of 10 standard proteins of molecular weights from 10 to 250 kD (Precision Plus Protein Standards: unstained, BioRad) was used in order to identify the various protein types separated by the electrophoresis. The migration was carried out at 80 V for 30 min, then at 100 V until the end of the migration (approximately 1 h). After protein separation, the gel was stained by a Coomassie blue D mixture (0.2 % m/v), diluted in a methanol/water solution (1/1 v/v) with 7.5 % of acetic acid, for at least 2 h. Discoloration was done in a methanol/water/acetic acid solution (30/62.5/7.5 v/v/v): several successive baths were used until the desired discoloration was achieved. The gel was then vacuum dried (gel dryer) for at least 1 hour.

The densitometric analysis of the electrophoresis gels used a Gel-Doc 2000 apparatus (BioRad). The surface values corresponding to the different protein types were calculated. On this basis and knowing the total amount of proteins interacting with the oil interface, the percentage of protein types adsorbed at the fat globule surface was calculated.

### X.3. Fluorescence method

Fluorescence measurements were related to the presence of tryptophan residues (Trp) naturally occurring in the protein mixtures used. Fluorescence measurements were performed on diluted **emulsions,** that is without a centrifugation step.

Fluorescence measurements were carried out using a fluorimeter SPEX Fluorolog 3 (Jobin Yvon, Horiba group) equipped with a charge-coupled device (CCD) camera that allowed instant emission spectra registration. The fluorimeter was equipped with a front-surface accessory. The reflected angle was set at 22.5°. This device allowed investigation of the fluorescence of powdered, turbid and concentrated samples in order to limit the problems of light diffusion. The emission spectra of Trp of proteins were recorded between 300 and 365 nm at an excitation wavelength of 290 nm. The slits of excitation and emission were fixed at 0.4 and 1 nm, respectively. Measurements were carried out whilst stirring at a specific temperature, maintained thanks to the temperature controlled sampler (Neslab RTE-7 Digital plus, Thermo Neslab). Measurements were performed at 20°C on fresh emulsions and at 4°C on aged emulsions. Moreover, a SMP solution (3 mg.ml$^{-1}$) prepared with the same procedure as that used for

emulsion preparation was diluted (0 - 0.5 $mg.ml^{-1}$) and analyzed. One spectrum of each sample (emulsions and SMP solutions) resulted from ten accumulated spectra.

Previous studies have pointed out that front-face fluorescence allowed distinguishing between Trp in hydrophilic and hydrophobic environments (Rampon, *et al.*, 2001; Rampon, *et al.*, 2004). This distinction was not possible directly with the fluorescence spectra. Only the fourth-derivative of the spectra allowed distinguishing between the two types of Trp. The mathematical treatment of the fluorescence emission spectra was performed in collaboration with J. Toutain (TREFLE UMR 5508, ENSAM, ENSCPB, Bordeaux 1 University). Each experimental fluorescence spectrum was composed of 1024 measurements for the wavelengths varying from 300 to 365 nm. The noise distribution was considered to be Gaussian. Based on the hypothesis that the spectrum and its derivatives with respect to the wavelength were continuous functions, the fluorescence spectrum was first smoothed. The smoothing (or filtering) algorithm was derived from the approximation theory by feed-forward neural network with a single hidden layer and a linear output layer on noisy scattered data. Any continuous function may be closely approximated as a linear combination of sigmoids, defined by the following equation:

$$f_N(\lambda) = a_0 \sum_{k=1}^{N} \Phi_k(\lambda)$$

With $\Phi_k(\lambda) = \dfrac{a_k}{1 + \exp(b_k \times \lambda + c_k)}$ and $\lambda$ the wavelength.

This so-called Tikhonov regularization problem consisted in minimizing a target function *Z* of parameters *a*, *b* and *c*, expressed by:

$$Z(a,b,c) = \sum_{i=1}^{1024} (Fs(\lambda_i) - f_N(\lambda_i))^2$$

thanks to an experimental data set at every experimental wavelength $\lambda_i$. This led to a least-square problem that was solved by a Levenberg-Marquardt algorithm, known to be efficient and robust.

The resulting fourth-derivative of the spectra revealed several peaks characterized by a wavelength, and an area corresponding to the different Trp environments. The peak at 334 nm was assumed to be correlated with Trp in a hydrophilic environment, *i.e.*, with the amount of proteins in the aqueous phase.

Whey proteins contain, on average, more Trp residues than caseins (casein $\alpha$ = 2 residues, caseins $\beta$ and $\kappa$ = 1 residue, $\beta$ lactoglobulin = 2 residues, $\alpha$ lactalbumin = 4 residues and BSA = 2 residues). The calculation of the Trp content of the different milk proteins leads to 1.0 g Trp / 100 g of total protein and 0.44 g Trp / 100 g of total protein, for caseins and whey proteins,

respectively. The weight ratio caseins / whey proteins in SMP is 80 / 20 and the ratio Trp in caseins / Trp in whey proteins is 70 / 30. Thus, protein quantification using Trp residue fluorescence slightly underestimates the casein fraction compared with whey protein fraction.

## XI. ICE CREAM FLUORESCENCE ANALYSIS

Fluorescence measurements on ice cream were carried out as previously described for mix samples (§ B.X.3.) The samples stored at -20°C were directly placed in a quartz cell. For each sample, 3 spectra were measured. Measurements were carried out at 1°C, maintained thanks to the temperature controlled sampler. The fluorescence emission spectra were smoothed to facilitate statistical multivariate analysis. The smoothing algorithm was similar to that used in the case of the emulsion analysis.

## XII. ICE CREAM SENSORY ANALYSIS

The sensory analysis aimed at characterizing the organoleptic properties (texture) of ice creams in order to highlight possible differences between the various compositions. It consisted in making taste coded samples of a product under fixed conditions using a list of descriptors. The development of this list by the group of tasters proceeded in 5 stages (Depledt and Strigler, 1998): 1. Research of the greatest number of descriptors; 2. Qualitative selection; 3. Quantitative selection; 4. Statistics selection using a hierarchical ascendant clustering (HAC) and a correspondence analysis (CA); 5. Drive of the group to the use of the reduced list.

In the case of ice cream, four descriptors were defined and studied:

- the "**airiness**" of the product, quantified by the evaluation of the quantity of air present in ice cream by observing the porosity, the air cells, and the weight of the product;
- the "**unctuousness**" (creaminess) characteristic, determined by the evaluation of the aptitude of ice cream to cover the palate, by the melting time in the mouth as well as by the sensation of fattiness that the product may cause;
- the perception of "**coldness**", evaluated by the time of diffusion of the cold sensation right behind the teeth;

- the "**firmness**" in mouth, defined as the compressive force necessary to crush the ice cream sample in the mouth between the tong and the palate.

The assessors must note the intensity of the various descriptors by coding the intensity on a horizontal scale. The establishment of the texture sensory profile of ice creams was evaluated on the selected criteria, thanks to a scale broken up into six levels (0 to 5), by the 16 assessors and in an individual way. The frozen samples, conditioned in a constant serving size (50 ml plastic packaging placed in isothermal boxes), were maintained at a constant and identical temperature during all the test duration. The products were presented in an identical packaging and were coded. The subjects received, in each session, four samples, all together in order to be able to return to one of the samples during analysis. The evaluation followed a defined test plan (each taster received a randomized order for tasting) and the definitions of the different attributes were always available during the session.

## XIII. STATISTICAL ANALYSIS

Analysis of variance of all data, using Fisher's least significant difference (LSD) procedures (Stat Graphic Plus software, Manugistics), was carried out on the different parameters of each analysis among the different formulations. Different parameters were correlated with the response variables of the different emulsions, mixes or ice creams, by linear regression. Correlation coefficients ($r^2$) were determined from the regression line.

Two kinds of statistical treatments were applied to the **emission fluorescence spectra**: principal component analysis (PCA) and partial least squares (PLS) discriminant analysis. PCA and PLS discriminant analysis were run on The Unscrambler® v8.0 (Camo Process AS, Oslo, Norway). PCA (Jolliffe, 1986) allows investigating the main differences between formulations in terms of fluorescence emission. PCA is a factorial method for exploratory statistics which can easily summarize great amounts of multidimensional data by means of simple 2-D representations called similarity maps. In the present application, these representations showed what kind of ice cream formulations were best discriminated by fluorescence data.

PLS discriminant analysis was chosen to investigate the ability of fluorescence data to distinguish between the different ice cream ingredients, *i.e.*, proteins, fats and emulsifiers. PLS regression techniques (Martens and Naes, 1989; Tenenhaus, 1998; Esbensen, 2001) have been initially designed in the context of modelisation and prediction of quantitative variables when

strong relationships exist between endogenous variables. For this reason, PLS regression was well-adapted to the case of fluorescence spectra since a strong correlation existed between the consecutive emission wavelengths. PLS discriminant analysis (Tenenhaus, 1998) is an adaptation of PLS regression in the case of categorical variables. In our case, the categorical variables were the dummy variables describing the membership of the formulations with the different categories: protein mixture (SMP or FDP); fat (hydrogenated coconut oil, refined coconut oil, refined palm oil or anhydrous milk fat) and emulsifier (saturated MDG or partially unsaturated MDG). PLS discriminant analysis consisted of finding models for the dummy variables. These models, called discriminant functions, were the linear combinations of the endogenous variables (*i.e.* the wavelength responses) that best modeled the dummy variables. As a result of PLS discriminant analysis, spectral patterns that represented the regression coefficients of the designed discriminant functions were obtained. These spectral patterns showed the emission wavelengths that best discriminated each protein mixture, each fat or each emulsifier. In order to carry out PLS discriminant analysis, a large amount of data was required. For this purpose, for each ice cream formulation, three different productions were carried out. Among each production, three samples were analyzed by fluorescence. The fluorescence emission spectra were split into a calibration set and a validation set. For the 3 fluorescence spectra recorded corresponding to the three different samples, two spectra were assigned to the calibration set, and the third one was assigned to the validation set. The calibration set was used to derive the PLS discriminant functions of all protein, fat and emulsifier categories. The validation set was used to produce classification matrices that allowed the verification of the relevance of the models in order to determine the discrimination of the different ingredients.

For **sensory analysis**, it was first necessary to undertake a statistical study in order to select relevant and discriminating attributes. A HAC was carried out in order to determine groups of attributes with similar meanings. Then, a CA was performed to determine the most relevant and discriminating attribute in each group. These tests were carried out using the software StatBox version 6.22. In a second step, the analysis of the sensory data aimed to provide information on the assessors' performance, the characterization of the products or the connection between the sensory data. One of the methods usually used to this end is the generalized procrustes analysis (GPA) (Gower, 1975). We applied the method suggested by Kunert and Qannari (Kunert and Qannari, 1999; Qannari and Meyners, 2001). The latter is presented like a simpler alternative of the GPA in terms of its ease of setup. This method consisted of two stages. Initially the data were pretreated in order to be free from the differences between the assessor

notation methods. It acted to some extent as a standardization of the individual configurations. Then, a PCA was carried out on the corrected data.

In summary, the different analysis performed on the different systems, *i.e.*, raw materials, model systems, emulsions, mixes and ice creams, are reported in Table 12.

**Table 12: Summary of the analysis carried out on the different systems.**

| Methods | Data obtained | Raw material | Model system | Emulsion | Mix | Ice cream |
|---|---|---|---|---|---|---|
| Gas chromatography | Composition of fat and emulsifiers | ✖ | | | | |
| Spectrophotometry | Protein partitioning at the oil in water interface | | | ✖ | | ✖ |
| Interfacial tension | Interfacial behavior of proteins and emulsifiers | | ✖ | | | |
| $^{1}$H-NMR | Fat and emulsifier crystallization | ✖ | | ✖ | | |
| Viscosity | Measurement of the fluid's resistance to flow | | | ✖ | ✖ | |
| Dynamic rheology | Textural properties, structural networks | | | ✖ | | ✖ |
| Light scattering | Fat droplet size evolution | | | ✖ | ✖ | ✖ |
| Melting time | Melting behavior | | | | | ✖ |
| DSC | Fat and emulsifier thermal behavior | ✖ | | | ✖ | ✖ |
| Fluorescence | Molecular data on protein environment | | | ✖ | | ✖ |
| Sensory analysis | Map making | | | | | ✖ |

# C. RESULTS AND DISCUSSION

The characteristics of ice cream structure are important to know since they condition the textural and sensory properties of the final product. These characteristics are influenced, at least partly, by the ingredient selection. This work focused on the roles of surface-active molecules on ice cream formation and properties. The interactions of proteins and low molecular weight emulsifiers between each other and with other ingredients, such as fat, were also investigated. We have considered the product in the different steps of processing and, in particular, before and after the homogenization steps, during the ageing period and after freezing. However, because some ice cream mix properties could be difficult to characterize due to the presence of sugars and polysaccharides, simplified systems such as oil-in-water emulsions were also studied. It is worth noting that, regardless the system considered, *i.e.*, oil-in-water emulsion, ice cream mix or ice cream product, the same apparatus and process parameters were used. Likewise, the same ingredients were chosen. They present an industrial interest for their wide use in food industry and especially in ice cream formulation. Thus, the purpose of this work was to master the influence of the formulation, *i.e.*, the protein mixture, the nature of the low molecular weight emulsifier and / or the fat characteristics on the physicochemical properties of the three systems studied. Correlations between the different methods of characterization were assayed.

# I. OIL-IN-WATER EMULSION

## I.1. Résumé

L'étude des émulsions huile dans eau permet d'appréhender le comportement de la crème glacée dans ses premières étapes de fabrication et, en particulier, la mise en place et l'évolution de l'interface formée autour des gouttelettes d'huile. Pour réaliser cette étude, nous considérons différentes émulsions à base de deux mélanges protéiques (poudre de lait écrémé (SMP) et protéines laitières fonctionnalisées (FDP)), deux types de mono- et diglycérides (MDG) (saturé et partiellement insaturé), trois matières grasses (deux huiles de coprah, l'une hydrogénée et l'autre raffinée, et une huile de palme raffinée). Cette sélection d'ingrédients devrait mettre en évidence les interactions se produisant entre la phase grasse, les émulsifiants de faible poids moléculaire et les protéines. Les émulsions sont caractérisées par le diamètre des globules gras, des paramètres rhéologiques et la couverture protéique à l'interface des globules gras. Deux méthodes différentes

sont utilisées pour la quantification des protéines adsorbées à la surface des globules gras : une méthode dite « classique » de centrifugation suivie d'un dosage colorimétrique et une méthode utilisant la fluorescence du tryptophane présent dans les protéines.

Après homogénéisation, le **diamètre des globules gras** mesuré pour les émulsions à base de FDP est élevé et reflète la présence d'agrégats. Avec ce type de protéines, les caractéristiques de taille des globules gras dépendent de la nature de la matière grasse et des émulsifiants présents dans la formulation. En revanche, les émulsions à base de SMP sont caractérisées par de faibles proportions de particules agrégées quelle que soit la formulation. L'étape de maturation (24 h à 4°C) entraîne un phénomène de désagrégation des globules dans les formulations à base de FDP, alors que les émulsions à base de SMP sont stables. Les **paramètres rhéologiques** (viscosité, modules élastique et visqueux) varient très faiblement avec la composition du système et ne dépendent pas d'un ingrédient spécifique de la formulation.

Après l'étape d'homogénéisation, la **quantité de protéines adsorbées à la surface** des globules gras varie en fonction de la nature de la matière grasse et/ou du type d'émulsifiant. Une adsorption sélective des caséines par rapport aux protéines de lactosérum est mise en évidence dans le cas des formules à base de FDP. L'étape de maturation favorise le phénomène de désorption protéique de la surface des globules gras, suggérant une rupture des interactions interfaciales entre les protéines. Cette désorption protéique est plus nettement prononcée dans les émulsions à base de SMP comparée à celle observée dans les émulsions à base de FDP. Dans les deux cas, ce phénomène est plus ou moins influencé par la nature de l'émulsifiant et de la matière grasse. Ces résultats de désorption protéique sont corrélés aux mesures de tension interfaciale. Par exemple, dans le cas d'un système à base de SMP et de MDG saturé, tant que la température de 20°C n'est pas atteinte, la protéine impose sa tension interfaciale. Quand la température décroît (ce qui correspond à l'étape de maturation), l'émulsifiant en cristallisant impose sa tension interfaciale au système. Ces résultats suggèrent fortement un phénomène de compétition de l'émulsifiant et de la protéine pour l'interface.

## I.2. Introduction

The study of oil-in-water emulsions allows the consideration of the ice cream product in the first step of processing and, in particular, the characterization of the adsorbed layer formed around the fat droplets in the emulsions. During the homogenization step, the preparation is subjected to high shear. This results in the disruption of the fat phase into small oil droplets. Surface-active components of the emulsion adsorb onto the nascent interface, lowering the

interfacial tension and, thus, stabilizing the droplets. As already mentioned (§ A.I.1.), the exact composition of the interfacial layer depends on the proportions of each type of surface-active component and their relative surface activities. Moreover, proteins that initially adsorb at the fat droplet interface are partially squeezed out from the surface during the low temperature ageing step, through the competition with low molecular weight emulsifiers (§ A.I.2.2.).

The purpose of this first part was to characterize some physicochemical properties of oil-in-water emulsions. Physical properties were assayed through fat particle size and viscosity measurements. The amount of proteins and the protein types adsorbed onto the fat globule surface was quantified thanks to different methods. In particular, a direct method of protein quantification in emulsified systems that avoided the use of centrifugation, namely front-face fluorescence, was developed. The stress was put on the behavior of protein and low molecular weight emulsifier at the oil-water interface and their interactions with the fat used. Interfacial surface activity measurements of protein and low molecular weight emulsifier were performed to interpret the surfactant behavior as a function of temperature.

The competitive adsorption between milk proteins and low molecular weight emulsifiers was first investigated through **interfacial tension measurements** as a function of different vegetable fats (oleic oil, hydrogenated and refined coconut oils, and refined palm oil) at oil / water interface. The surface active molecules considered were two MDG (saturated and partially unsaturated), and SMP. The obtained results (**Granger *et al.*, 2003**, Appendix 2) suggested that, at room temperature, the interfacial tension value was influenced, at least partly, by both the chain length and the unsaturation degree of the oil phase fatty acids (Table 13). Some interactions between the oil and the lipid emulsifier would occur through the hydrophobic parts of the molecules. Thus, surface activity could be favored when the fatty acid chain length of the fat fitted that of the emulsifier, as in the case of refined coconut oil and MDG. In contrast, increasing the unsaturation degree of the oil phase fatty acids, like in refined palm oil, reduced the possibility of interaction since the cis double bond created a bend in the fatty acid structure that would enable the rest of the molecule to interact with the emulsifier. However, these hydrophobic interactions only explained partly the results obtained. For instance, irrespectively of the MDG used, systems based on hydrogenated and refined coconut oils were characterized by significantly different surface tension values, although these two oils only differed by 8 % of oleic acid. Thus, the behavior of the systems with different oil phases could also be relevant of different crystallization behavior of the fats. Indeed, responses to changes in interfacial tension could be

**Table 13: Effect of fat type and emulsifier nature on interfacial tensions of oil / water interfaces.**

| Interfacial tension (mN/m) [1] | | |
|---|---|---|
| Mono- diglycerides | Fat [2] | 20°C |
| Saturated | Hydrogenated coconut oil | 6.6 ± 0.2 |
| | Refined coconut oil | 3.3 ± 0.1 |
| | Refined palm oil | 4.6 ± 0.1 |
| Partially unsaturated | Hydrogenated coconut oil | 5.0 ± 0.2 |
| | Refined coconut oil | 2.9 ± 0.0 |
| | Refined palm oil | 4.1 ± 0.1 |

[1] Each listed value is the average of, at least, triplicate sets of measurements ± standard deviation.

[2] Each fat is a mixture of sunflower oil with the fat listed in the table at a ratio of 80/20.

associated with morphology of the fat crystals (Ogden and Rosenthal, 1997). In the case of the combination of hydrogenated coconut oil and partially unsaturated MDG, the formation of platelet fat crystals that behave very differently from the other crystallized globular fat (Goff, personal communication) could account for the specific behavior of this fat. Thus, each fat would lead to specific interfacial value dependent on the MDG. Results of interfacial tension obtained with SMP mixed with emulsifiers showed that, as long as the temperature of 20°C was not reached, SMP imposed its interfacial tension. Upon cooling, the interfacial activity of MDG dominated.

It is known that monoglycerides destabilize protein-based emulsions, *i.e.*, monoglycerides squeeze out proteins from the interface at or below the critical temperature for monoglyceride crystallization (Krog and Larsson, 1992). Thus, in order to correlate the variation of the surface activity of the emulsifier with temperature and with the protein load at the fat globule surface, the amount and types of adsorbed proteins were quantified before and after the ageing process (**Granger *et al.*, 2003**, Appendix 2; **Granger *et al.*, 2005a,** § C.I.3.). At 20°C, after the homogenization step, emulsions were mostly stabilized by proteins. A displacement of the proteins adsorbed onto the oil droplet interface by the lipid surfactant was a consequence of the ageing step, suggesting a disruption of the interfacial protein interactions. This disruption was more marked with SMP than with FDP and, in both cases, was more or less influenced by the emulsifier and fat natures. A competitive adsorption of caseins over whey proteins was demonstrated in the case of FDP but not in the case of SMP. Because, the quantification of the protein load at the fat globule surface required a step of centrifugation that could induce artifacts in the protein partitioning, we developed a direct technique of characterization that avoided the use of centrifugation (**Granger *et al.*, 2005b,** § C.I.4.). Front-face fluorescence spectroscopy allows investigation of turbid samples like emulsions. The protein coverage of oil-in-water emulsions was followed through the presence of intrinsic tryptophan residues in proteins. However, because the variations in the emission spectra with the emulsion formulation were too small to allow the accurate discrimination of proteins at the interface or in the aqueous phase, we investigated the fourth derivative spectra. Indeed, it was reported (Rampon, *et al.*, 2001; Rampon, *et al.*, 2003), that the fourth derivative tryptophan emission spectrum differentiated tryptophan in hydrophobic and hydrophilic environments. The mathematical treatment of the fluorescence emission spectra developed in collaboration with J. Toutain (Bordeaux 1 University, ENSAM, ENSCPB, Trefle UMR 5508) allowed the quantification of the amount of proteins non adsorbed

onto the fat globules. This amount was well correlated with that found after centrifugation and a classical spectrophotometric method of protein quantification ($r^2 = 0.91$, $p<0.05$).

In parallel, the physical characterization of the emulsions pointed out that, at 20°C, after the homogenization step, the fat globule size was mostly dependent of the protein mixture used, *i.e.*, low mean diameter and low aggregation for SMP-based emulsions and high diameter and high aggregation phenomenon for FDP-based emulsions. Only for FDP-based formulations, the fat globule size characteristics were dependent on the emulsifier and fat types present in the formulation. While for SMP-based formulation the ageing period (24h at 4°C) had no effect on the mean diameter characteristics, some disaggregation was observed in the case of FDP emulsions.

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# Physicochemical behavior of oil-in-water emulsions: influence of milk protein mixtures, glycerol ester mixtures and fat characteristics

C. Granger[a,b], P. Barey[b], P. Veschambre[c], M. Cansell[a,*]

[a] Laboratoire "Milieux Dispersés Alimentaires: Physico-Chimie, Formulation et Vectorisation Nutritionnelle", ISTAB, Avenue des Facultés, F-33405 Talence Cedex, France
[b] Degussa Food Ingredients, Business Line Texturant Systems France SAS, France
[c] Laboratoire "Biosciences de l'aliment", ISTAB, Avenue des Facultés, F-33405 Talence Cedex, France

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**Abstract**

Different emulsions based on two protein mixtures (skim milk powder (SMP) and functional dairy proteins (FDP)), two mono-di-glyceride mixtures (MDG) (saturated and partially unsaturated), three fats (hydrogenated and refined coconut oils and refined palm oil) were studied to investigate the interactions occurring between the oil phase, low molecular weight emulsifiers and proteins. Immediately following the emulsification process, high diameters of fat globules were obtained in FDP-based systems, relevant of an aggregation phenomenon. At this stage, the fat globule size characteristics were dependent on the emulsifier and fat types present in the formulation. In contrast, SMP-based emulsions were characterized by low proportions of aggregated particles regardless the formulations. Ageing (24 h at 4 °C) promoted disaggregation in FDP formulations, while SMP emulsions were well stabilized. Just after the homogenization step, less proteins were required to stabilize the globule interface in FDP systems as compared to SMP ones. Only with SMP, the amount of protein load at the fat globule surface was influenced by the oil nature and/or by the emulsifier type. A competitive adsorption of caseins, over whey proteins, was demonstrated in the case of FDP. The ageing period promoted a displacement of the proteins adsorbed at the oil droplet interface, suggesting a disruption of the interfacial protein interactions. This disruption was more marked with SMP than with FDP and, in both cases, was more or less influenced by the emulsifier and oil phase natures. The variations of the viscosity and rheological parameters (elastic and viscous moduli) were not dependent on one specific component of the formulation.


*Keywords:* Oil-in-water emulsion; Vegetable oils; Milk proteins; Mono-di-glycerides; Protein displacement; Stability

## 1. Introduction

Numerous studies on ice creams or ice cream emulsions have pointed the influence of formulation on the emulsion stability [1–4] and ice cream characteristics [1,5,6]. In the ice cream mix, a variety of amphiphilic molecules, such as milk proteins and low molecular weight emulsifiers, contribute to the properties of the interface layer around the fat globules. The association of low molecular weight emulsifiers with milk proteins takes benefit of the emulsifying properties of each type of molecule, at the different steps of ice cream process. Indeed, it is usually admitted that emulsifiers, such as mono-di-glycerides (MDG) are not required to aid emulsification, but rather participate in the destabilization of fat globules during the whipping and freezing steps [7–10]. More precisely, during the low temperature ageing step, a competitive adsorption between the various molecules present at the fat globule interface occurs, related, at least partly, to lipid surfactant interfacial crystallization [2,11] and solid fat content [4,12,13]. As a result, part of the proteins that were initially adsorbed at the fat droplet interface is squeezed out from the surface and released into the aqueous phase [9,14,15]. This displacement reduces the

* Corresponding author. Tel.: +33 5 40 00 34 53; fax: +33 5 40 00 24 72.
*E-mail address:* m.cansell@istab.u-bordeaux1.fr (M. Cansell).

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stability of the fat globules leading to partial coalescence [8,16].

In order to gain in beneficial interfacial properties, different MDG varying in fatty acid chain length or unsaturation degree have been used. In comparison with saturated MDG, unsaturated fatty acids in MDG were better at inducing shear-sensitivity in emulsions and led to higher melting resistance in ice cream products [2,7]. Concerning the proteins, different types of milk derivates, such as whey protein concentrates [1,14,17–19], milk protein mixtures varying in their casein to whey protein ratio [3], and partially denatured proteins [20] have been used to improve the emulsion stability. Emulsions containing proteins were stable against coalescence but depending on protein content and composition, aggregation between fat droplets could be observed. Moreover, the amount of proteins adsorbed at the fat globule surface was a function of the protein type used in the formulation. Besides the roles of MDG and milk proteins on the ice cream emulsion properties, the oil characteristics used are often suggested to influence the adsorbed protein layer [2,4,5,13,21]. However, little is known about the influence of the polarity of the oil phase, the crystallization and melting behaviors, or the presence of small impurities in the oil on the resulting interfacial parameters.

Since the protein–MDG–fat combination is a key factor in development of strategies for controlling emulsion stability or instability, the aim of the present study was to improve understanding of the complex interactions occurring between these different mix components during the homogenization and ageing steps of ice cream process. For this purpose, results present, in a single work, the influence of both the MDG and the protein mixtures in relation with fatty acid oil characteristics constituting the fat droplets. The amount of proteins and the protein types adsorbed onto the surface of fat globules were quantified. The emulsions were also characterized in terms of fat particle size and rheological properties. The results obtained for emulsions were discussed in terms of the degree of influence of each component and, in terms of interactions that may occur between them.

## 2. Experimental procedures

### 2.1. Materials

Three different vegetable fats, i.e., hydrogenated coconut oil (HCO), refined coconut oil (RCO) and refined palm oil (RPO) (SIO, Bougival, France) were used for the preparation of oil-in-water emulsions. Table 1 presents the physicochemical characteristics of the fats. Skim milk powder (SMP) (Coopérative d'Isigny Saint-Mère, Isigny Sur Mer, France) and functional dairy proteins (FDP) (Bel Industries, Vincennes, France) were used. The functionalization consisted in a partial protein denaturation by a heat treatment performed by the supplier. Chemical compositions of the two protein mixtures are presented in Table 2. Relatively to the total protein content, SMP contained 80 wt.% of caseins and 20 wt.% of whey proteins. In the case of FDP, whey proteins represented 60 wt.%. Two lipid emulsifiers consisting of a mixture of MDG (60%, min. monoglycerides) were supplied by Degussa Food Ingredients (Baupte, France). The partially unsaturated MDG contained oleic acid (18:1; 20 wt.% of total fatty acids) and linoleic acid (18:2; 3 wt.% of total fatty acids). The saturated MDG comprised only palmitic acid (16:0; 56 wt.% of total fatty acids) and stearic acid (18:0; 42 wt.% of total fatty acids). Maximum iodine values of the saturated MDG and partially unsaturated MDG were equal to 2 and 19, respectively.

Table 1
Physicochemical characteristics of the three vegetable fats used for the preparation of oil-in-water emulsions

| | Hydrogenated coconut oil | Refined coconut oil | Refined palm oil |
|---|---|---|---|
| Solid fat content (%) | | | |
| 5 °C | 91.6 | 86.4 | 54.5 |
| 20 °C | 51.3 | 36.3 | 30.6 |
| Fatty acids (%) | | | |
| 12:0 | 45 | 45 | – |
| 14:0 | 19 | 19 | – |
| 16:0 | 10 | 11 | 44 |
| 18:0 | 13 | 3 | 5 |
| 18:1 | – | 8 | 38 |
| 18:2 | – | – | 10 |

### 2.2. Emulsion preparation

Oil-in-water emulsions were composed as follows: 8 wt.% vegetable fat, 10 wt.% SMP or FDP, 0.3 wt.% saturated or partially unsaturated MDG adjusted to 100% with water. Emulsions were prepared as described in Granger et al. [4]. Briefly, two premixes, one containing the fat and the emulsifier, the other the aqueous protein solution, were preheated separately at 70 °C, mixed, brought to 90 °C and immediately homogenized under constant stirring (two-step homogenizer (Manton

Table 2
Chemical characteristics of the two protein mixtures used for the preparation of oil-in-water emulsions

| Composition (wt.%) | SMP[a] | FDP[b] |
|---|---|---|
| Proteins | 35 | 20 |
| Caseins | 28 | 8 |
| Whey proteins | 7 | 12 |
| Moisture | 4.0 | 3.5 |
| Fat | 1.5 | 1.5 |
| Lactose | 52.0 | 62.0 |
| Ashes | 8.1 | 8.5 |
| Calcium | 1.30 | 1.01 |
| Potassium | 1.55 | 2.31 |
| Sodium | 0.60 | 0.56 |
| Phosphorus | 1.05 | 0.79 |
| Chlorides | 1.10 | 1.47 |

[a] SMP: skim milk powder.
[b] FDP: functional dairy protein.

Gaulin SA 1200 AB, Holland), 17.5 MPa first stage, 3.0 MPa second stage). After homogenization, the emulsions were first cooled to 20 °C, then, aged for 24 h at 4 °C under stirring. Each formulation was prepared at least in duplicate.

### 2.3. Rheological studies

Viscosity was recorded on a Physica Modular Compact Rheometer 300 (Anton-Paar, Germany) with a cone (7.5 cm diameter, 0.992° angle, 0.05 mm gap) and a plate geometry with smooth surfaces. Viscosity measurements were performed on emulsions at 20 °C after homogenization and on preparations at 4 °C after a 24-h ageing period. Samples were transferred to the rheometer plate set at the working temperature and left 5 min in the rheometer before beginning the experiment. Viscosity was recorded at a shear rate of 1 $s^{-1}$ and measured for 3 min. The dynamical rheological parameters, i.e., the storage modulus ($G'$), the loss modulus ($G''$), and the damping factor ($\tan\delta = G''/G'$) were recorded by using the same equipment as that used for viscosity measurements. Dynamic parameters versus frequency (from 5 to 0.05 Hz at 2% strain) were determined on aged samples. The amplitude was chosen on the basis of the determination of the linear viscoelastic regime of emulsion by a deformation amplitude sweep test.

### 2.4. Particle size analysis

The fat globule size distribution of the emulsions was obtained by integrated light scattering by using a Mastersizer 2000 (Malvern Instruments SA, Orsay, France). A relative refractive index of 1.456 and an absorptive index of 0.003 were used to calculate the particle size distribution from the measured scattering pattern. The emulsions were pre-diluted (obscuration 10–20%) prior to measurement. The samples were characterized by the mean particle size diameter (as evaluated by the volume weighted average diameter $d_{4,3}$) and the volume proportion (%) of fat droplets having a diameter greater than 2 μm ($d > 2$ μm). The measurements were carried out at room temperature on the freshly made emulsions or on samples stored at 4 °C during 24 h. In some cases, sodium dodecyl sulfate (SDS, 1 wt.%) was used to discriminate between coalescence and flocculation in the emulsion [4,10,14]. From these experiments, the aggregation factor expressed as the ratio of the apparent and real mean droplet diameters was calculated [20]. All analyses were performed at least in duplicate.

### 2.5. Protein analysis

Freshly homogenized emulsions and aged preparations were centrifuged in order to separate the oil phase and the aqueous phase [22]. The centrifugation parameters (15,000 × $g$, 30 min, 20 or 4 °C, Suprafuge 22, Heraeus Sepatech) were chosen so that the fat globule size was not drastically changed after centrifugation. Total protein content of the aqueous phase was determined by using the Bradford method [23]. The amount of fat matter remaining in the serum after centrifugation was checked by quantitative fat determination in this phase [24]. It was found below 15% of the initial fat content, irrespectively of the formulation. The percentage of proteins associated with the fat globule surface was calculated as the difference between total protein percent and protein percent in the aqueous phase, after correction for the volume fraction of fat. Relative proportions of the different protein types were obtained after separation by using SDS–polyacrylamide gel electrophoresis (15% acrylamide gels, Precast gel system, Bio-Rad). Samples were prepared to reach a final protein concentration of 1 mg/ml. Visualization of the bands was accomplished by Coomassie staining. Relative protein proportions were obtained by densitometry determination (Gel-Doc 2000, Bio-Rad). Analyses were performed at least in duplicate with a fresh emulsion.

### 2.6. Statistical analysis

Statistical analysis was performed by using Stat-Graphic Plus software (Manugistics, Maryland). Multifactor ANOVA and analysis of variance Fisher's least significant difference (LSD) procedures were used to determine which factors had a statistically significant effect and to compare means values.

## 3. Results and discussion

### 3.1. Particle size analysis

Fig. 1 presents the particle size distribution in the emulsions based on refined palm oil, partially unsaturated MDG,

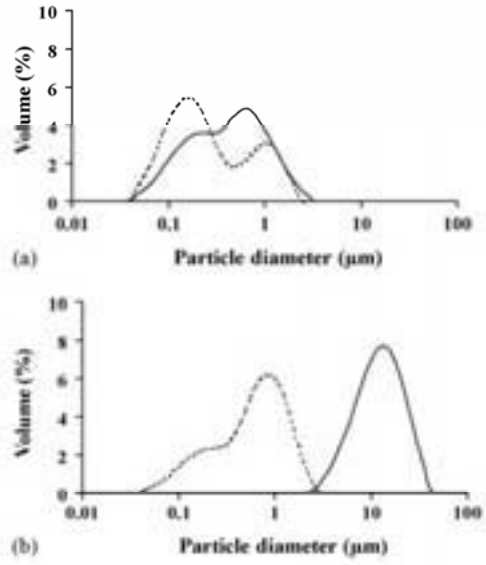


Fig. 1. Particle size distribution for emulsions based on refined palm oil, partially unsaturated MDG and (a) SMP; (b) FDP. Dispersion in water (—), or in SDS dissociative solution (- - -).

and the two protein mixtures. With SMP, the analysis of droplet size distribution revealed the existence of two overlapping populations, between 0.03–0.3 μm and 0.3–3 μm (Fig. 1a). The presence of FDP in the emulsion formulation led to an unimodal distribution with droplet sizes ranging from 2 to 40 μm (Fig. 1b). After dispersion of the samples in 1 wt.% SDS solution, changes in the shape of particle size distributions and displacement towards lower sizes were observed, for the two protein mixtures used. However, the displacement was more drastic in the case of FDP than in the case of SMP. The aggregation factor calculated for the two emulsions was equal to 1.3 and 19.3 for SMP and FDP-based systems, respectively. These results strongly suggested that high particle sizes principally corresponded to flocculated fat droplets, which interacted through non-covalent bonds. Although a greater amount of flocs was measured in the case of FDP, compared with SMP-based emulsions, it was worth noting that, after emulsion dispersion in SDS solution, all droplet sizes were below 2 μm.

Fat globule size analysis was performed on emulsions based on various fat, protein and emulsifier compositions. Mean particle diameters and the percentages of particles with sizes higher than 2 μm are presented in Table 3. At 20 °C, the protein type was the main factor determining the size characteristics of the emulsions containing various protein–MDG–fat combinations. The use of FDP led, independently of the MDG and the fat considered, to significantly higher fat globule diameters as compared with SMP. Thus, while SMP-based emulsions exhibited mean diameters ranging from 0.5 to 1 μm, for emulsions containing FDP, droplet sizes varying from 12 to 26 μm were observed. These high values corresponded to high proportions of $d > 2$ μm suggesting that aggregation occurred in the presence of FDP, irrespective of the formulation. The ageing process influenced differently these two families of emulsions. While for SMP-based emulsions, ageing did not modify the fat globule size characteristics, the droplet mean diameters decreased, in relative proportions ranging from 19 to 51%, depending on the fat-emulsifier composition, for FDP-based aged systems. This decrease in size might correspond to a partial disaggregation of the oil droplets.

Even though the mixture of proteins used in the formulation was the main ingredient influencing the emulsion size properties, for a given mixture (FDP or SMP), other variables individually influenced emulsion properties. As already reported [4], in SMP-based formulations, the size behavior of emulsions seemed to be slightly dependent on the emulsifier type (Table 3). The influence of the oil nature was only illustrated with the partially unsaturated MDG (Table 3). For freshly prepared emulsions containing FDP, the apparent droplet size depended on the fat nature used, but not on the MDG type. In contrast, for aged emulsions, the types of fat and lipid emulsifier used influenced the mean diameters, independently of the decrease in size observed, so that emulsions which exhibited similar droplet sizes before ageing (i.e., those based on the saturated MDG or hydrogenated coconut oil and refined palm oil) were different after ageing. In contrast, size differences that were measured in some formulations just after homogenization (emulsions based on the saturated MDG and refined coconut oil or refined palm oil) tended to vanish after ageing.

These results provided evidence that the two protein mixtures acted differently on homogenized fat globule stabilization. The stability of SMP-based emulsions in the presence of competitively adsorbing surfactants and the flocculation phenomenon found in the case of emulsions containing FDP were consistent with other data [1,3]. In particular, our results agreed with the fact that droplets were less aggregated in emulsions containing the highest proportion of caseins [3]. The presence of caseins in the micellar form, at the surface

Table 3
The effect of milk protein, emulsifier and fat types on particle size characteristics of the oil-in-water emulsions, before and after ageing (24-h storage at 4 °C)

| Protein | MDG | Fat | $d_{4,3}$[a] (μm) | | $d > 2$ μm[a,b] (%) | |
|---|---|---|---|---|---|---|
| | | | 20 °C | 4 °C | 20 °C | 4 °C |
| SMP | Saturated | HCO | 0.5 ± 0.0 | 0.5 ± 0.0 | 1 ± 0 | 1 ± 0 |
| | | RCO | 0.5 ± 0.0 | 0.5 ± 0.0 | 1 ± 0 | 1 ± 0 |
| | | RPO | 0.5 ± 0.0 | 0.5 ± 0.0 | 1 ± 0 | 2 ± 0 |
| | Partially unsaturated | HCO | 1.0 ± 0.1 | 0.9 ± 0.0 | 15 ± 1 | 9 ± 1 |
| | | RCO | 0.5 ± 0.0 | 0.6 ± 0.0 | 1 ± 0 | 2 ± 0 |
| | | RPO | 0.5 ± 0.0 | 0.5 ± 0.1 | 2 ± 1 | 1 ± 0 |
| FDP | Saturated | HCO | 12.6 ± 3.1 | 6.2 ± 1.7 | 100 ± 0 | 77 ± 5 |
| | | RCO | 25.8 ± 1.0 | 13.6 ± 3.5 | 94 ± 2 | 91 ± 0 |
| | | RPO | 12.3 ± 3.4 | 10.0 ± 3.5 | 93 ± 2 | 85 ± 1 |
| | Partially unsaturated | HCO | 19.4 ± 1.6 | 14.4 ± 1.1 | 98 ± 2 | 85 ± 6 |
| | | RCO | 23.1 ± 9.2 | 17.5 ± 5.0 | 97 ± 3 | 93 ± 1 |
| | | RPO | 15.8 ± 3.9 | 11.2 ± 4.2 | 96 ± 4 | 86 ± 5 |

MDG: mono-di-glycerides; HCO: hydrogenated coconut oil; RCO: refined coconut oil; RPO: refined palm oil; SMP: skim milk powder; FDP: functional dairy proteins.

[a] Values are means ± S.D. ($n = 2$).

[b] Percentage of particles with mean diameters higher than 2 μm.

of oil droplets, creates a steric stabilizing layer that maintains the repulsion between fat globules and protects the droplets against destabilization [25]. In contrast, whey proteins exhibit compact globular structures. They form close-packed protein monolayers, described as dense-dimensional assemblies of highly interacting deformable particles [26]. This molecular arrangement may prevent a sufficient protein surface loading. Thus, the protein electric charges turning towards the water phase cannot provide enough interglobular repulsion [1]. In addition, the use of whey proteins partially denatured by heat treatment, may favor the formation of polymers at the oil–water interface through weak interactions between whey protein polymers and disulphide groups of other protein molecules (bridging flocculation) [27]. Partial protein denaturation may also lead to higher droplet sizes due to a higher water binding capacity [20]. This last point would explain, at least partially, the dependence of the droplet size upon ageing since water binding was expected to vary as a function of temperature [28].

Besides the drastic protein influence on the morphological behavior of the emulsions, some differences were observed as a function of the types of fat and emulsifier used. The peculiar emulsion size behavior of some formulations towards the presence of MDG or fat may illustrate the competitive adsorption of milk proteins (see below) and MDG as well as complex interactions that may take place between these two components and the fat.

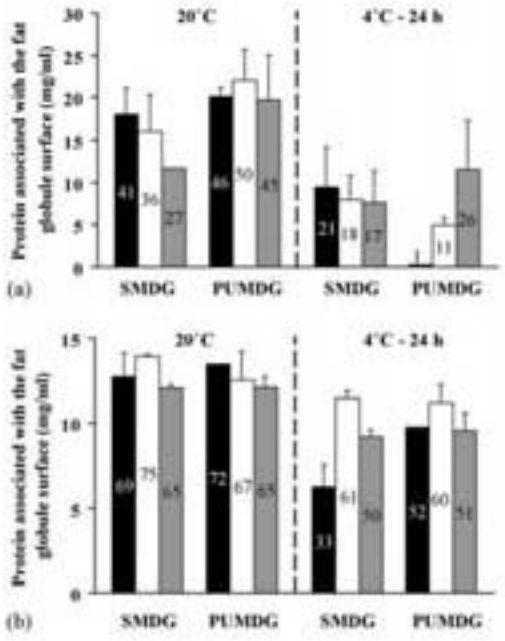


Fig. 2. Fat globule protein coverage (mg/ml of emulsion) for different compositions of emulsions just before and after ageing (4 °C, 24 h): (a) based on SMP; (b) based on FDP and containing saturated MDG (SMDG) or partially unsaturated MDG (PUMDG) and hydrogenated coconut oil (■); refined coconut oil (□); refined palm oil (▩ ). Data are means ± S.D. of duplicate samples and at least two separate preparations. Percentages of proteins adsorbed are reported in the bar histograms.

### 3.2. Effect of ageing process on adsorbed proteins

The influence of ageing on emulsions with various fat, emulsifier and protein compositions was assessed by the quantification of proteins associated with the fat globule surface (Fig. 2a and b). Results are presented in terms of quantity and percentage of proteins adsorbed at the interface since the amount of proteins in SMP and FDP mixtures was different. As already reported [4] for SMP-based emulsions, at 20 °C, just after the homogenization step, the quantity of proteins associated with the fat phase was lower when using the saturated MDG instead of the partially unsaturated MDG (Fig. 2a). Regardless of the formulation, the ageing period reduced the quantity of proteins associated with the droplet interface. However, desorption was less drastic with the saturated MDG than with the partially unsaturated one. Moreover, the influence of the fat composition was principally revealed in the case of the partially unsaturated MDG after ageing: the more unsaturated fatty acids composing the oil phase, the more proteins were adsorbed. In the case of FDP-based products (Fig. 2b), the influence of formulation was only marked for hydrogenated coconut oil and saturated MDG after ageing. At 20 °C, the amount of proteins loaded at the fat globule surface was lower than that observed with the equivalent SMP systems. In contrast, after ageing, FDP-based formulations led to equivalent or higher amount of protein coverage. However, at 20 and 4 °C, a higher proportion of FDP proteins interacted with the droplet interface. Irrespective of the formulation, protein desorption occurred to a lesser extent than that observed with SMP.

In order to investigate the competitive adsorption between caseins and whey proteins, these two types of proteins were quantified at the fat globule interface in the formulations based on refined palm oil (Table 4). At 20 °C, just after preparation, for SMP-based emulsions, no selective adsorption of caseins over whey proteins was found, in comparison with the proportion of these proteins in the initial SMP mixture. It was worth noting that the casein/whey protein ratio was quite similar irrespective of the emulsifier used, although the amounts of proteins associated with the fat globule interface were significantly different (Fig. 2a). In contrast, a selective adsorption of caseins over whey proteins was observed for FDP-based emulsions, regardless of the emulsifier used. The competitive adsorption between caseins and whey proteins at the oil–water interface has been well studied [18,29]. The interfacial structures of the emulsions are closely dependent on the purity of the milk protein fractions used. This makes the results all the more difficult to interpret when industrial emulsions are studied. Nevertheless, the competitive protein adsorption process is affected by different factors [29]: (i) the relative affinity of the proteins for the surface, (ii) the probability that a protein meets a fat globule in a turbulent flow in relation to its size; (iii) the residence times of individual protein molecules at the interface in relation to their rate of unfolding. The higher adsorption capacity for caseins compared to whey proteins observed in FDP-based formulations could

Table 4
The effect of milk protein and emulsifier types on protein distribution at the fat globule surface of the refined palm oil-based emulsions, before and after ageing (24-h storage at 4 °C)

| MDG | Protein type | SMP | | FDP | |
|---|---|---|---|---|---|
| | | 20 °C | 4 °C | 20 °C | 4 °C |
| SMDG | Total adsorbed proteins (mg/ml[a]) | 11.7 ± 0.0 | 7.6 ± 3.8 | 12.1 ± 0.2 | 9.3 ± 0.4 |
| | Caseins[b,d] (%) | 81.2 ± 16.4 | 81.8 ± 16.1 | 59.6 ± 12.0 | 67.0 ± 13.5 |
| | Whey proteins[c,d] (%) | 18.8 ± 5.2 | 18.2 ± 3.6 | 40.4 ± 8.0 | 33.0 ± 6.6 |
| PUMDG | Total adsorbed proteins (mg/ml[a]) | 19.7 ± 5.3 | 11.5 ± 5.7 | 12.1 ± 0.6 | 9.6 ± 1.0 |
| | Caseins[b,d] (%) | 71.5 ± 14.3 | 86.7 ± 17.2 | 64.1 ± 12.8 | 60.0 ± 12.0 |
| | Whey proteins[c,d] (%) | 28.5 ± 5.8 | 13.3 ± 2.6 | 35.9 ± 7.1 | 40.0 ± 8.0 |

SMDG: saturated mono-di-glycerides; PUMDG: partially unsaturated mono-di-glycerides; SMP: skim milk powder; FDP: functional dairy proteins.
[a] Values are given in mg/ml of emulsion.
[b] Values corresponding to the quantification of α-, β- and κ-caseins.
[c] Values corresponding to the quantification of α-lactalbumin, β-lactoglobulin and serum albumin.
[d] Percentages of each protein type were calculated relatively to the quantity of total adsorbed proteins.

account for a quasi-equal proportion of both types of proteins in the initial mixture. This was not the case for SMP-based systems, probably due to the high initial amount of caseins. After the ageing period, for SMP-based emulsions, regardless of the emulsifier type, the casein/whey protein ratio adsorbed at the fat globule surface remained constant. Quantification of individual casein species (α-, β- and κ-caseins) did not show that a particular casein type was removed to the benefit of another (results not shown). Similar results were found with whey proteins (α-lactalbumin, β-lactoglobulin and serum albumin). In the case of FDP-based formulations, the selective adsorption of caseins remained, in agreement with the low protein desorption phenomenon observed. Whey proteins are known to displace caseins from emulsion surfaces upon temperature variations [18] or reduction in pH [29]. The absence of protein displacement could be interpreted by: (i) in the case of SMP, the amount of whey proteins was too low to compete with caseins; (ii) in the case of FDP, whey proteins led to the formation of a rather stable protein network during the emulsification process that was only slightly modified by the ageing conditions; (iii) the casein micelles were tightly and closely adsorbed at the fat globule surface preventing their displacement by whey proteins; (iv) working with protein mixture may result in different emulsion interfacial characteristics from that obtained with the purified fractions used in other studies.

Besides protein–protein competition, proteins can be removed from the interface as a consequence of competition with small-molecule surfactants (replacement mechanism). Displacement of proteins arises because surfactants lower the interfacial free energy more effectively than proteins at low temperature [4,11,29]. Fat crystallization in emulsion may also promote the positioning of the emulsifier at the interface of the droplets to the detriment of proteins [12,30]. In the case of FDP-based emulsions, upon ageing, protein desorption was correlated to the size modification, desorption being all the more important than decrease in size was marked (Fig. 3). This type of correlation suggested strongly that the main parameter influencing the emulsion characteristics was the proteins at the interface and their behavior upon temperature. In other words, the FDP droplet coverage characteristics were not directly influenced by fat and emulsifier structural modifications related to partial crystallization, and to fat and emulsifier fatty acid compositions. However, the interactions between fat, protein and the low molecular weight emulsifier might have occurred during the homogenization step as illustrated by the variations of the size parameters upon fat formulation. The poor variation of protein droplet coverage upon ageing was also in good agreement with the existence of a protein network around fat globules with weak direct interactions with the interface. In contrast, in SMP systems, the dependence of the protein amount at the fat globule surface on the emulsifier type and the oil phase nature may account for specific interactions between fat and emulsifiers probably occurring by the hydrophobic parts of the molecules. It is possible that an optimal fit between the fatty acids of the fat and that of the emulsifier involved more surface-active crystals [11] and/or a different organization of the emulsifier at the interface leading to more protein desorption [2]. The

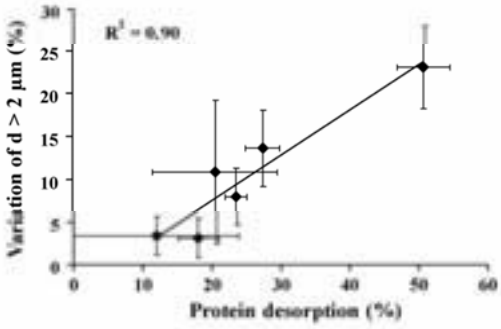


Fig. 3. Correlation between the variation of the percentage of particles with diameters greater than 2 µm ($d > 2$ µm) and the protein desorption for FDP-based formulations. The variation of $d > 2$ µm was calculated from data in Table 3, considering the values at 20 °C and after the ageing period (4 °C, 24 h). The protein desorption corresponded to the variation of the protein associated at the fat globule surface before and after the ageing period (data in Fig. 2b).

presence of increased amount of kinks in the fatty acid chains of the oil, due to double bonds, would decrease the fit with the emulsifier leading to a low influence of the emulsifier nature on protein desorption (Fig. 2a).

### 3.3. Rheological parameters of the oil-in-water emulsions

The viscosity was measured on emulsions formulated with the various fat, emulsifier and protein types, after homogenization at 20 °C and after ageing (24 h at 4 °C). At 20 °C, emulsions exhibited low viscosities regardless of their compositions (Table 5). These viscosity values were neither well correlated with the oil droplet size of emulsions nor to the percentage of particles with diameters greater than 2 μm that reflected droplet aggregation. The ageing period induced a drastic increase in the viscosity in all emulsions. It was worth noting that irrespective of the protein type considered, the increase in viscosity could not be attributed to a variation of the globule size in the emulsion. In the case of SMP-based emulsions, the different factors influencing the increase of viscosity were presented in Granger et al. [4]. In particular, the increased protein content in the water phase induced by protein desorption, in the presence of a low molecular weight emulsifier, could result in depleted flocculation of the emulsion droplets and thus, to an increase in the viscosity [31]. The increase in viscosity observed with FDP could be due to an increase of droplet concentration due to disaggregation but cannot be attributed to a variation of the aqueous phase viscosity itself, since no (or few) protein desorption was observed. Moreover, it was worth noting that the emulsion composed of FDP-partially unsaturated MDG-refined coconut oil exhibited a peculiar behavior with a high viscosity. Thus, viscosity values resulted not only from one parameter (droplet size, amount of protein in the aqueous phase, crystallized fat matter) but were rather the consequence of complex protein–emulsifier–fat interactions.

Table 5
The effect of milk protein, emulsifier and fat types on viscosity (Pa s) (shear rate, 1 $s^{-1}$) of the oil-in-water emulsions before and after ageing (24-h storage at 4 °C)

| Protein | MDG | Fat | Viscosity (Pa s) | |
|---|---|---|---|---|
| | | | 20 °C[a] | 4 °C[a] |
| SMP | Saturated | HCO | 0.1 ± 0.1 | 0.7 ± 0.2 |
| | | RCO | 0.2 ± 0.2 | 0.7 ± 0.1 |
| | | RPO | 0.1 ± 0.1 | 0.9 ± 0.1 |
| | Partially unsaturated | HCO | 0.3 ± 0.0 | 0.7 ± 0.2 |
| | | RCO | 0.2 ± 0.0 | 0.6 ± 0.2 |
| | | RPO | 0.1 ± 0.1 | 0.7 ± 0.1 |
| FDP | Saturated | HCO | 0.1 ± 0.1 | 0.7 ± 0.1 |
| | | RCO | 0.1 ± 0.0 | 0.8 ± 0.3 |
| | | RPO | 0.1 ± 0.0 | 0.8 ± 0.2 |
| | Partially unsaturated | HCO | 0.4 ± 0.0 | 1.2 ± 0.3 |
| | | RCO | 0.2 ± 0.1 | 2.4 ± 0.5 |
| | | RPO | 0.5 ± 0.1 | 0.6 ± 0.0 |

MDG: mono-di-glycerides; HCO: hydrogenated coconut oil; RCO: refined coconut oil; RPO: refined palm oil; SMP: skim milk powder; FDP: functional dairy proteins.
[a] Values are means ± S.D. ($n = 2$).

The storage ($G'$) and loss ($G''$) moduli were determined on aged emulsions for the different formulations. Following the rheological behavior allowed detecting stable and unstable emulsions in a defined frequency range. Typical curves of these two types of emulsions based on hydrogenated coconut oil and SMP are reported in Fig. 4. Stable emulsions were characterized by $G'$ values being always higher than $G''$ values. These emulsions exhibited an elastic behavior. In contrast, unstable emulsions were elastic at high frequencies ($G' > G''$) and more viscous at low frequencies ($G'' > G'$). Table 6 reports the stability characteristics of the various emulsions according to the relative position of $G'$ and $G''$ as a function of frequency in the range of 0.05–5 Hz. Emulsion stability was related to the formulation. Most of the emulsions containing the partially unsaturated emulsifier could be considered as stable in the frequency range studied, whereas the majority of the preparations based on the saturated emulsifier were unstable. The analysis of damping factor ($\tan\delta = G''/G'$) was interpreted in terms of "solid-like" emulsions ($\tan\delta < 1$) and "liquid-like" emulsions ($\tan\delta > 1$). At high frequencies (2.7 Hz), all formulations exhibited solid-like behaviors (Table 6). This behavior may be related to protein gelation and/or an aggregation of denatured molecules with a certain degree of order, resulting in the formation of a continuous network [32]. However, the formulation based on FDP-partially unsaturated MDG-refined coconut oil was characterized by at least tenfold higher $G'$ and $G''$ values than the other formulations (results not shown). In this case, higher amounts of non-covalent bonds among proteins around the fat globules (e.g., hydrogen bonding, hydrophobic interactions) may help in stabilizing and strengthening the gel structure. This hypothesis was supported by the fact that high droplet mean diameters were measured after ageing (Table 3) and that no significant protein desorption was observed (Fig. 2b). The fact that this behavior was found for refined coconut oil, which was intermediate in terms of fatty acid chain length and unsaturation degree, suggested that specific interactions

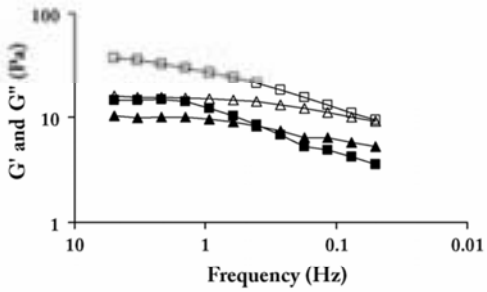


Fig. 4. Typical dynamic storage modulus (squares) and loss modulus (triangles) as a function of frequency for emulsions based on hydrogenated coconut oil, SMP and saturated MDG: (■) and (▲) and unsaturated MDG: (□) and (△).

Table 6
The effect of milk protein, emulsifier and fat types on rheological characteristics of the oil-in-water emulsions after ageing (24-h storage at 4 °C)

| Protein | MDG | Fat | Stability characteristics[a] | tan δ (2.7 Hz)[b] |
|---|---|---|---|---|
| SMP | Saturated | HCO | Unstable | 0.7 ± 0.0 |
| | | RCO | Stable | 0.4 ± 0.0 |
| | | RPO | Unstable | 0.4 ± 0.0 |
| | Partially unsaturated | HCO | Stable | 0.4 ± 0.0 |
| | | RCO | Stable | 0.3 ± 0.0 |
| | | RPO | Stable | 0.4 ± 0.0 |
| FDP | Saturated | HCO | Unstable | 0.5 ± 0.1 |
| | | RCO | Unstable | 0.6 ± 0.1 |
| | | RPO | Stable | 0.4 ± 0.0 |
| | Partially unsaturated | HCO | Unstable | 0.6 ± 0.0 |
| | | RCO | stable | 0.3 ± 0.0 |
| | | RPO | stable | 0.4 ± 0.1 |

MDG: mono-di-glycerides; HCO: hydrogenated coconut oil; RCO: refined coconut oil; RPO: refined palm oil; SMP: skim milk powder; FDP: functional dairy proteins.

[a] Stability was determined by relative position of $G'$ and $G''$ as a function of frequency (0.05–5 Hz). Stable emulsions were characterized by $G'$ values being always higher than $G''$ values, in this frequency range. Otherwise, emulsions were unstable.

[b] $\tan\delta = G''/G'$. Values are means ± S.D. ($n = 2$).

may occur with a particular amount of unsaturated fatty acids that vanished when this amount became too high.

## 4. Conclusions

Our results showed that interactions actually occurred between the different components of the emulsion. These interactions took place at different steps of the process, i.e., during the homogenization and/or ageing phases. In the case of FDP-based formulations, the interactions were mainly illustrated by the fat globule size parameters just after emulsification and by the rheological behavior after ageing. In contrast, in SMP systems, only the characterizations of initial protein adsorption and desorption upon ageing pointed out the influence of formulation. These interactions resulted in: (i) different protein organization at the interface; (ii) the competitive adsorption of milk proteins and MDG; (iii) the affinity of the hydrophobic parts of the molecules and especially the fit between the fatty acids of the MDG and the oil phase. The occurrence of supplementary aeration and freezing steps in ice cream process should again modify these interactions so that, the influence of formulation on the final characteristics and stability of aerated frozen desserts is also worth investigating.

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# Direct quantification of protein partitioning in oil-in-water emulsion by front-face fluorescence: Avoiding the need for centrifugation

C. Granger[a,b], P. Barey[b], J. Toutain[c], M. Cansell[a,*]

[a] Laboratoire "Milieux dispersés alimentaires: physico-chimie, formulation et vectorisation nutritionnelle", ISTAB, Avenue des Facultés, F-33405 Talence Cedex, France
[b] Degussa Food Ingredients, Business Line Texturant Systems France SAS, France
[c] Laboratoire TREFLE U.M.R. 8508 site Université Bordeaux 1, ENSAM, ENSCPB, 16 Avenue Pey Berland, F-33607 Pessac Cedex, France

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**Abstract**

The quantification of proteins adsorbed at the oil-in-water interface is often difficult since it requires separation of fat globules from the aqueous phase that may damage the fat globule size and/or modify the interfacial composition. Front-face fluorescence spectroscopy was used to characterize the protein partitioning between the aqueous and oil phases of emulsions without separating these two phases. Different emulsions based on skim milk powder (SMP), two mono- and di-glyceride (MDG) mixtures (saturated and partially unsaturated), and three fats (hydrogenated and refined coconut oils and refined palm oil) were studied. The impact of an ageing period (24 h at 4 °C) was also investigated to typify the first step of ice cream processing. The emulsions were characterized for protein partitioning, immediately following emulsification and after ageing, using the Bradford spectrophotometric method, applied to the aqueous phase recovered after emulsion centrifugation. In parallel, the emulsions were characterized by their tryptophan emission fluorescence spectra. The area of the peaks at 333 nm, of the fourth-derivative fluorescence spectra corresponding to the amount of proteins present in the aqueous phase of emulsions, was well correlated with the Bradford measurements ($r^2 = 0.91$). This amount was also calculated from the fluorescence calibration curve obtained with SMP in solution. In conclusion, front-face fluorescence spectroscopy appeared to be a powerful and simple technique allowing the quantification of different populations of protein in an emulsified system, i.e., in the aqueous phase and loaded at the fat globule interface.


*Keywords:* Oil-in-water emulsion; Front-face fluorescence spectroscopy; Protein quantification

## 1. Introduction

Protein adsorption and desorption are key stages in ice cream processing since they influence the final product characteristics. Indeed, it is generally agreed that, during the homogenization step, proteins initially adsorb at the fat droplet interface. However, they are partially squeezed out from the surface during the low temperature ageing step, through a competitive mechanism with low molecular weight emulsifiers [1]. Numerous studies have demonstrated that the amount of proteins loaded at the fat globule interface depended on the formulation, i.e., the protein type [2], the nature of the low molecular weight emulsifier [1–6] and/or the fat characteristics [3,5,7–9]. Several methods are available to quantify the amount of proteins present in aqueous phase, but they generally require the separation of the fat fraction by centrifugation when emulsified systems are studied. Although centrifugation is a well-documented method in the case of emulsions and mixes [3,5,6], the centrifugation parameters have to be carefully checked, otherwise the values obtained can be underestimated due to the effect of partial coalescence during the separation step.

* Corresponding author. Tel.: +33 5 40 00 34 53; fax: +33 5 40 00 24 72.
*E-mail address:* m.cansell@istab.u-bordeaux1.fr (M. Cansell).

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In this context, the choice of a direct method that would provide information on the presence of adsorbed and/or free proteins is worthwhile. For several years, fluorescence spectroscopy has been used to characterize protein structure and protein interactions [10,11]. More recently, the development of front-face fluorescence spectroscopy allowed the characterization of powdered, turbid and concentrated samples that were either model emulsions [12–15] or food samples such as milk emulsions [16–18], cheese products [19], and meat emulsions [20]. In particular, it was shown, using the fourth-derivative of the emission spectra, that the intrinsic fluorescence of tryptophanyl residues was sensitive to its aqueous and hydrophobic environments [14].

The aim of the present study was to validate the use of front-face fluorescence spectroscopy in emulsion and to quantify, by this method, the amount of protein adsorbed at the oil-in-water interface and/or that present in the aqueous phase of the emulsion. Oil-in-water emulsions were formulated with ingredients classically used in the ice cream domain. In order to beneficiate a large range of adsorbed protein concentrations, different emulsion formulations were studied, i.e., based on two mono- and di-glycerides (MDG) mixtures, varying in fatty acid chain length or unsaturation degree, and three vegetable fats characterized by different triacylglyceride composition. The effect of temperature known to be involved in the protein desorption process was also investigated.

## 2. Experimental procedures

### 2.1. Materials

Three different vegetable fats, i.e., hydrogenated coconut oil, refined coconut oil and refined palm oil (SIO, Bougival, France) were used for the preparation of oil-in-water emulsions. Skim milk powder (SMP) (Coopérative d'Isigny Saint-Mère, Isigny Sur Mer, France) contained 39 wt.% proteins represented by caseins (80 wt.%) and whey proteins (20 wt.%). Two lipid emulsifiers consisting of a mixture of MDG (60% min. mono-glycerides) were supplied by Degussa Food Ingredients (Baupte, France). The partially unsaturated MDG contained oleic acid (18:1; 20 wt.% of total fatty acids) and linoleic acid (18:2; 3 wt.% of total fatty acids) in contrast to the saturated MDG which comprised mostly palmitic acid (16:0; 56 wt.% of total fatty acids) and stearic acid (18:0; 42 wt.% of total fatty acids).

### 2.2. Emulsion preparation

Oil-in-water emulsions were composed as follows: 8 wt.% vegetable fat, 10 wt.% SMP, 0.3 wt.% saturated or partially unsaturated MDG adjusted to 100% with water. Emulsions were prepared as described in [3]. Briefly, two premixes, one containing the fat and emulsifier, the other the aqueous protein solution, were pre-heated separately at 70 °C. After, they were mixed, brought to 90 °C and immediately homogenized under constant stirring with a two-step homogenizer (Manton Gaulin SA 1200 AB, Holland) operating at a pressure of 17.5 MPa first stage, 3.0 MPa second stage. After homogenization, the emulsions were first cooled to 20 °C, then, aged for 24 h at 4 °C under stirring. Each formulation was prepared in triplicate at least. When used as a standard, SMP solution was prepared following the same experimental procedure.

### 2.3. Protein quantification

Freshly homogenized emulsions and aged preparations were centrifuged in order to separate the oil phase and the aqueous phase (15,000 × *g*, 30 min, 20 or 4 °C, Suprafuge 22, Heraeus Sepatech) [21]. Total protein content of the aqueous phase was determined using the Bradford method [22]. Analyses were performed in duplicate at least on each fresh emulsion.

Fluorescence spectra were recorded using a Fluorolog®-3 spectrofluorimeter (Jobin Yvon, Horiba, France) mounted with a front-face accessory. The incidence angle of excitation radiation was set at 22.5°. Emission spectra were recorded using a charged-coupled device (CCD) detector (270 M imaging spectrograph, Jobin Yvon, Horiba, France) with excitation and emission slits set at 0.4 and 1 nm, respectively. Samples were placed in a quartz cell with 1 mm optical pathway. The spectra were collected at $20.0 \pm 0.2$ and $4.0 \pm 0.2$ °C. Temperature regulation was ensured by a thermostatically controlled accessory (Neslab RTE-7 Digital plus, Thermo Neslab, France). Emission spectra of tryptophan were recorded from 310 to 360 nm with the excitation wavelength set at 290 nm. Ten fluorescence spectra were accumulated on each sample. The measurements were made in quadruplicate on the different samples. Emulsions were diluted 1:40 or 1:50 with water before analysis.

Calculation of the fourth-derivative of each experimental fluorescence spectrum was performed as described in [23]. Briefly, the fluorescence spectra were first smoothed using a filtering algorithm derived from the approximation theory by feed-forward neural network with a single hidden layer and a linear output layer on noisy scattered data. Under the reasonable hypothesis that the spectrum with respect to the wavelength was a continuous function, it was approximated as a linear combination of sigmoids. This so-called "Tikhonov regularization problem's" consisted in minimizing a target function thanks to an experimental data set at every experimental wavelength. This led to a least-square problem which was solved by a Levenberg–Marquardt algorithm, known to be efficient and robust. After derivation, the fourth-derivative spectra revealed the existence of several peaks. The area under each peak was calculated. This mathematical treatment was applied on fluorescence spectra obtained for emulsion and SMP in solution. For emulsion, the amount of proteins in the aqueous phase was calculated using the SMP calibration curve. In this case, a corrective factor equal to 2 was applied irrespective of the formulation and the temperature studied.

Table 1
Effect of fat type and emulsifier nature used in formulation on protein amount in the aqueous phase of oil-in-water emulsions

| Proteins in the aqueous phase ($g\,l^{-1}$)[a] | | Spectrophotometry[b] | | Fluorescence | |
|---|---|---|---|---|---|
| MDG[c] | Fat | 20 °C | 24 h at 4 °C | 20 °C | 24 h at 4 °C |
| Saturated | Hydrogenated coconut oil | 24.6 ± 2.6 | 32.5 ± 2.1 | 25.9 ± 0.6 | 30.4 ± 1.1 |
| | Refined coconut oil | 24.2 ± 0.3 | 31.7 ± 0.4 | 25.5 ± 0.2 | 29.8 ± 0.4 |
| | Refined palm oil | 23.4 ± 3.5 | 27.1 ± 0.5 | 24.9 ± 0.0 | 29.2 ± 0.7 |
| Partially unsaturated | Hydrogenated coconut oil | 21.2 ± 3.0 | 37.7 ± 2.2 | 24.1 ± 0.8 | 31.9 ± 0.6 |
| | Refined coconut oil | 20.7 ± 2.8 | 39.1 ± 0.6 | 23.7 ± 0.6 | 32.6 ± 0.1 |
| | Refined palm oil | 21.4 ± 2.7 | 27.0 ± 1.8 | 24.1 ± 0.9 | 29.6 ± 0.9 |

[a] Each listed value is the average of, at least, triplicate sets of measurements ± standard deviation ($n = 3$).
[b] The amount of proteins in the aqueous phase was measured using the Bradford method after the removal of the fat phase by centrifugation.
[c] Mono- and di-glycerides.

## 3. Results and discussion

Table 1 presents the amount of proteins in the aqueous phase quantified using the Bradford method after separation of the fat droplets by centrifugation. These results are in agreement with that of a previous study that demonstrated that the amount of proteins adsorbed at the oil-in-water interface and/or desorbed during the ageing step depended on the emulsion formulation [3]. Briefly, when the saturated MDG was considered, at 20 °C just after homogenization, the amount of proteins present in the aqueous phase slightly varied from 24.6 $g\,l^{-1}$ with the more saturated fat (hydrogenated coconut oil) to 23.4 $g\,l^{-1}$ for the more unsaturated oil (refined palm oil). With the partially unsaturated MDG, the protein droplet coverage was not influenced by the fat composition (about 21 $g\,l^{-1}$ of proteins in the aqueous phase). After the ageing period (24 h at 4 °C), for the saturated MDG-based formulations, the amount of proteins in the aqueous phase was dependent on the lipid phase used (between 27.1 and 32.5 $g\,l^{-1}$) but to a lesser extent than with the partially unsaturated MDG (between 27.0 and 37.7 $g\,l^{-1}$). In this latter case, protein desorption ranged from 32% for refined palm oil to more than 92% for hydrogenated and refined coconut oils. These results were interpreted in terms of interactions between the oil phase, low molecular weight emulsifier and protein as discussed elsewhere [3].

The purpose of the present study was to validate the use of fluorescence spectroscopy in the front-face mode by correlating fluorescence parameters with the data obtained from the Bradford test. The presence of an intrinsic fluorescent probe in milk proteins, i.e., tryptophanyl residues, prevented the use of an external dye and, therefore, the occurrence of additional interactions between the interfacial components and the fluorophore. The tryptophanyl residues present in bovine serum albumin have been shown to be accurate fluorescent dyes to follow protein structural changes and to investigate protein interactions at the hydrophobic interface [14,15]. More precisely, front-face fluorescence allowed distinguishing tryptophanyl residues in hydrophilic and hydrophobic environments, assigned to proteins loaded at the oil–water interface and proteins in the aqueous environment, respectively. In this work, we hypothesized that trypophanyl residue fluorescence could actually probe milk proteins on the whole since these residues were present in the different protein types, i.e., α-, β- and κ-caseins and whey proteins (α-lactalbumin, β-lactoglobulin, serum albumin).

Fig. 1 presents the front-face emission spectra (excitation wavelength: 290 nm) of an emulsion based on refined palm oil and saturated MDG just after homogenization and after ageing (24 h at 4 °C). The wavelengths of the maximum fluorescence intensity were located at 336.4 and 335.4 nm, for the emulsions at 20 and 4 °C, respectively. Moreover, fluorescence intensity measured in the aged emulsion was significantly higher than that measured in the emulsion at 20 °C. These spectral differences were observed irrespective of the emulsion formulation (results not shown). The temperature-dependence of both the maximum fluorescence intensity wavelength and the spectral intensity is a well-known phenomenon [10,11]. This accounts, at least partly, for an incomplete solvent relaxation prior to emission when the solvent viscosity increases at low temperature [10]. Besides this temperature effect, protein desorption that occurred during the ageing process placed part of the tryptophanyl residues in a more hydrophilic environment and, thus, should also influence the spectral patterns as already reported in the case of bovine serum albumin [14,15].

At a given temperature, even for emulsions characterized by a different partitioning of the proteins between the

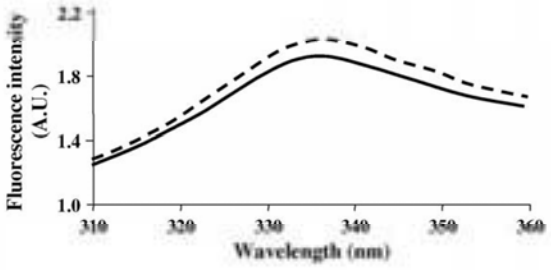


Fig. 1. Front-face emission fluorescence spectra of tryptophanyl residues of milk proteins-stabilized emulsion based on refined palm oil and saturated MDG, at 20 °C, just after homogenization (continuous line) or at 4 °C, after a 24 h ageing period (dashed line).

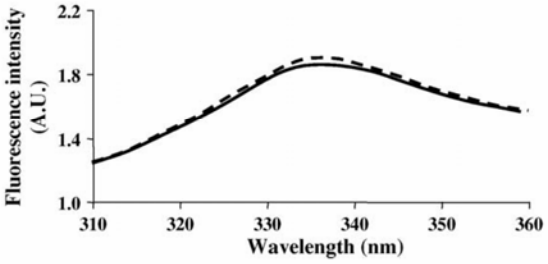


Fig. 2. Front-face emission fluorescence spectra of tryptophanyl residues of milk proteins-stabilized emulsions based on the partially unsaturated MDG and refined palm oil (27.0 g $l^{-1}$ of proteins in the aqueous phase) (continuous line) or hydrogenated coconut oil (37.7 g $l^{-1}$ of proteins in the aqueous phase) (dashed line). Spectra are obtained on aged emulsions (24 h at 4 °C).

aqueous and the fat phases, the fluorescence intensity spectra of the tryptophanyl residues in emulsions appeared very similar both in intensities and maximum fluorescence intensity wavelengths. As an example, Fig. 2 presents the emission fluorescence spectra, at 4 °C, for an emulsion based on the partially unsaturated MDG and refined palm oil (27.0 g $l^{-1}$ of proteins in the aqueous phase) or hydrogenated coconut oil (37.7 g $l^{-1}$ of protein in the aqueous phase). Thus, direct spectral analysis did not allow the quantification of the amount of proteins adsorbed onto the fat globule surface and/or in the aqueous solution. As already reported [13,14], only the fourth-derivative spectra clearly differentiated tryptophanyl residues in hydrophilic and hydrophobic environments. Typical fourth-derivative spectral patterns are given, in Fig. 3, for the two emulsions mentioned above. The peak at 333 nm was assigned to tryptophanyl residues of proteins in an aqueous environment, whereas the peak at 319 nm was attributed to tryptophanyl residues in a more hydrophobic environment, corresponding to proteins interacting with the oil interface. In order to quantify these two protein populations, the area under each peak was determined. The areas corresponding to the peak at 319 nm could not be accurately correlated with the amount of proteins loaded at the fat globule surface determined with the Bradford test. In contrast, for the different formulations tested, a good correlation ($r^2 = 0.91$) was found between the areas of the peak at 333 nm and the amount of

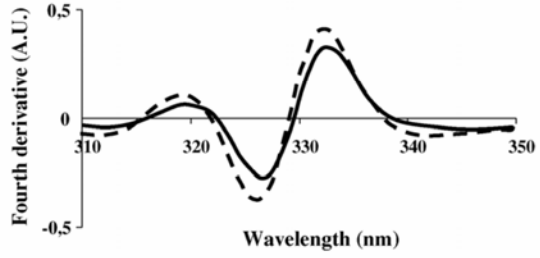


Fig. 3. Fourth-derivative emission fluorescence spectra of tryptophanyl residues of milk proteins-stabilized emulsions based on the partially unsaturated MDG and refined palm oil (27.0 g $l^{-1}$ of proteins in the aqueous phase) (continuous line) or hydrogenated coconut oil (37.7 g $l^{-1}$ of proteins in the aqueous phase) (dashed line). Spectra are obtained on aged emulsions (24 h at 4 °C).

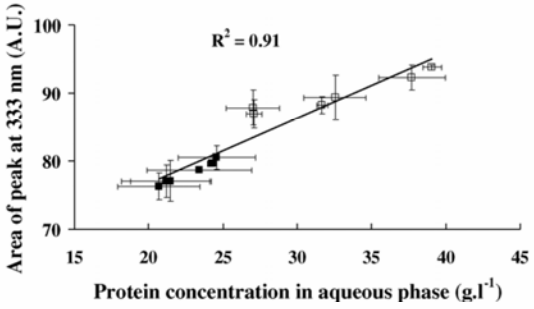


Fig. 4. Correlation between the areas of the peak at 333 nm determined by the fourth-derivative emission fluorescence spectra of oil-in-water emulsions and the amounts of proteins in the aqueous phase determined by the Bradford test after centrifugation. Emulsions just after homogenization at 20 °C (■). Emulsions after the ageing period 24 h at 4 °C (□).

proteins in the aqueous phase measured by spectrophotometry (Fig. 4).

In parallel, areas of the peak at 333 nm of the fourth-derivative emission fluorescence spectra were obtained for SMP solutions at 20 and 4 °C. Fig. 5 presents the corresponding calibration curve. It is worth noting that, in the protein concentration range studied, a single curve was obtained irrespective of the temperature. The amount of proteins present in the aqueous phase of stabilized emulsions was calculated from the SMP calibration curve and the areas of the peak at 333 nm of the fourth-derivative emission fluorescence spectra of the emulsions. Results are reported in Table 1. When compared with the values obtained with the Bradford method, for each emulsion formulation, similar results were found regardless of the temperature.

In conclusion, several studies have previously demonstrated that front-face fluorescence was an accurate method to distinguish between tryptophanyl residues in different environments, i.e., related to proteins loaded at the fat globule interface and in solution. To our knowledge, these studies did not manage to quantify the proportions of these two protein types. The development of a powerful mathematical treatment of the emission fluorescence spectra allowed determination of: (i) the area corresponding to the peak attributed to proteins in the aqueous phase, and (ii) the corresponding quantity using a SMP fluorescence calibration curve. Thus,

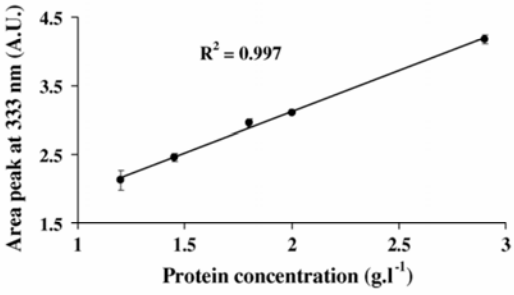


Fig. 5. Variation of the area of the peak at 333 nm determined by the fourth-derivative emission fluorescence spectra of SMP solutions as a function of the total protein concentration.

the protein partitioning was quantified directly on the emulsion without the possible artifacts of centrifugation.

## Acknowledgments

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## I.5. Conclusion

The results obtained on oil-in-water emulsions suggested that not only the surfactant molecules, *i.e.*, emulsifier and protein, but also the fat used in the formulation participated in the development of the characteristics of the oil droplet interface and of the rheological emulsion properties. Several methods characterizing the fat globule size distribution, the amount of protein adsorbed at the fat globule surface and some rheological parameters were used to assess the oil-in-water emulsion properties. Each method enlightened on the role of one or more specific ingredients (protein mixture, emulsifier type, fat nature) or of a process parameter (ageing time or temperature). As a result, no correlation could be found between the different methods used, for the entire set of formulations studied. However, for selected formulations like those based on FDP, protein desorption was correlated with the variation in mean diameters of fat globules, desorption being all the more important than the decrease in size was marked. Thus, it seemed necessary to cross different methods in order to better characterize the oil-in-water emulsion on the whole.

As expected from the chemical composition and the heat treatment, the two protein mixtures were well discriminated. Although the protein amount was not held constant for SMP-, and FDP-based formulations, the values obtained for the total adsorbed proteins at the fat globule interface showed that protein amount was not limiting with FDP for fat globule coverage. Thus, the differences between FDP and SMP were more likely to be due to different protein network formation. For **SMP-based formulations**, protein desorption during the ageing period was influenced by both the emulsifier type and the fat nature. Regardless of the fat used, partially unsaturated MDG was more effective at displacing milk proteins from the droplet surface than the saturated emulsifier. However, as the fatty acid unsaturation degree of the fat increased, the influence of the emulsifier type on protein desorption tended to vanish. This suggested that SMP allowed the development of fat/emulsifier interactions at the expense of fat/protein interactions. For **FDP-based formulations**, the protein network limited the apparent protein desorption phenomenon during ageing. It could be hypothesized that the protein/protein interactions prevailed over the protein/emulsifier or protein/fat interactions. It is worth noting that some formulations exhibited unexpected behaviors. Formulation based on hydrogenated coconut oil / saturated MDG showed significant lower amount of adsorbed protein at the fat globule surface. The formulation based on refined coconut oil / partially unsaturated MDG developed a high viscosity.

# II. ICE CREAM MIXES

## II.1. Résumé

Au niveau de sa composition, le mix de crème glacée se différencie de l'émulsion huile dans eau par la présence de sucres et de polysaccharides. En revanche, les proportions d'émulsifiant, de protéine et de matière grasse restent inchangées comparées à celles des émulsions. Etant donné que le process de fabrication du mix de crème glacée comprend un refroidissement rapide à 4°C après pasteurisation, les analyses sur le mix sont réalisées uniquement à 4°C, et non plus à 20°C comme dans le cas des émulsions. Les mixes sont caractérisés par la distribution granulométrique des globules gras et leurs propriétés rhéologiques. La cristallisation de la matière grasse du mix est étudiée par analyse thermique différentielle.

Pour les mixes à 4°C, la nature du mélange protéique (SMP ou FDP) est le facteur déterminant la **distribution granulométrique** des globules gras. Les formulations à base de SMP sont caractérisées par des globules gras de plus petite taille et un pourcentage plus faible de particules ayant un diamètre supérieur à 2 μm que les formulations à base de FDP qui présentent des proportions de particules agrégées élevées. De plus, le temps de maturation (jusqu'à 16 h) n'influence pas de manière significative les paramètres de taille des globules gras pour les formulations à base de SMP indiquant que les mixes sont stables. Les mixes à base de FDP maturés présentent des évolutions variables selon les formulations, *i.e.*, une agrégation persistante voire amplifiée ou une désagrégation partielle. De manière générale, pour une matière grasse donnée, les formulations contenant le MDG insaturé contiennent des globules gras de plus grand diamètre que celles préparées avec le MDG saturé. De plus, quel que soit l'émulsifiant, les matières grasses composées d'acides gras saturés, *i.e.*, huiles de coprah hydrogénée et raffinée conduisent à des tailles de globules gras plus importantes.

A 4°C, les mixes développent de faibles **viscosités apparentes**, entre 69 et 118 cP, quelle que soit la composition du mix. Aucune influence significative du type de matière grasse, d'émulsifiant ou de protéine n'est mise en évidence. Après maturation, la viscosité apparente augmente, généralement, de 20 % par rapport à la valeur à 4°C. Cependant, les mixes composés d'huiles de coprah, de MDG partiellement insaturé et de FDP présentent un comportement particulier avec une augmentation de la viscosité d'un facteur 10 au minimum.

L'émulsification de la matière grasse entraîne une modification des **profils de cristallisation** par rapport à la matière grasse en phase continue. La dispersion de la matière grasse dans les mixes de crème glacée conduit à l'apparition d'un nouveau pic de cristallisation au-dessus de 30°C qui pourrait correspondre à la cristallisation du MDG en association avec la matière grasse. Pour certaines formulations notamment à base de SMP, une diminution de la température du pic principal de cristallisation est observée par rapport à la cristallisation de la phase continue. Le remplacement des protéines du lait natives par des protéines fonctionnalisées augmente généralement la proportion de matière grasse cristallisée et lisse les différences de comportement entre la matière grasse sous forme dispersée et en phase continue.

## II.2. Introduction

Ice cream mixes only differed from oil-in-water emulsions by the presence of sugars and polysaccharides. The proportions between emulsifier, protein and fat remained unchanged compared with emulsion systems. As ice cream process consisted of a rapid cooling at 4°C after pasteurization, the analyses were performed only on mix at 4°C, and no more at 20°C as for emulsions. Mixes were characterized by droplet size distribution and rheological properties. Mix crystallization was also investigated using DSC. The purpose if this second part, was to study a system that was actually implied in ice cream processing, and to assay the differences if any between mix and emulsion with the aim to use simplified emulsion systems instead of real systems.

For mixes at 4°C, it is clear from Tables 14 and 15, that the protein mixture (SMP or FDP) was the main factor determining the **droplet size distribution** in ice cream mixes composed of various fat, emulsifier, and protein combinations. Concerning the influence of **the protein mixture**, compared with FDP, the presence of SMP in the formulations led to smaller droplet sizes and a lower percentage of $d > 2$ μm. With SMP, the droplet size distribution followed unimodal distribution with droplet sizes ranging from 0.03-10 μm. The presence of FDP in the mix formulation revealed the existence of two overlapping fat globule populations between 0.03-2 μm and 2-20 μm. After dispersion of the aggregates with an SDS solution, the particle size distribution displaced towards lower sizes close to those found for SMP-based formulations. Thus, the high particle sizes found for FDP-based systems could correspond to flocculated fat droplets. Concerning the influence of **the fat nature or the emulsifier type**, in the case of SMP-based systems, only small variations were observed, suggesting that, the homogenization

**Table 14: Effect of milk protein mixture, emulsifier and fat types on particle mean diameter (µm) of ice cream mixes before and after ageing (16 h-storage at 4°C).**

| Protein | MDG | Fat | Particle mean diameter[1] (µm) | |
|---|---|---|---|---|
| | | | 4°C - 0h | 4°C - 16h |
| SMP | Saturated | AMF | 0.6 ± 0.1 | 0.6 ± 0.0 |
| | | HCO | 0.7 ± 0.0 | 0.7 ± 0.0 |
| | | RCO | 0.7 ± 0.0 | 0.7 ± 0.0 |
| | | RPO | 0.6 ± 0.0 | 0.6 ± 0.0 |
| | Partially unsaturated | AMF | 0.8 ± 0.1 | 0.8 ± 0.1 |
| | | HCO | 0.7 ± 0.1 | 1.7 ± 0.9 |
| | | RCO | 0.8 ± 0.0 | 0.7 ± 0.0 |
| | | RPO | 0.9 ± 0.1 | 1.0 ± 0.1 |
| FDP | Saturated | AMF | 7.2 ± 0.4 | 6.7 ± 1.0 |
| | | HCO | 11.0 ± 1.71 | 12.9 ± 1.8 |
| | | RCO | 8.4 ± 2.3 | 13.7 ± 1.1 |
| | | RPO | 13.8 ± 1.2 | 9.6 ± 0.8 |
| | Partially unsaturated | AMF | 14.2 ± 0.8 | 12.9 ± 1.3 |
| | | HCO | 8.0 ± 0.4 | 21.9 ± 7.0 |
| | | RCO | 11.8 ± 0.5 | 24.9 ± 0.3 |
| | | RPO | 16.6 ± 0.4 | 14.2 ± 0.7 |

[1] Values are means of ± SD (n=6).

MDG: mono- and diglyceride mixture; AMF: anhydrous milk fat; HCO: hydrogenated coconut oil; RCO: refined coconut oil; RPO: refined palm oil; SMP: skim milk powder; FDP: functional dairy proteins.

**Table 15: Effect of milk protein mixture, emulsifier and fat types on the proportion of particles with diameters higher than 2 µm (d > 2 µm) (%) in ice cream mixes before and after ageing (16-h storage at 4°C).**

| Protein | MDG | Fat | d > 2 µm[1] (%) | |
|---|---|---|---|---|
| | | | 4°C - 0h | 4°C - 16h |
| SMP | Saturated | AMF | 4.8 ± 0.5 | 4.3 ± 0.5 |
| | | HCO | 5.3 ± 0.3 | 4.7 ± 0.7 |
| | | RCO | 4.4 ± 0.3 | 4.4 ± 0.0 |
| | | RPO | 4.5 ± 0.3 | 4.6 ± 0.2 |
| | Partially unsaturated | AMF | 5.9 ± 1.1 | 5.1 ± 0.8 |
| | | HCO | 5.9 ± 0.8 | 9.5 ± 3.2 |
| | | RCO | 5.6 ± 0.3 | 4.4 ± 0.1 |
| | | RPO | 4.6 ± 0.3 | 4.0 ± 0.5 |
| FDP | Saturated | AMF | 89.4 ± 8.4 | 77.7 ± 2.9 |
| | | HCO | 99.9 ± 0.1 | 87.6 ± 2.2 |
| | | RCO | 89.8 ± 4.6 | 84.4 ± 2.3 |
| | | RPO | 87.1 ± 1.7 | 73.9 ± 1.3 |
| | Partially unsaturated | AMF | 98.4 ± 3.4 | 98.0 ± 0.4 |
| | | HCO | 99.8 ± 0.1 | 90.4 ± 0.2 |
| | | RCO | 93.9 ± 1.9 | 92.0 ± 0.1 |
| | | RPO | 86.7 ± 0.8 | 79.2 ± 1.1 |

[1] Values are means of ± SD (n=6).

MDG: mono- and diglyceride mixture; AMF: anhydrous milk fat; HCO: hydrogenated coconut oil; RCO: refined coconut oil; RPO: refined palm oil; SMP: skim milk powder; FDP: functional dairy proteins.

pressure was the dominating factor in ice cream mix formation that determined the droplet size distribution. In the case of FDP-based formulations, mean diameters ranged from 7.2 to 24.9 μm and, in all cases, more than 74 % of the particles were involved in flocculated aggregates. For freshly prepared ice cream mixes containing FDP, the apparent droplet size varied without any specific influence of the fat and emulsifier types. The **ageing time** (until 16 h) did not influence significantly the size parameters for SMP-based formulations indicating that the ice cream mixes were well stabilized. This stability suggested that the entire oil-water interface was covered by a sufficient amount of surfactants so that, upon time, aggregation and coalescence of the fat globules were prevented. In contrast, for aged mixes containing FDP and for a given fat, partially unsaturated emulsifier-based formulations led to higher fat globule sizes than saturated ones. Moreover, the more saturated the oils (refined and hydrogenated coconut oils), the higher mean diameters observed, regardless of the emulsifier type used.

**Viscosity** was measured on the ice cream mixes before and after the ageing step. **Before ageing, at 4°C**, mixes exhibited low apparent viscosities, between 69 and 118 cP, regardless of their compositions (Table 16). No significant influence of fat, emulsifier or protein mixture was found. With the **ageing time,** the apparent viscosity increased by 20 ± 5 % in average, for almost all formulations. However, it was worth noting that the mixes composed of FDP-partially unsaturated MDG-coconut oils exhibited a peculiar behavior with a high apparent viscosity after ageing. Apparent viscosity was increased by a factor 10.1 and 12.7 for hydrogenated coconut oil and refined coconut oil, respectively. Considering the ageing time, the viscosity values were neither correlated with the oil droplet size of the ice cream mixes nor with the percentage of d > 2 μm that reflected aggregation.

The **crystallization behavior** of the different fats used was studied by DSC in bulk samples and in aged mixes. Rapid cooling conditions were applied to mimic the thermal events that occurred during ice cream processing. Compared with bulk fat, emulsification led to a modification of the DSC profiles in the case of SMP- and FDP-based formulations (Figure 4). All the mix thermograms were characterized by a small additional crystallization event above 30°C (peak 2) that was not present in the bulk samples. Table 17 presents the onset temperature and enthalpy values determined on peaks 1 and 2, for SMP or FDP and saturated MDG-based mixes. Results concerning SMP-based formulations are presented in **Granger *et al.*, 2005c** (§ C.II.3.).

**Table 16: Effect of milk protein mixture, emulsifier and fat types on apparent viscosity (cP) of ice cream mixes before and after ageing (16 h-storage at 4°C).**

| Protein | MDG | Fat | Apparent viscosity[1] (cP) | |
|---|---|---|---|---|
| | | | 4°C - 0h | 4°C - 16h |
| SMP | Saturated | AMF | 88 ± 11 | 108 ± 3 |
| | | HCO | 76 ± 16 | 101 ± 4 |
| | | RCO | 85 ± 0 | 115 ± 0 |
| | | RPO | 73 ± 4 | 85 ± 0 |
| | Partially unsaturated | AMF | 106 ± 27 | 131 ± 36 |
| | | HCO | 80 ± 0 | 124 ± 1 |
| | | RCO | 84 ± 5 | 125 ± 0 |
| | | RPO | 74 ± 5 | 104 ± 1 |
| FDP | Saturated | AMF | 69 ± 2 | 79 ± 4 |
| | | HCO | 96 ± 34 | 108 ± 3 |
| | | RCO | 75 ± 7 | 93 ± 10 |
| | | RPO | 74 ± 2 | 80 ± 3 |
| | Partially unsaturated | AMF | 74 ± 5 | 91 ± 9 |
| | | HCO | 86 ± 9 | 875 ± 45 |
| | | RCO | 118 ± 4 | 1495 ± 115 |
| | | RPO | 71 ± 1 | 114 ± 6 |

[1] Values are means of ± SD (n=2).

MDG: mono- and diglyceride mixture; AMF: anhydrous milk fat; HCO: hydrogenated coconut oil; RCO: refined coconut oil; RPO: refined palm oil; SMP: skim milk powder; FDP: functional dairy proteins.

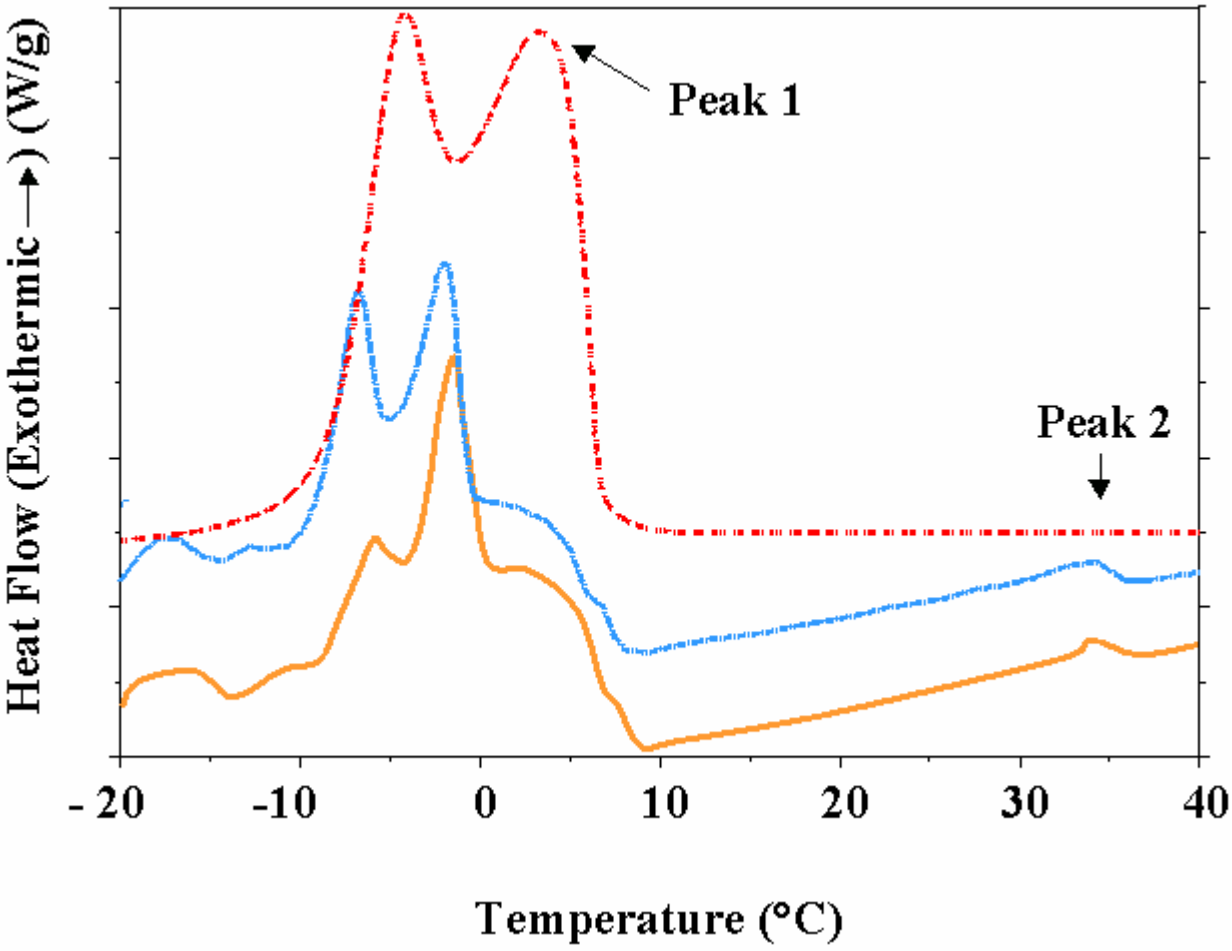


**Figure 4: Exothermic peaks obtained by DSC during cooling of refined coconut oil in the bulk phase ( – – – –) and in emulsions based on saturated mono- and diglyceride mixture and functional dairy proteins (——) and skim milk powder (·····).**

**Table 17: Effect of fat type and protein mixture on crystallization temperatures and enthalpies of DSC cooling curves of saturated MDG-based aged mixes.**

| Fat / Physical form | | Crystallized matter (%) [a] | Peak 1 [b] | | | Peak 2 [b] | |
|---|---|---|---|---|---|---|---|
| | | | Onset temperature (°C) [c] | End of peak (°C) [c] | Area (J.g-1) c | Onset temperature (°C) [c] | Area ($J.g^{-1}$) [c] |
| **Refined coconut oil** | | | | | | | |
| Bulk | | | 10.0 ± 1.8 | –18.5 ± 1.0 | 97.81 ± 2.90 | - | - |
| Emulsified | SMP | 34 ± 4 | 7.9 ± 0.4 | –14.8 ± 3.3 | 2.65 ± 0.39 | 36. 1 ± 0.2 | 0.18 ± 0.01 |
| | FDP | 37 ± 0 | 9.0 ± 0.0 | –19.1 ± 3.3 | 2.92 ± 0.08 | 38.1 ± 1.2 | 0.12 ± 0.03 |
| **Hydrogenated coconut oil** | | | | | | | |
| Bulk | | | 19.3 ± 1.1 | –22.2 ± 1.5 | 103.07 ± 1.90 | - | - |
| Emulsified | SMP | 29 ± 4 | 16.7 ± 2.8 | –14.6 ± 3.1 | 2.61 ± 0.55 | 36.3 ± 0.9 | 0.15 ± 0.02 |
| | FDP | 49 ± 6 | 21.5 ± 0.1 | –18.7 ± 0.4 | 4.00 ± 0.49 | 39.0 ± 0.9 | 0.09 ± 0.01 |
| **Refined palm oil** | | | | | | | |
| Bulk | | | 20.7 ± 0.0 | –25.3 ± 0.0 | 50.62 ± 3.80 | - | - |
| Emulsified | SMP | 57 ± 6 | 22.6 ± 0.5 | –16.0 ± 1.2 | 2.00 ± 0.25 | 38.9 ± 0.3 | 0.14 ± 0.00 |
| | FDP | 64 ± 3 | 24.1 ± 0.6 | –17.4 ± 0.0 | 2.25 ± 0.11 | 42.2 ± 0.9 | 0.08 ± 0.02 |
| **Anhydrous milk fat** | | | | | | | |
| Bulk | | | 18.0 ± 0.0 | –26.1 ± 0.0 | 59.76 ± 0.70 | - | - |
| Emulsified | SMP | 60 ± 4 | 20.9 ± 0.6 | –16.6 ± 1.1 | 2.57 ± 0.16 | 41.3 ± 1.8 | 0.14 ± 0.01 |
| | FDP | 57 ± 1 | 21.6 ± 0.2 | –13.2 ± 0.7 | 2.41 ± 0.04 | 40.9 ± 0.2 | 0.10 ± 0.00 |

[a] The percentage of crystallized fat contained in mixes was calculated according to:

$$\frac{area\ of\ \ emulsified\ \ fat\ (peak\,1)}{area\ of\ \ bulk\ \ fat\ (peak\,1)\times 0.08}\times 100$$

8 % fat content in the formulations. The amount of fat associated with MDG crystallization (peak 2) was neglected.

[b] Peaks 1 and 2 are defined as in figure 4 and in **Granger *et al.*, 2005c** (§ C.II.3.)

[c] Values are means of ± SD (n=2).

SMP: skim milk powder; FDP: functional dairy proteins.

In the following, we focus on the **influence of the protein mixture** on the mix thermal behavior. Expect in the case of anhydrous milk fat, the presence of FDP enlarged the principal peak of crystallization (peak 1) and was therefore associated with an increase in the amount of crystallized matter. This was especially the case for the formulation based on the totally saturated fat (hydrogenated coconut oil). Crystallization always occurred at a higher temperature for the FDP-based mixes compared with the SMP ones. The supercooling effect observed with emulsified refined and hydrogenated coconut oils and SMP was less observed with FDP. This agrees with the hypothesis put forward for emulsions that FDP interacted poorly with fat and, thus, influenced to a lesser extent fat crystallization in the emulsified state. However, the differences found in crystallization onset temperature as a function of protein mixture used in mixes may also account for higher fat droplet aggregation (higher values of particle mean diameter and % of particles d > 2 μm) in the case of FDP-based mixes (Tables 14 and 15), as droplet-droplet interaction may influence fat crystallization (Relkin, *et al.*, 2003a).

The **additional exotherm (peak 2)** found in all emulsified systems could be attributed principally to MDG crystallization. Indeed, MDG crystallize themselves at higher temperatures than the fats used in this work (59 and 46°C for onset crystallization temperatures for the saturated MDG and partially unsaturated MDG, respectively). The fact that the characteristics of this peak both in terms of temperature and areas varied with the ingredient formulation strongly suggested the occurrence of fat/emulsifier and/or fat/protein interactions. The influence of the MDG type in SMP-based formulation is presented in **Granger *et al.*, 2005c** (§ C.II.3.). Fat/protein interactions were revealed by the analysis of peak 2 characteristics when the protein mixture was varied. For saturated MDG-based formulations, the onset temperatures of peak 2 ranged from 36 to 41°C with SMP and from 38 to 42°C with FDP (Table 17). For a given fat, compared with SMP, FDP-based mixes presented higher onset temperature of peak 2 (except for anhydrous milk fat) and lower crystallized matter. These results suggested once again that FDP poorly interacted with fat, and that, when these interactions occurred, they should involve lower amount of triacylglycerols than in SMP mix.

## II.3. C. Granger, A. Schöppe, A. Leger, P. Barey, M. Cansell (2005c). Influence of formulation on the thermal behavior of ice cream mix and ice cream. JAOCS, 82, 427-431.[3]

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# Influence of Formulation on the Thermal Behavior of Ice Cream Mix and Ice Cream

C. Granger[a,b], A. Schöppe[c], A. Leger[b], P. Barey[b], and M. Cansell[a,*]

[a]Laboratoire "Milieux dispersés alimentaires: physico-chimie, formulation et vectorisation nutritionnelle," Institut des Sciences et Techniques des Aliments de Bordeaux (ISTAB), F-33405 Talence Cedex, France, [b]Degussa Food Ingredients, Business Line Texturant Systems France SAS, F-92035 Paris La Défense, France, and [c]Degussa Food Ingredients, GmbH, D-85354 Freising, Germany

**ABSTRACT:** The influence of fat and emulsifier types on particle size and thermal behavior of aged mixes and the corresponding ice creams was investigated. Mixes and ice creams based on partially unsaturated monodiglycerides (MDG) were characterized by an increased percentage of agglomerated fat globules compared with saturated MDG-based systems. DSC thermograms obtained for refined coconut oil in mix showed a displacement of the main crystallization event toward lower crystallization temperatures compared with fat in the bulk phase. This supercooling effect was more or less pronounced for the three other fats used (hydrogenated coconut oil, refined palm oil, and anhydrous milk fat). In emulsified systems, an additional exotherm was observed that was interpreted in terms of MDG crystallization. The fact that this peak appeared at different temperatures ranging from 32 to 41°C as a function of the fat selection suggested that different fat–emulsifier interactions would occur. In the case of ice creams, although the water peak interfered with the fat peak, melting DSC curves allowed the discrimination between the fat types used in the formulation.

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**KEY WORDS:** Fat, ice cream, ice cream mix, lipid emulsifier, thermal behavior.

Ice cream is a complex polyphasic food system in which part of the dispersed phase consists of fat globules in a crystalline state. These globules are organized in a partially coalesced/agglomerated continuous 3-D network formed during the freezing and whipping steps of ice cream processing. This fat globule organization supports other microstructural elements in ice cream, such as air bubbles, and contributes greatly to the quality of the final product. The partial coalescence phenomenon and the extent of fat globule instability are greatly influenced by the amount of crystallized matter (1,2), the process (3), the size and shape of the fat crystals (2,4), the orientation of the crystals at the interface, and the surfactant type and concentration (5–8). Since each type of fat exhibits a specific polymorphism function of its TAG composition, the thermal behavior of fats during ice cream processing should influence the physicochemical properties of the intermediate and final products.

*To whom correspondence should be addressed at Laboratoire "Milieux dispersés alimentaires: physico-chimie, formulation et vectorisation nutritionnelle," ISTAB, Ave. des Facultés, F-33405 Talence Cedex, France. E-mail: m.cansell@istab.u-bordeaux1.fr

In previous studies, we showed that the nature of the fat and the degree of unsaturation of the emulsifier led to different properties of the oil-in-water emulsions (9) and ice creams (10). Although particle size characterization was relevant to discriminate between emulsifiers, this method gave poor information regarding the influence of fat type in oil-in-water emulsions and ice creams. In contrast, determination of rheological parameters and melting times appeared to be two convenient methods to illustrate the influence of fat nature although melting times were related only to room temperature. In particular, a high linear correlation was found between the storage modulus measured at 20°C and the melting time (10).

The aim of the present study was to investigate the effects of fat and emulsifier selection on the thermal characteristics of mixes and ice creams. Four types of fat and two monodiglyceride (MDG) mixtures—saturated (SMDG) and partially unsaturated (PUMDG)—were chosen since they are commonly used in ice cream products. Mixes were studied under rapid cooling conditions to mimic the thermal events that occurred during ice cream processing. The corresponding ice creams were analyzed as their temperature increased since ice creams are subjected to warming during their consumption. The mix and ice cream structures were also characterized by particle size analysis to illustrate the partial coalescence and/or agglomeration of fat globules.

## MATERIALS AND METHODS

*Ice cream preparation.* Eight different ice cream mixes were prepared based on fat (8 wt%; hydrogenated coconut oil, refined coconut oil, refined palm oil, or anhydrous milk fat), emulsifier (0.3 wt%; SMDG or PUMDG—60% α-MG), skim milk powder (10 wt%), sucrose (12 wt%), corn syrup solids (6 wt%; 40 dextrose equivalent, 80 Brix solid content), guar gum and locust bean gum mixture (0.2 wt%). All ingredients, except the fat, were dry blended, mixed with water, and immediately blended at 65°C with the melted fat portion for 15 min. Mixes were homogenized at 70°C, 170/30 bar, and pasteurized at 85°C for 30 s, cooled, and immediately stored at 4°C in a water bath. They were aged for one night at 4°C under stirring. Batches of ice cream mix (20 L) were frozen in a continuous freezer (overrun: 100%, outlet temperature: −5°C). Ice creams were hardened at −40°C and stored at −25°C. For each recipe, at least two different batches were prepared.

*Particle size analysis.* Particle size distribution of aged mixes and ice creams was measured by integrated light scattering using a Mastersizer S (Malvern Instruments, Malvern, United Kingdom). Samples were directly diluted in the sample chamber with water at approximately 1:1000. Water temperature used for dilution was at 13°C. Ultrasonication was used on ice cream samples to ensure the absence of air bubbles. Particle mean diameter (evaluated by the volume weighted average diameter, $d_{4,3}$) and the cumulative percentage of the particles with diameters greater than 2.0 μm (% particles >2 μm) were determined.

*Thermal analysis.* A TA Instruments differential scanning calorimeter (model MDSC 2920; Guyancourt, France) was used. Mixes (≈10 mg) aged at 4°C for 16 h were sealed in aluminum pans. The samples were first rapidly heated to 60°C and then cooled from 60 to −20°C at 5°C·min$^{-1}$. Ice creams (≈80 mg) stored at −20°C were equilibrated at −30°C for 10 min in the DSC instrument. The samples were heated from −30 to 65°C at 1°C·min$^{-1}$. Heat flow was recorded as a function of increasing or decreasing temperature. For each mix and ice cream preparation, the experiment was conducted at least in duplicate. The percentage of the crystallized matter in ice cream mixes was calculated, according to the thermogram of fat in the mix, on the one hand, and the thermogram of the fat in the bulk phase, on the other hand, from the ratio of the areas under the mix peak and the bulk peak. This latter was balanced by the proportion of fat in the formulation. This calculation was based on the hypothesis that no event other than fat crystallization occurred in the temperature range considered. Similar calculation was performed from ice cream melting DSC thermograms.

*Statistical analysis.* ANOVA of all data, using Fisher's LSD procedures, was made on $d_{4,3}$, cumulative % particles >2 μm, onset temperatures, and areas under the DSC peaks to discriminate among the eight formulations.

## RESULTS AND DISCUSSION

*Influence of fat and emulsifier selection on mix and ice cream particle size.* The influence of fat nature and emulsifier type on particle size analysis was studied on mixes after aging (16 h at 4°C, under stirring) and on the corresponding ice creams (Table 1). The $d_{4,3}$ was chosen to follow changes in particle size diameter. The % particles >2 μm was calculated to characterize fat globule agglomeration and/or partial coalescence (11). In the case of SMDG-based mixes, the fat nature did not influence the droplet mean diameters, which were associated with a low % particles >2 μm. With PUMDG, higher $d_{4,3}$ values were observed. These average values corresponded to a biphasic distribution (Fig. 1) and an increase in the % particles >2 μm (Table 1). Admittedly, emulsifiers do not play a major role in ice cream mix stabilization just after homogenization at high temperatures due to an excess of protein in the mix formulation. Thus, the stabilization of the fat globules against coalescence is achieved thanks to the presence of sufficient protein to cover the droplet surface fully (9,12). The influence of MDG type could account for different protein coverage of the fat droplets in the presence of competitively adsorbing emulsifiers, as observed in emulsified systems (9,12).

Irrespective of the formulation, the whipping/freezing steps led to an increase in $d_{4,3}$ in ice creams compared with the corresponding mixes (Table 1, Fig. 1). For a given fat, PUMDG was associated with a greater increase in particle size in ice creams than SMDG, which corresponded to a two- or threefold increase in % particles >2 μm. This suggested that PUMDG in ice cream was more efficient in promoting aggregation and/or partial coalescence of fat droplets than SMDG (11–14). The increase in size between mixes and ice cream was independent of fat type used for SMDG-based products, i.e., close to 64% on average. In contrast, it was influenced by the degree of unsaturation of fat,

**TABLE 1**
**Effect of Fat Type and Emulsifier Nature on Particle Size Characteristics in Aged Mixes and Ice Creams**

| | Aged mixes | | Ice creams | |
|---|---|---|---|---|
| Fat/emulsifier | $d_{4,3}$ (μm)[a] | % particles >2 μm[a,b] | $d_{4,3}$ (μm)[a] | % particles > 2 μm[a,b] |
| Refined coconut oil | | | | |
| SMDG | 0.58 ± 0.01 | 1.6 ± 0.1 | 1.43 ± 0.02 | 14.6 ± 1.3 |
| PUMDG | 1.50 ± 0.23 | 15.5 ± 2.6 | 2.53 ± 0.49 | 36.7 ± 2.1 |
| Hydrogenated coconut oil | | | | |
| SMDG | 0.58 ± 0.03 | 1.8 ± 0.3 | 1.30 ± 0.08 | 14.3 ± 1.2 |
| PUMDG | 1.72 ± 0.05 | 11.2 ± 2.1 | 2.10 ± 0.40 | 24.7 ± 4.6 |
| Refined palm oil | | | | |
| SMDG | 0.60 ± 0.00 | 2.2 ± 0.4 | 2.15 ± 0.40 | 25.1 ± 4.6 |
| PUMDG | 0.99 ± 0.07 | 6.9 ± 2.0 | 3.29 ± 0.57 | 43.7 ± 6.6 |
| Anhydrous milk fat | | | | |
| SMDG | 0.58 ± 0.01 | 3.6 ± 1.9 | 1.60 ± 0.19 | 13.6 ± 1.7 |
| PUMDG | 0.96 ± 0.21 | 5.8 ± 1.8 | 2.80 ± 0.15 | 39.1 ± 2.2 |

[a]Values are means ± SD ($n$ = 2).
[b]Percentage of particles with diameters greater than 2 μm. SMDG, saturated monodiglyceride mixture; PUMDG, partially unsaturated monodiglyceride mixture.

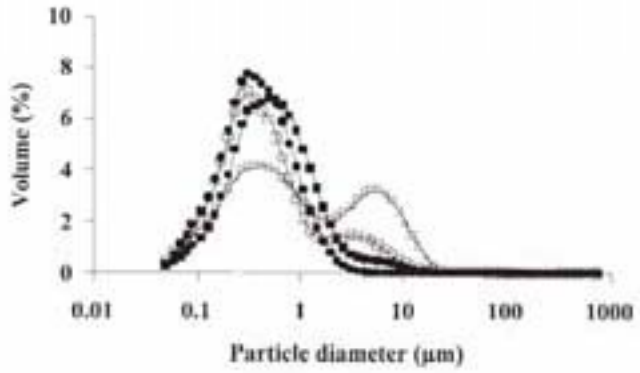


**FIG. 1.** Particle size distribution observed after dispersion in distilled water of mixes (closed symbols) and ice creams (open symbols) based on refined coconut oil and saturated monodiglycerides (●, ○) or partially unsaturated monodiglycerides (■, □).

which, in the case of PUMDG, ranged from 20 to 70%. Previous results obtained with vegetable fats suggested that fat globule destabilization was all the more important since the FA chains of the fat were unsaturated and long (10). The results obtained with anhydrous milk fat were in good agreement with this statement. They illustrated that globule stabilization occurred through interactions between the fat and the lipid emulsifier. These interactions would be favored with SMDG, i.e., when the largest hydrophobic parts of the FA chains of emulsifier and fat would fit with each other.

*Mix crystallization.* DSC crystallization curves were obtained on aged mixes and compared with that of fat in bulk phase. The first step was to warm the mix before cooling it. Fat crystallization can be influenced by fat droplet size (8) and emulsion stability (15). For each formulation, we checked that the particle size in the mix remained unchanged in the conditions of the thermal analysis (results not shown). Thus, it could be assumed that the thermal reactions of fat were not related to a modification of the interfacial composition and/or mix destabilization. Moreover, rapid cooling conditions ($5°C \cdot min^{-1}$) were used to mimic the thermal events that occurred during ice cream processing.

The amount of crystallized matter in aged mixes was calculated (Table 2). For fats composed mostly of saturated FA (coconut oils), this amount was low, i.e., close to 30%. In contrast, for refined palm oil and anhydrous milk fat, the crystallization level was high, ranging from 50 to 65%. This may be related to different crystallization kinetics between bulk and emulsions (16). Typical crystallization curves obtained for refined coconut oil in bulk phase or in emulsified systems are shown in Figure 2. In the bulk phase, refined coconut oil crystallized in a principal peak (peak 1) composed of two overlapping exothermic peaks. This suggested that some partitioning of TAG species should occur during the crystallization process (17). In emulsified systems, a small crystallization event occurred above 30°C (peak 2) that was not detected in the bulk fat thermogram. Moreover, a displacement of peak 1 toward lower crystallization temperatures was observed for mixes compared with the bulk phase. The presence of an extra thermal event around −15°C enlarged the crystallization profile of emulsified systems whereas almost all fat in bulk phase was crystallized at −10°C. The influence of MDG type on the cooling curves was illustrated by different peak 1 profiles and different onset temperatures of peak 2.

Table 2 reports the onset temperatures and enthalpy values determined for peaks 1 and 2 for both bulk and emulsified fats.

**TABLE 2**
**Effect of Fat Type and Emulsifier Nature on Crystallized Fat, Crystallization Temperatures, and Enthalpies of DSC Cooling Curves for Bulk and Emulsified Fats in Aged Mixes**

| | | | Peak 1[b] | | | Peak 2[b] | |
|---|---|---|---|---|---|---|---|
| Fat/physical form | | Crystallized matter (%)[a] | Onset temperature (°C)[c] | End of peak (°C)[c] | Area ($J \cdot g^{-1}$)[c] | Onset temperature (°C)[c] | Area ($J \cdot g^{-1}$)[c] |
| Refined coconut oil | | | | | | | |
| Bulk | | | 10.0 ± 1.8 | −18.5 ± 1.0 | 97.81 ± 2.90 | — | — |
| Emulsified | SMDG | 34 ± 4 | 7.9 ± 0.4 | −14.8 ± 3.3 | 2.65 ± 0.39 | 36.1 ± 0.2 | 0.18 ± 0.01 |
| | PUMDG | 31 ± 1 | 8.9 ± 0.0 | −17.6 ± 2.4 | 2.55 ± 0.08 | 31.9 ± 0.8 | 0.20 ± 0.02 |
| Hydrogenated coconut oil | | | | | | | |
| Bulk | | | 19.3 ± 1.1 | −22.2 ± 1.5 | 103.07 ± 1.90 | — | — |
| Emulsified | SMDG | 29 ± 4 | 16.7 ± 2.8 | −14.6 ± 3.1 | 2.61 ± 0.55 | 36.3 ± 0.9 | 0.15 ± 0.02 |
| | PUMDG | 37 ± 1 | 17.0 ± 1.4 | −16.0 ± 0.4 | 2.86 ± 0.05 | 33.7 ± 0.1 | 0.05 ± 0.00 |
| Refined palm oil | | | | | | | |
| Bulk | | | 20.7 ± 0.0 | −25.3 ± 0.0 | 50.62 ± 3.80 | — | — |
| Emulsified | SMDG | 57 ± 6 | 22.6 ± 0.5 | −16.0 ± 1.2 | 2.00 ± 0.25 | 38.9 ± 0.3 | 0.14 ± 0.00 |
| | PUMDG | 65 ± 3 | 22.3 ± 1.7 | −13.2 ± 0.1 | 2.62 ± 0.11 | 36.6 ± 0.3 | 0.07 ± 0.02 |
| Anhydrous milk fat | | | | | | | |
| Bulk | | | 18.0 ± 0.0 | −26.1 ± 0.0 | 59.76 ± 0.70 | — | — |
| Emulsified | SMDG | 60 ± 4 | 20.9 ± 0.6 | −16.6 ± 1.1 | 2.57 ± 0.16 | 41.3 ± 1.8 | 0.14 ± 0.01 |
| | PUMDG | 50 ± 3 | 16.8 ± 3.2 | −18.4 ± 1.5 | 2.41 ± 0.16 | 36.6 ± 1.7 | 0.07 ± 0.01 |

[a]The percentage of the crystallized matter contained in mixes was calculated according to: (area under peak 1 in mix × 100)/(area under peak 1 in bulk fat × 0.08). The value 0.08 is derived from the 8% fat content in the formulations. The area under peak 2 in mix was neglected.
[b]Peaks 1 and 2 are defined as in Figure 2.
[c]Values are means ± SD ($n = 2$). SMDG, saturated monodiglyceride mixture; PUMDG, partially unsaturated monodiglyceride mixture.

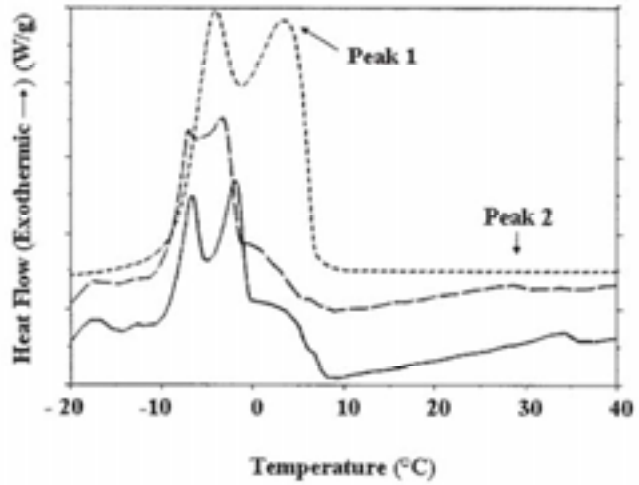


**FIG. 2.** Exothermic peaks obtained by DSC during cooling of refined coconut oil in the bulk phase (- - - -) and in emulsions based on saturated monodiglycerides (———) or partially unsaturated monodiglycerides (— — —).

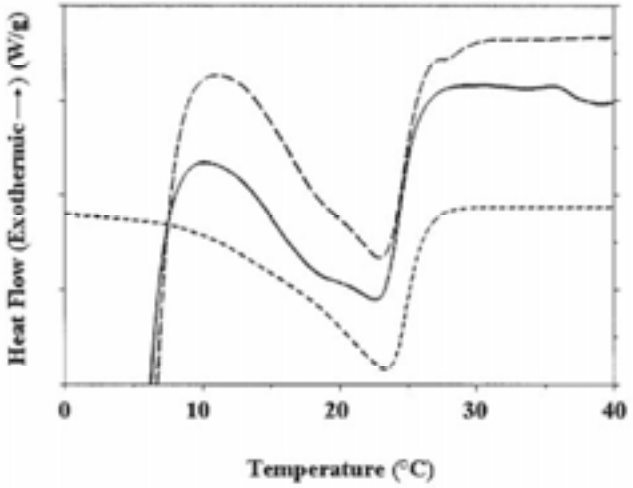


**FIG. 3.** Endothermic peaks obtained by DSC during melting of refined coconut oil in the bulk phase (- - - -) and in ice cream based on saturated monodiglycerides (———) or partially unsaturated monodiglycerides (– – –).

For fats principally composed of saturated FA, such as refined and hydrogenated coconut oils, a displacement of the onset temperature of peak 1 toward lower crystallization values was observed in mixes. This agrees with crystallization of emulsified oil requiring a degree of supercooling (2,16). The delayed fat crystallization occurring in mix systems compared with the bulk fat sample could be explained by homogeneous nucleation through spontaneous formation of nuclei in the case of emulsions, whereas in bulk oil systems nucleation is predominantly heterogeneous (16,18,19). A noncatalytic action of adsorbed molecular species also has been proposed (14). In the case of refined palm oil and anhydrous milk fat, supercooling in mix samples was not observed probably due to the larger range of crystallization temperatures of these fats. The additional exotherm (peak 2), found in all emulsion systems, could be attributed mainly to MDG crystallization. Indeed, MDG crystallize at higher temperatures than the fats used in this work. The onset temperature of peak 2 was higher in SMDG-based systems than in PUMDG ones. This agrees with saturated FA that begin to crystallize at higher temperatures than unsaturated FA. However, since different onset temperature values and enthalpy values were recorded as a function of the fat used, specific interactions between the emulsifier and the fat could not be excluded. This was especially the case for mix where PUMDG was associated with refined coconut oil (Table 2).

*Ice cream fusion.* DSC was used to characterize the thermal behavior of ice cream upon heating. Regardless of ice cream formulation, DSC patterns exhibited a peak ranging from −30 to 10°C that was attributed, to a large extent, to free water (20). When compared with the fusion thermograms of bulk fats, i.e., neglecting the emulsifying effects, the part of melted fat overlapped by the broad water melting peak in ice cream could be estimated at 11% for both refined and hydrogenated coconut oils, 38% for anhydrous milk fat, and 60% for refined palm oil. The greater overlapping obtained for the anhydrous milk fat and refined palm oil was due to their large temperature melting range (17,21). Thus, DSC thermograms were analyzed only for the two types of coconut oil in the temperature range between 10 and 40°C. Typical melting profiles are presented in Figure 3 for refined coconut oil-based ice creams formulated with SMDG or PUMDG. Melting of refined coconut oil bulk phase is also reported. The profiles of fat melting in ice cream and bulk system were similar although a shoulder in the main peak and a small thermal event around 30°C were detected in ice cream curves. In particular, nearly identical maximum melting temperatures (≈23°C) were found for bulk and emulsified systems, regardless of emulsifier nature. The difference in curve shapes between fat in bulk phase and in ice cream samples could reveal some TAG partitioning in ice cream and/or a more complex TAG polymorphism in emulsified system. When compared with the crystallization curves of mixes based on refined coconut oil (Fig. 2), the maximum melting temperatures of the main thermal event were shifted positively. This indicated that, starting from melted fat, a supercooling is needed to initiate crystallization (14).

Although it is well accepted that the type of fat phase influences ice cream characteristics, very few data actually demonstrate this effect. The eight different recipes herein highlighted the effect of fat and emulsifier selection on mix and ice cream properties. Particle size determination was relevant to discriminate between emulsifiers but gave only poor information regarding the influence of fat type in ice cream products. On the other hand, DSC profiles of emulsified fat in the mix allowed discrimination between the formulations. Crystallization patterns of mix could reveal the existence of different interactions between fat FA and emulsifier specific to each fat–emulsifier couple selected. Concerning ice creams, their melting behavior was also characteristic of the fat used in the formulation. After hardening at −40°C and storage at −20°C, the fat globules in ice cream regained the thermal properties of the bulk fat. Nevertheless,

although the differences observed between the emulsified fat and bulk fat melting profiles were quite small, they suggested that the freezing/whipping steps could induce some differences in fat organization.

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## II.4. Conclusion

As reported in the case of oil-in-water emulsion study, the necessity of confronting different methods for an overall characterization of the mix was pointed out since no correlation was found between particle size distribution, viscosity measurements and DSC analysis. It is worth noting that DSC proved to be a powerful method to investigate the fat/emulsifier and fat/protein interactions during crystallization through the variations of the onset temperatures and enthalpy areas when the mix formulations were varied.

**Similarities in the behavior of ice cream mix and emulsion** were found in: i) the **droplet size stability** of SMP-based ice cream mixes in the presence of competitively adsorbing surfactants and the flocculation phenomenon found in the case of mixes containing FDP. In particular, our results agreed with the fact that oil droplets were less aggregated in systems containing the highest proportion of caseins (Berger and White, 1971; Sourdet, *et al.*, 2002). In contrast, whey proteins partially denatured by heat treatment may favor the formation of polymers at the oil-water interface through bridging flocculation (Segall and Goff, 1999) and may also lead to a higher water binding capacity (Sünder, *et al.*, 2001); ii) the **increase in viscosity** after the ageing period. In particular, the association of FDP, partially unsaturated MDG and coconut oils led to high viscous emulsions and ice cream mixes. Viscosity values resulted not only from one parameter (droplet size, crystallized fat matter) but were rather the consequence of complex protein-emulsifier-fat interactions. It can be hypothesized that the fatty acid chain length and unsaturation degree of refined coconut oil would fit well with the characteristics of the partially unsaturated emulsifier (fatty acid chain length and unsaturation degree) at the fat globule interface. In presence of this peculiar association, refined coconut oil-partially unsaturated MDG, FDP would be able to interact more with the fat globules through non-covalent bonds than with other fat-emulsifier combination and would lead to a strong gel structure.

However, **differences in the behavior of ice cream mix and emulsion** were also pointed out in: i) the **disaggregation phenomenon** observed in the case of emulsion systems during the ageing period for the FDP-based formulations that was not revealed in all ice cream mixes. This may account, at least partly, for a different reference temperature before ageing (20°C and 4°C for emulsions and mixes, respectively) so that protein desorption might have already begun in the

case of mix. The presence of hydrocolloids in the aqueous phase may also influence the colloidal stability (Dickinson, 2003). Hydrocolloids can modify the interfacial characteristics either by adsorption at the interface or by complexation with proteins. This could result in difference in stability with respect to aggregation when emulsion and mix are compared; ii) the **rheological behavior** of some formulations. Samples that exhibited higher viscosities in the emulsion system were even more individualized in the ice cream mix system. For example, after the ageing period, the viscosity of the mix based on FDP, hydrogenated coconut oil and partially unsaturated MDG was three times higher than that of the corresponding emulsion. When hydrogenated coconut oil was replaced by refined coconut oil, the viscosity was multiplied by a factor 16 in the mix compared with the corresponding emulsion.

On the whole, the results obtained on ice cream mixes provided evidence that oil-in-water emulsions could not always simulate the phenomena occurring in more complex systems, so that both systems were worth studying.

# III. ICE CREAM

## III.1. Résumé

Les étapes concomitantes de foisonnement / congélation constituent les dernières étapes du procédé de préparation de la crème glacée et permettent le développement final de la structure et des propriétés organoleptiques du produit. La diminution de la température à -5°C entraîne la formation de petits cristaux de glace de sorte qu'environ 50 % de l'eau se trouve sous forme cristallisée. Cette cristallisation s'accompagne d'un phénomène de cryo-concentration de la phase aqueuse. La diminution de la température permet également à la cristallisation de la matière grasse et de l'émulsifiant, qui a commencé pendant l'étape de maturation, de se poursuivre. L'incorporation d'air entraîne une expansion en volume du produit. Les étapes de foisonnement / congélation s'effectuent sous cisaillement, ce qui favorise la collision des globules gras. En conséquence, de nouvelles interfaces (cristaux de glace / phase aqueuse, air / globules gras, air / phase aqueuse), et des réseaux (gras, protéique, de cristaux de glace partiellement fusionnés) sont

formés. Il est important de maîtriser la formation de ces structures parce qu'elles influencent les propriétés texturales de la crème glacée.

Ce troisième chapitre est centré sur l'influence de la nature de la phase grasse, du type d'émulsifiant de faible poids moléculaire et du type de protéine sur les différentes structures présentes dans les crèmes glacées. Pour réaliser cette étude, seize formulations, correspondant à quatre types de matière grasse (huiles de coprah hydrogénée ou raffinée, huile de palme raffinée et beurre), deux mélanges de MDG (saturé et partiellement insaturé) et deux types de mélanges protéiques (SMP et FDP) sont sélectionnées pour présenter a priori des caractéristiques structurales différentes. Les crèmes glacées sont caractérisées par des techniques physicochimiques classiques telles que la distribution en taille des globules gras, et la quantité de protéines présente dans la phase aqueuse. D'autres techniques ont demandé une mise au point comme l'analyse thermique ou la caractérisation rhéologique des crèmes glacées pour pouvoir analyser les produits de manière reproductible aux températures négatives. Les spectres de fluorescence du tryptophane sont enregistrés directement à partir des échantillons de crème glacée. La capacité discriminante des données est étudiée par une analyse en composantes principales et par une analyse discriminante des moindres carrés partiels. Enfin, les études de résistance à la fonte et l'analyse sensorielle permettent de répondre aux attentes du consommateur. Les objectifs de ce chapitre donc sont multiples. Il s'agit : i) de déterminer l'influence d'un type d'ingrédient sur une ou plusieurs caractéristiques du produit fini ; ii) de savoir si il est possible de prédire les propriétés des crèmes glacées connaissant celles du mix ; ii) d'établir d'éventuelles corrélations entre les différentes méthodes d'analyse effectuées.

De manière générale, les formulations de crèmes glacées à base du MDG insaturé contiennent un plus grand pourcentage de globules gras agglomérés, sont plus fermes (valeurs élevées du module élastique) et sont caractérisées par un temps de fonte long. Quelques formulations présentent des comportements spécifiques, notamment celle à base d'huile de coprah raffinée, de MDG partiellement insaturé et de FDP (temps de fonte et module élastique particulièrement élevés). L'étude rhéologique des crèmes glacées met bien en évidence l'influence d'un type d'ingrédient dans une plage de température définie, ce qui permet d'individualiser les formulations les unes par rapport aux autres. L'analyse en composantes principales des spectres de fluorescence du tryptophane réalisés sur les crèmes glacées permet une discrimination claire entre les deux mélanges protéiques. De plus, en considérant chaque mélange protéique séparément, l'analyse discriminante des moindres carrés partiels met en évidence une discrimination en fonction du type de matière grasse. Par contre, aucune

discrimination n'est trouvée en fonction du type d'émulsifiant. Les caractéristiques de texture des crèmes glacées évaluées par l'analyse sensorielle permettent d'obtenir une cartographie des produits finis. Seule l'influence du mélange protéique est révélée par cette analyse.

## III.2. Introduction

The next stage of ice cream structure development occurs during the concomitant whipping and freezing steps. Decreasing the temperature down to -5°C allows the formation of small ice crystals. In ice cream, about 50 % of the initial water is in the form of ice. This results in the cryo-concentration of the aqueous phase. The temperature decrease leads also to fat and emulsifier crystallization although this crystallization has already begun during the ageing step. Air incorporation leads to a volume expansion of the extruded product while shearing favored fat globule collision. As a result, new interfaces, *i.e.*, aqueous phase / ice crystals, fat globules / air, aqueous phase / air, and networks of partially coalesced fat, proteins and ice crystals are formed. All these structures are important to control because they are determining factors influencing ice cream structure and texture as well as ice cream time stability. For example, it is well known that the fat globule network obtained during the freezing stage is involved in ice cream dryness (Kloser and Keeney, 1959), shape retention after scraped surface freezing, slowness of meltdown (John and Sherman, 1962) and smooth eating textural properties after hardening (Berger and White, 1971).

In this third chapter, the different phases present in ice cream were characterized using different methods. Classical methods such as fat globule mean diameter and melting time were used. Some methods, like thermal analysis and rheological characterization were adapted to the ice cream product. Front-face fluorescence was applied to ice cream and multivariate analysis of the fluorescence spectra was developed thanks to the collaboration with J.-P. Da Costa (ENITA de Bordeaux). Finally, ice creams were evaluated through sensory analysis. Correlation between physicochemical characteristics and consumer perception was assayed.

**Fat globule size distribution and melting times** of ice creams pointed out the influence of the emulsifier, and, to a lesser extent, the influence of the fat type (**Granger *et al.*, 2005d,** § C.III.3. and **Granger *et al.*, 2005e**, § 0.). Saturated MDG led to lower fat globule mean diameter, fat agglomeration and melting time values compared with the corresponding ice creams based on partially unsaturated MDG. For a given MDG, small differences in particle size distribution and melting time were observed when the fat type varied. The protein mixture used in ice cream

formulation had no significant effect on the physical characteristics of ice cream although the formulation based on partially unsaturated MDG, refined coconut oil and FDP led to a peculiar high melting time value.

Ice creams were characterized by the storage modulus (G') and the damping factor (tan δ). This **rheological analysis** allowed the underlining of the different structures present in the ice cream product when considering specific temperature ranges. Thus, –10 to –2°C was relevant of the melting of ice crystals, 17 to 30°C of the fat globule network and 43 to 55°C of the protein network destruction (**Granger *et al.*, 2004,** Appendix 3, and **Granger *et al.*, 2005d**, § C.III.3.). In particular, for temperatures between 17 to 30°C, G' variations were dependent on the fat nature so that G' decrease was less pronounced when the melting temperature range of the vegetable fats used was extended. Partially unsaturated MDG-based formulations were associated with higher initial G' values than saturated MDG-based ones. Since G' may be interpreted in terms of system rigidity, firmer ice creams should be obtained when partially unsaturated MDG was used. Moreover, the presence of the partially unsaturated emulsifier in the formulation was revealed by two peaks of the damping factor observed at 20-21 and 27°C, while only the peak at 27°C was observed when the saturated MDG was used. Additional experiments performed on ice cream based on the two protein mixtures consolidated the use of rheology to characterize the protein network (Figures 5 a and b). Between 5 and 20°C, FDP led to higher G' values compared with SMP-based samples. Above 45°C, the storage modulus values of FDP-based formulations were higher than that of SMP, suggesting that some structural network remained in the corresponding heated product. FDP-based ice creams seemed more resistant to temperature increase than SMP-based ones, especially when the saturated MDG was present.

**Thermal properties** of ice cream were analyzed upon heating. Although the water peak interfered with the fat peak, melting DSC curves of ice creams allowed the discrimination between the fat types used in the formulation but were not significantly influenced by the emulsifier type (**Granger *et al.*, 2005c,** § C.II.3). After the whipping and freezing steps (hardening at -40°C and storage at -20°C), the fat globules in ice cream regained the thermal properties of the bulk fat.

The **front-face fluorescence data** were analyzed through the fourth derivative tryptophan emission spectrum as already performed on emulsions. It was not possible to differentiate tryptophan in hydrophilic and hydrophobic environments probably due to the complexity of the final product. There could be an overlapping of the tryptophan responses corresponding to each

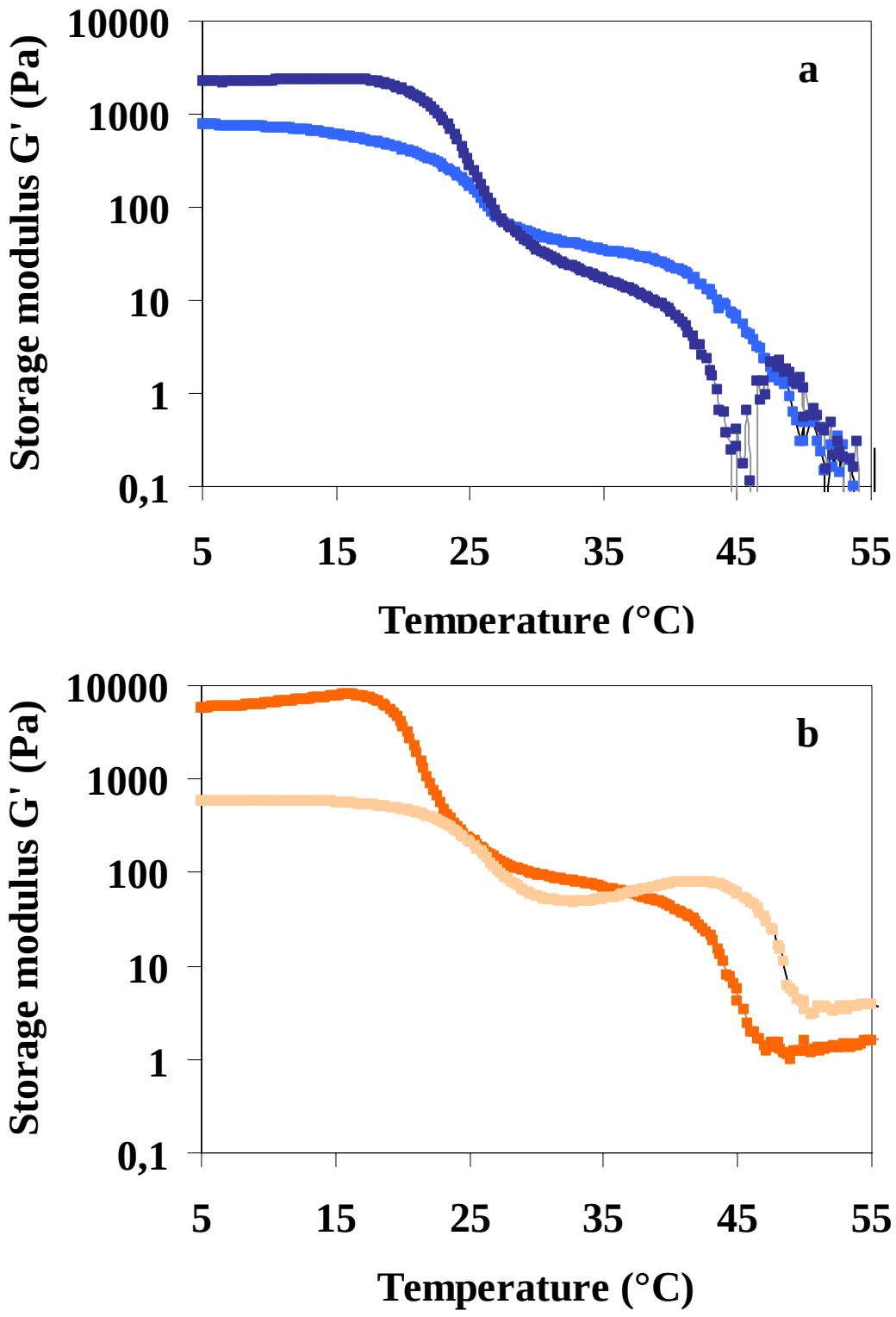


**Figure 5: Storage modulus (G') of ice cream product based on refined coconut oil as a function of the temperature (ω = 1 Hz, heating rate = 0.5°C/min). a: Ice cream based on skim milk powder; b: Ice cream based on functional dairy protein. Saturated mono- and diglyceride mixture (light symbols) and partially unsaturated mono- and diglyceride mixture (dark symbols).**

type of interface present in ice cream. Thus, the discriminant ability of the fluorescence spectra was investigated by PCA and PLS discriminant analysis. The spectral patterns associated with the first axis of PCA pointed out that the wavelengths involved in the discrimination of the different fat type formulations were different from that found for protein discrimination. Thus, besides the identification, at a molecular level, of different protein structures, the statistical approach of the fluorescence data allowed the underlining of different interactions occurring between one type of protein mixture and the various fats (Granger *et al.*, 2005e, § 0.).

## III.3. C. Granger, A. Leger, P. Barey, V. Langendorff, M. Cansell (2005d). Influence of formulation on the networks in ice cream, International Dairy Journal, 15, 255-262.[4]

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# Influence of formulation on the structural networks in ice cream

C. Granger[a,b], A. Leger[b], P. Barey[b], V. Langendorff[b], M. Cansell[a,*]

[a] *Laboratoire Milieux dispersés alimentaires: physico-chimie, formulation et vectorisation nutritionnelle, ISTAB, Avenue des Facultés, F-33405 Talence cedex, France*

[b] *Degussa Food Ingredients, Business Line Texturant Systems France SAS, 32, Place ronde, F-92035 Paris La Défense, France*

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**Abstract**

Six different formulations, corresponding to three types of fat (hydrogenated or refined coconut oils or refined palm oil) and two mixtures of mono- and di-glycerides, namely saturated and partially unsaturated were chosen to investigate the influence of the oil phase nature and the low molecular weight emulsifier type on the networks present in ice cream. Ice creams were characterized for particle size distribution of fat globules, melting resistance and amount of proteins in the aqueous phase. Variation of rheological parameters as a function of temperature allowed following the ice network melting, the fat globule aggregation state and destabilization, and the structural arrangement of proteins. Presence of unsaturated fatty acids in the emulsifier promoted an increased percentage of agglomerated fat globules, increased melting time and higher storage modulus values at 5 °C. The influence of the fat type on ice cream characteristics was mainly illustrated by different rheological parameters and, to a lesser extent, by melting time, whereas the amount of proteins in the aqueous phase did not allow discriminating among the formulations.


*Keywords:* Ice cream; Networks; Rheology; Lipid emulsifier; Vegetable fat

## 1. Introduction

Ice cream structure determines several important sensorial parameters in the final product such as stiffness, dryness, melt resistance and texture. The setting up of ice cream structure comes from the manufacturing process that includes the steps of preheating, homogenization, pasteurization, ageing, freezing and hardening, as well as from the various components used in the formulation. In particular, fat appears to contribute largely to the properties of ice cream during freezing and whipping especially through the partially coalesced/agglomerated continuous three-dimensional network of homogenized globules (Goff, 1997). Indeed, part of the fat globules surrounds air bubbles and, thus, participates in the air phase stabilization (Goff, Verespej, & Smith, 1999). Moreover, increased levels of fat aggregation are also correlated to improved melting resistance (Bolliger, Goff, & Tharp, 2000). In addition to fat, low molecular weight emulsifiers play an important role in ice cream structure by promoting partial coalescence of fat globules. During cold ageing, lipid emulsifiers displace some of the proteins from the fat droplet interface (Barfod, Krog, Larsen, & Buchheim, 1991; Krog & Larsson, 1992; Gelin, Poyen, Courthaudon, Le Meste, & Lorient, 1994; Gelin, Poyen, Rizzotti, Courthaudon, & Lorient, 1996; Granger, Barey, Combe, Veschambre, & Cansell, 2003a) leading to reduced stability of the fat globules during the whipping and freezing process (Gelin et al., 1994; Goff et al., 1999). Besides the competitive protein displacement, the extent of fat globule instability also depends on the fat crystal type and/or amount (Davies, Dickinson, & Bee, 2001). As a consequence of lipid emulsifier action in ice cream, some of the proteins, including both casein micelles and whey proteins, are distributed in the

*Corresponding author. Tel.: +33-5-57-96-3453; fax: +33-5-56-84-2472.
*E-mail address:* m.cansell@istab.u-bordeaux1.fr (M. Cansell).

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cryo-concentrated phase. These unabsorbed molecules contribute also to the final product structure (Goff, 2002; Granger, Barey, Renouf, Langendorff, & Cansell, 2003b; Granger, Langendorff, Renouf, Barey, & Cansell, 2004). Finally, hydrocolloid stabilizers added in certain ice cream formulations, are involved in increasing the mix viscosity (Goff, Freslon, Sahagian, Hauber, Stone & Stanley, 1995) and in the limitation of the growth rate of ice crystals during re-crystallization (Bolliger et al., 2000). In addition to the role of each component in the ice cream structure, interactions between the various ingredients also take place, making it all the more difficult to predict the different structural networks on the basis of formulation alone. While several studies have focused on the nature of the emulsifier (Bolliger et al., 2000) or of the protein (Gelin et al., 1996; Sourdet, Relkin, & Cësar, 2003), only a few investigations pointed out the role of the fat type (Abd El-Rahman, Madkor, Ibrahim, & Kilara, 1997; Adapa, Dingeldein, Schmidt, & Herald, 2000; Granger et al., 2003a).

The aim of the present study was to investigate the fat/emulsifier interactions in ice creams. Three types of vegetable fats were tested. The low molecular weight emulsifiers used consisted of a mixture of mono- and diglycerides (MDG) with different unsaturation degree; saturated (SMDG) and partially unsaturated (PUMDG). The ice cream structure was characterized by classical methods, i.e., fat globule particle size, protein quantification; and melting time. In parallel, oscillation thermo-rheometry was used to illustrate the different networks present in the ice creams.

## 2. Materials and methods

### 2.1. Ice cream preparation

Ice cream mix formulations were based on 8% vegetable fat, 10% skim milk powder (SMP; 35% proteins, Coopérative d'Isigny Saint-Mère, Isigny sur mer, France), 12% sucrose (Eurosucre, Paris, France), 6% corn syrup solids (40 DE, 80 Brix solid content, Cerestar, Haubourdin, France), 0.2% stabilizer (guar gum and locust bean gum, Degussa Food Ingredients, Baupte, France) and 0.3% lipid emulsifier. Three different fats were used, i.e., hydrogenated or refined coconut oils or refined palm oil (Société Industrielle des Oléagineux, Bougival, France). The lipid emulsifiers were a mixture of MDG (60% alpha mono-glycerides, Degussa Food Ingredients, Baupte, France). SMDG and PUMDG (23% of unsaturated fatty acids) were used.

All ingredients except the vegetable fat were dry blended, mixed with water, and immediately blended at 65 °C with the melted fat portion, for 15 min. Mixes were homogenized, at 70 °C, at 170/30 bar (Manton Gaulin Lab 60, double piston two-stage homogenizer, APV France, Evreux, France), pasteurized at 90 °C for 30 s (APV Junior, APV France), cooled and immediately stored at 4 °C in a water bath and aged for one night at 4 °C under stirring. Batches of ice cream mix (20 L) were frozen in a continuous freezer (WCB ice cream CS 100, Waukesha Cherry Burrell, Denmark; overrun: 100%, outlet temperature: −5 °C). Ice creams were hardened at −40 °C and stored at −25 °C. For each recipe, at least two different batches were prepared.

### 2.2. Fat particle size analysis

Fat particle size distributions in ice creams were measured by integrated light scattering using a Mastersizer S (Malvern Instruments SA, Orsay, France). Hardened ice cream samples were directly diluted in the sample chamber with water at approximately 1:1000 at 16–18 °C. Measurements were performed, at room temperature, using ultrasonication to ensure the absence of air bubbles. Particle mean diameter (evaluated by the volume-weighted average diameter $d_{4,3}$) and the cumulative percentage of the particles greater than 2.0 μm were recorded. Some ice creams were diluted (at approximately 1:1000) in a 1% (w/w) sodium dodecyl sulphate (SDS) solution. This dissociating medium was used to disperse the droplet aggregates formed (Gelin et al., 1994; Granger et al., 2003a).

### 2.3. Melting test

All the samples were stored at −25 °C before carrying out the melting test. Ice cream samples (270 ± 5 g) were placed on a mesh grid (mesh size 1 × 1 cm) and allowed to stand at 29 °C, at constant humidity. The weight of the material passing through the screen was recorded as a function of time. The time necessary to melt 30 g of product was recorded.

### 2.4. Oscillation thermo-rheometry

Dynamic rheological behaviour of ice cream products during melting was followed with a controlled stress rheometer Physica MCR 300 (Anton Paar: Ostfildern Germany) fitted with streaked parallel plates (1 mm gap) as described in Granger et al. (2004). Before placing onto the rheometer, ice creams were stored for 2 h at −10 °C. The sample was stabilized for 15 min in the rheometer before beginning the experiment. The evolutions of the storage ($G'$) and loss ($G''$) moduli and the damping factor, $\tan\delta$ ($G''/G'$), were measured as a function of temperature (heating rate: 0.5 °C min$^{-1}$) at two different deformation amplitudes, i.e., 0.05% between −10 and 5 °C and 0.1% between 5 and 50 °C, and at a frequency $\omega$ of 1 Hz. These amplitudes were

chosen on the basis of the determination of the linear viscoeslatic regime of ice cream by a deformation amplitude sweep test.

### 2.5. Quantification of proteins in the aqueous phase

Melted ice creams (4 °C for 1 h) were centrifuged in order to separate the oil phase and the aqueous phase (15,000 *g*, 30 min, 4 °C, Suprafuge 22, Heraeus Sepatech, Osterode, Germany) as already described (Granger et al., 2003a). The subnatant aqueous phase was recovered by removal with a syringe and stored at −20 °C awaiting protein analysis. Method of Bradford (1976) was used to quantify the total protein content in the aqueous phase. Bovine serum albumin was used as a standard.

### 2.6. Statistical analysis

Analysis of variance of all data, using Fisher's least significant difference (LSD) procedures (Stat Graphic Plus software, Manugistics, MD), was made on $d_{4,3}$, cumulative percentage of the particles greater than 2.0 μm, protein amount in the aqueous phase and rheological parameters to discriminate among the six formulations. The time necessary to melt 30 g of ice cream product from the two replicates of the six formulations was correlated with the response variables of the six ice creams, i.e., cumulative percentage of the particles greater than 2.0 μm and storage modulus at 20 °C, by linear regression. Correlation coefficients ($r^2$) were determined from the regression line.

## 3. Results and discussion

### 3.1. Influence of ice cream formulation on ice cream characteristics

Table 1 presents the effect of fat type and emulsifier nature used in ice cream formulations on fat droplet size characteristics. For all ice cream formulations considered, the average diameters of droplets resulted in the existence of two distinct populations, a major one between 0.05 and 2 μm and a minor one between 2 and 10 μm (Fig. 1a). The presence of the second peak in the particle size distributions was assigned to fat droplet coalescence and/or fat droplet aggregation occurring upon whipping/freezing (Gelin et al., 1994; Goff, 1997; Bolliger et al., 2000; Relkin, Sourdet, & Fosseux, 2003). In the presence of a dissociative medium (SDS), the percentage of particles with diameters greater than 2 μm

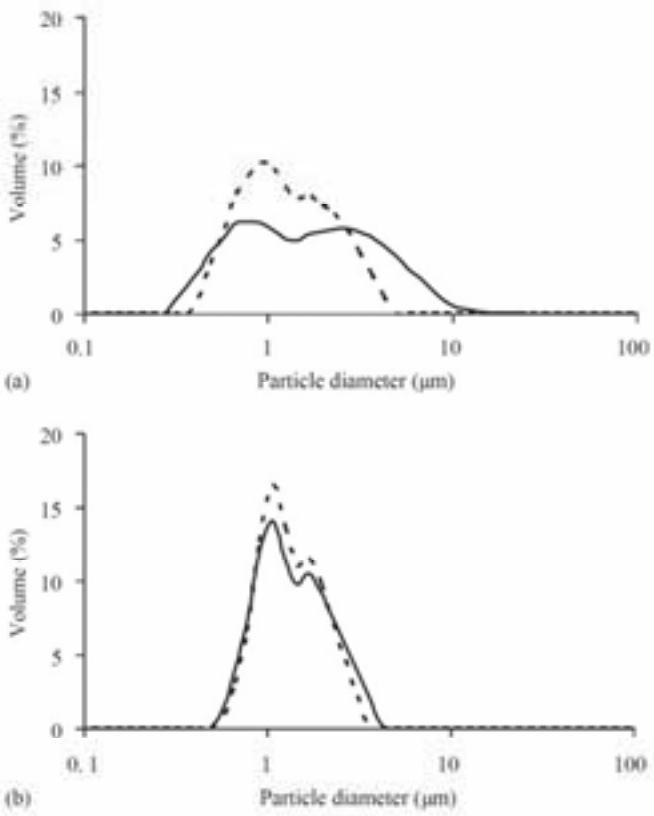


Fig. 1. Particle size distribution of fat droplets for ice cream based on hydrogenated coconut oil and saturated MDG (--) or partially unsaturated MDG (—). (a) Dispersion in water; (b) dispersion in SDS solution.

Table 1
Effect of fat type and emulsifier nature used in formulations on ice cream characteristics

| | Hydrogenated coconut oil | | Refined coconut oil | | Refined palm oil | |
|---|---|---|---|---|---|---|
| | SMDG[d] | PUMDG[e] | SMDG | PUMDG | SMDG | PUMDG |
| $d_{4,3}$ (μm)[a] | 1.3±0.1 | 2.1±0.4 | 1.4±0.1 | 3.0±0.1 | 2.2±0.4 | 3.3±0.6 |
| % particles >2 μm[a,b] | 12±1 | 21±5 | 12±1 | 33±1 | 21±4 | 38±7 |
| Melting time for 30 g of product (min)[a] | 52±7 | 106±5 | 57±2 | 123±4 | 62±8 | 100±3 |
| Proteins in the aqueous phase (wt%)[a,c] | 56±6 | 72±6 | 56±3 | 56±7 | 59±8 | 70±12 |

[a]Each listed value is the average of duplicate sets of measurements ± standard deviation ($n = 2$).
[b]Percentage of particles with mean diameters greater than 2 μm.
[c]Percentages of proteins represent the quantity of proteins in the aqueous phase relative to the 3.5% initially present in the ice cream mix.
[d]SMDG: saturated mono- and di-glycerides.
[e]PUMDG: partially unsaturated mono- and di-glycerides.

decreased (Fig. 1b). This decrease was dependent on the ice cream formulation. It is worth noting that, regardless of formulation, after SDS treatment, the $d_{4,3}$ and the percentage of particle greater than 2 μm tended to converge to similar values, i.e., around 1.5 μm and 10%, respectively (results not shown). Thus, partial coalescence occurred to a similar extent. In contrast, the aggregation phenomenon was dependent on the ingredients present.

The fat nature, as well as the emulsifier type, used in ice cream formulations were two parameters influencing the fat droplet size distribution in the final product (Table 1). Systematically, for a given fat, SMDG led to lower diameters of fat globules in ice cream compared with PUMDG. Increase in the mean diameters observed with PUMDG-based formulations corresponded to a higher percentage of particles >2 μm and, thus, to an increased aggregation of fat droplets (Table 1). Similar results showing the influence of the MDG type were already reported (Barfod et al., 1991). The fat type used in the ice cream formulation had also an impact on the particle size diameter. For a given MDG type, the more unsaturated and the longer the fatty acid chains of the vegetable fat used in the formulation, the more pronounced was the fat globule destabilization. Moreover, formulations based on hydrogenated and refined coconut oils and PUMDG were characterized by significantly different particle size diameters, although these two oils differed only by 8% of oleic acid. This suggested that specific interactions may occur between the fat and the lipid emulsifier. Indeed, fat and MDG could interact more or less tightly through the hydrophobic parts of the molecules (Davies et al., 2001; Granger et al., 2003a).

Melting times were dependent on the ice cream formulation and especially on the nature of the emulsifier (Table 1). The presence of PUMDG in ice creams caused a marked increase in the melting times compared with those measured for formulations containing the saturated emulsifier. The influence of the fat type on the melting times was less pronounced. Fat aggregation appeared to be the major contributor to the melting resistance of ice creams (Pelan, Watts, Campbell, & Lips, 1997; Bolliger et al., 2000; Goff & Spagnuolo, 2001) through the existence of different networks resulting from the presence of fat, proteins or other stabilizers. However, besides high melting times associated with elevated fraction of particles with diameters greater than 2 μm in the ice cream (Table 1), a low correlation ($r^2 = 0.42$) was found between these two parameters. Moreover, high melting times were not always associated with formulations characterized by a high solid fat content at 20 °C. For example, refined coconut oil which was in a fluid state at 25 °C (Granger et al., 2003a) led to ice cream with higher melting times compared to hydrogenated coconut oil formulation (Table 1). Thus, the melting behaviour did not simply reflect the molecular and supramolecular nature of one species but rather, was indicative of: (i) the development of interactions between the various ice cream ingredients; (ii) the existence of several structures, i.e., the partially coalesced/aggregated three-dimensional network of homogenized globules, the stabilized air phase, and the protein and polymer networks. These different factors contributed more or less to the ice cream global structure not only during the freezing and whipping steps but also under storage temperature variations.

The quantitative analysis of proteins at the droplet interface after the separation of the fat fraction by centrifugation is a well-documented method in the case of emulsions and mixes (Bolliger et al., 2000; Davies et al., 2001; Granger et al., 2003a). However, if the centrifugation parameters are not carefully checked, the values obtained could be underestimated due to the effect of partial coalescence during the separation step. In ice cream products, the creation of new interfaces due to incorporation of air, may compromise the accuracy of the protein quantification in the aqueous phase. We hypothesized that the proteins absorbed at the air interface were not recovered in the aqueous phase. Indeed, a similar amount of proteins in the aqueous phase was found after destabilization of the air phase by heating and/or sonication (results not shown). Based on the data in Table 1, and placing the issue of protein adsorption at the air interface aside, we inferred that the amount of proteins in the aqueous phase in melted ice cream products could vary upon formulation. Similar proportions of non-adsorbed proteins were determined for ice creams based on SMDG, irrespective of the fat type used. For hydrogenated coconut oil and refined palm oil-based formulations, there was a significant effect of the MDG type, i.e., a higher amount of proteins in the aqueous phase with PUMDG as compared with the saturated one. The case of ice creams based on refined coconut oil and PUMDG showed that proteins remained adsorbed at the hydrophobic interfaces (oil and/or air) although high fat globule destabilization occurred. This suggested that this type of oil may develop specific interactions with this particular emulsifier.

### 3.2. Dynamic rheological measurements

Characterization of ice cream products using oscillation thermo-rheometry allowed coupling mechanical and thermal analyses together in order to gain more information on the structural networks. Fig. 2 presents the variations of the real (elastic) part of the complex shear modulus, i.e., the storage modulus ($G'$), versus increasing temperature, in oscillation test, for an ice cream product based on refined coconut oil and SMDG. The damping factor was also plotted since this

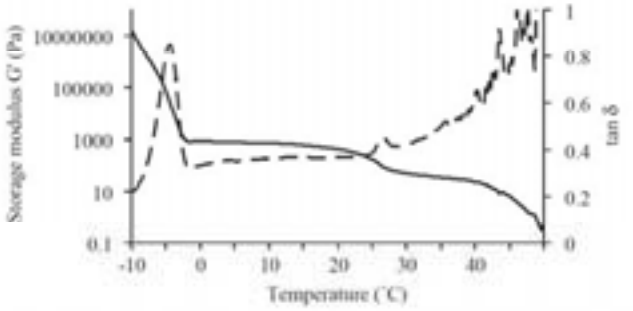


Fig. 2. Storage modulus ($G'$) (—) and damping factor ($\tan\delta$) (– –) for ice cream based on saturated MDG and refined coconut oil as a function of the temperature ($\omega = 1$ Hz, heating rate = 0.5 °C min$^{-1}$).

parameter is an important indicator of phase melting (Lucey, 2003). At −10 °C, the storage modulus value was high, whereas the $\tan\delta$ value was low. These values characterized a stiff and well-structured product. Increasing the temperature from −10 to −2 °C led to a steep decrease in $G'$ and to a peak of the damping factor. In this temperature range, $G'$ variations were attributed to a decrease in the ice fraction leading to a lower rigidity of the ice cream product, a dilution of the unfrozen phase and a modification of the microstructure arrangements (Goff et al., 1995; Wildmoser, Scheiwiller, & Windhab, 2003; Granger et al., 2004). It is worth noting that the temperature ending of the large $G'$ decrease, i.e., −2 °C, corresponded to the ice melting temperature of our system according to Bradley and Smith (1983). Between −2 and 15 °C, $G'$ values stabilized at a plateau. In this temperature range, all ice crystals were melted so that other compositional factors such as air bubbles, fat structure, proteins and polysaccharide stabilizers may in turn contribute to the magnitude of $G'$ (see below). Increasing the temperature from 15 to 50 °C led to two distinct decreases in the storage modulus. The first $G'$ decrease occurred between 15 and 30 °C and was concomitant, at least partly, with the fat melting, solid fat content of refined coconut oil decreasing from 65% to 0% in this temperature range (Granger et al., 2003a). This decrease could also be attributed to the fat globule network reorganization. Indeed, size measurements performed on samples submitted to a similar heating treatment as that applied in rheology showed that the $G'$ decrease was associated with a reduction of $d_{4,3}$ and of the percentage of particles with diameters greater than 2 μm (results not shown). The peak observed for $\tan\delta$ confirmed the fat globule network destruction. However, the fact that $G'$ still underwent variations beyond 25 °C, i.e., temperature at which all refined coconut oil should be in a melted state, may account for delayed structural rearrangements and/or a shift in the fusion temperature toward higher temperatures in the emulsified system as compared to the bulk phase. The second decrease in $G'$ occurred between 35 and 50 °C. In this temperature range, mainly the dispersed air and the aqueous phase (proteins and stabilizers) would have an impact on the rheological characteristics. In particular, for temperatures higher than 45 °C, $\tan\delta$ remained at values lower than 1, suggesting the existence of a residual network that could be attributed, at least partly, to the presence of proteins and their interactions with the other components (Granger et al., 2003b, 2004).

Since at negative temperatures, the rheological parameters were mainly influenced by the ice crystals present, the $G'$ and $\tan\delta$ variations are only present above 5 °C, i.e., where the influence of the fat nature and the emulsifier type could be revealed. The effect of the emulsifier type on the rheological behaviour of ice cream is shown in Fig. 3. At 5 °C, PUMDG-based sample demonstrated significantly higher storage modulus value than SMDG-based product. In contrast, $\tan\delta$ appeared to be less sensitive to the emulsifier type, suggesting that this rheological parameter was less pertinent at 5 °C to illustrate the impact of MDG on the organization level of the ice cream product. The structural differences in firmness and elasticity, as a function of the emulsifier type, could come from an increased fat globule destabilization within the system containing PUMDG (Table 1). Indeed, it was suggested that fat aggregates contribute greatly to the structure of ice cream by forming a three-dimensional network (Goff, 1997). Examining the region ranging from 5 to 15 °C showed constant values for both $G'$ and $\tan\delta$. Between 15 and 35 °C, the storage modulus decreased significantly, irrespective of the emulsifier type used in the formulation. In contrast with SMDG, in PUMDG-based formulation, the decrease in the storage modulus occurred in two distinct stages and two peaks for the damping factor were observed at two different temperatures, i.e., 22 and 26 °C (Fig. 3). The occurrence of the different peaks could be interpreted in terms of specific interactions of part of the fat with the saturated and unsaturated fractions present in PUMDG and/or a

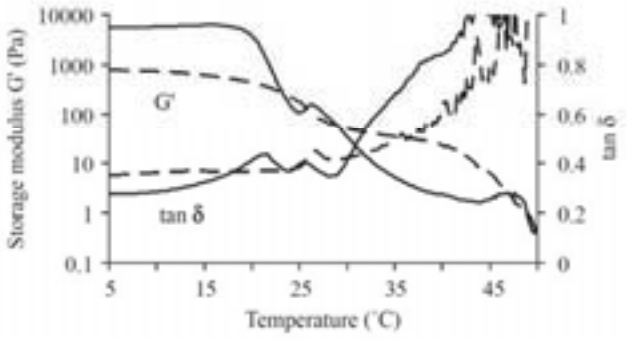


Fig. 3. Storage modulus ($G'$) and $\tan\delta$ as a function of the temperature ($\omega = 1$ Hz, heating rate = 0.5 °C min$^{-1}$) of ice cream products based on refined coconut oil and saturated MDG (– –), and partially unsaturated MDG (—).

difference in composition of adsorbed layers at the fat globule surface. Between 30 and 50 °C, with the two MDG-based ice creams, the storage modulus decreased regularly, although the shapes of the curves were different.

Measurements of the storage modulus and of the damping factor, as a function of the fat type for SMDG-based ice cream formulations upon melting, are reported in Figs. 4a and b, respectively. The influence of the fat nature was revealed between 15 and 40 °C (Fig. 4a). Decrease of the $G'$ with increasing temperatures followed quite well the melting temperature range of the vegetable fats used, i.e., the $G'$ decrease being even less pronounced when the melting temperature range was extended. The effect of fat type on the damping factor evolution is also shown in Fig. 4b. The peak in the damping factor values, found in the case of refined coconut oil formulations, was not observed in the case of hydrogenated coconut oil and refined palm oil-based products. As similar mean particle diameters were found with SMDG and the different types of fat (Table 1), the occurrence of the peak of $\tan\delta$ in the case of the refined coconut oil was not due to a different amount of destabilized fat. Therefore, $\tan\delta$ behaviour upon fat formulation could account for a difference in fat globule organization in the melting ice cream and/or for various amounts of triglyceride crystals.

The $G'$ and $\tan\delta$ variations of ice cream products based on PUMDG for the three fats used in the formulations are presented in Figs. 5a and b, respectively. At 5 °C, refined coconut oil-based formulations developed a greater stiffness than hydrogenated coconut oil and refined palm oil-based products. The amount of agglomerated fat globules measured in refined coconut oil-based ice cream (Table 1) could not totally explain the high $G'$ value, since higher aggregation was found with refined palm oil. Thus, it may be that specific interactions occurred between this fat and the emulsifier due to an adequate composition of the fatty acids of PUMDG and refined coconut oil. This would lead to an optimal fat network organization. With increasing temperatures, the refined coconut oil continued to be distinguished from the two other fats. In particular, two steep decreases of storage modulus, between 15 and 35 °C, were clearly observed and $G'$ value above 35 °C was lower (Fig. 5a). Concerning the two peaks of the damping factor observed with refined coconut oil product, they were less important and slightly shifted to higher temperatures in the case of hydrogenated coconut oil. They were completely removed in the case of palm oil (Fig. 5b). At temperatures higher than 35 °C,

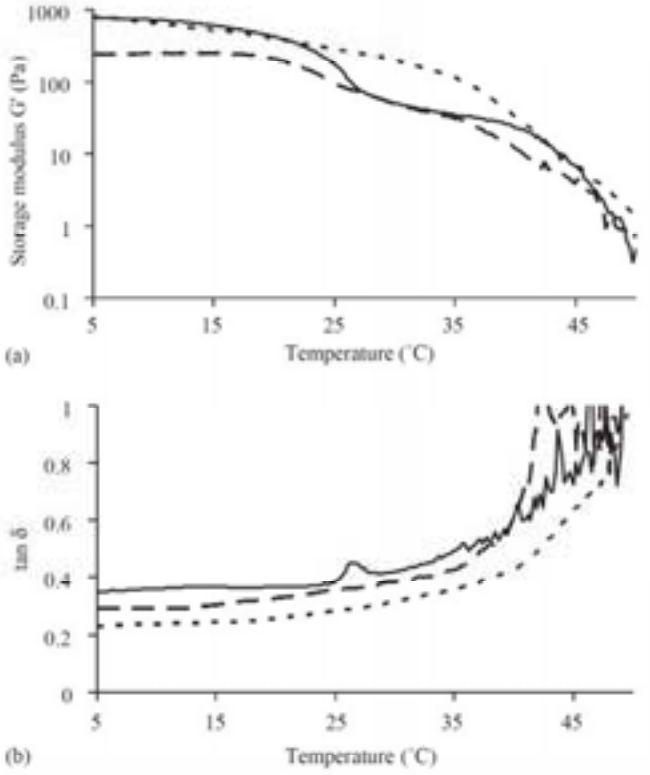


Fig. 4. Rheological behaviour of ice cream products based on saturated MDG and hydrogenated coconut oil (– –), refined coconut oil (—) and refined palm oil (· · · · · · · · ·), as a function of temperature ($\omega = 1$ Hz, heating rate = 0.5 °C min$^{-1}$). (a) Storage modulus ($G'$); (b) $\tan\delta$.

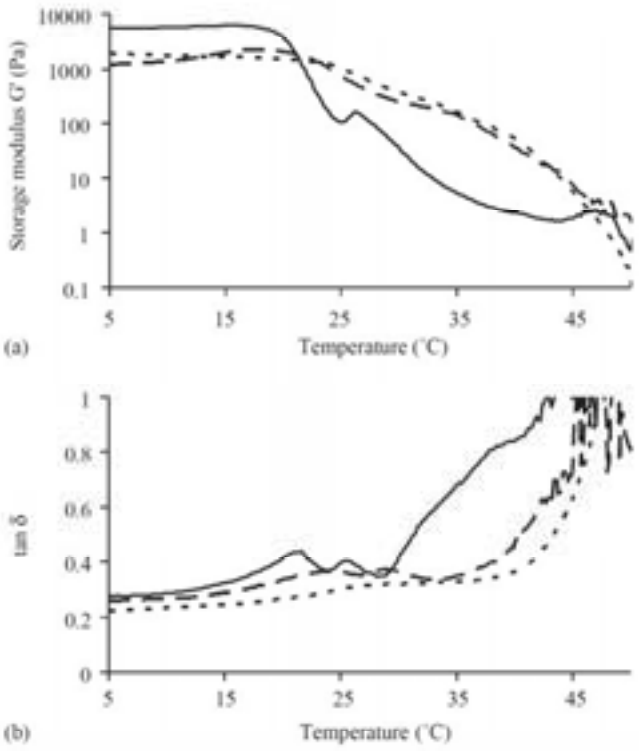


Fig. 5. Rheological behaviour of ice cream products based on partially unsaturated MDG and hydrogenated coconut oil (– –), refined coconut oil (—) and refined palm oil (· · · · · · · · ·), as a function of temperature ($\omega = 1$ Hz, heating rate = 0.5 °C min$^{-1}$). (a) Storage modulus ($G'$); (b) $\tan\delta$.

the tan $\delta$ values of refined coconut oil-based formulations were high in comparison with the two other formulations. In this temperature range, mainly the aqueous and air phases developed the viscoelastic properties of the melted ice creams. In fact, these differences in rheological behaviour may account for various amounts of proteins in the aqueous phase, i.e., 56% for refined coconut oil and 70–72% for both hydrogenated coconut oil and refined palm oil, respectively (Table 1).

## 4. Conclusion

Although it seems well plausible that the type of the fat phase would influence the ice cream characteristics, only limited data actually demonstrated this effect. Among the different methods of characterization used in this work to discriminate among the different formulations, some, e.g., size determination of fat droplets, were relevant to distinguish between emulsifiers, but gave less information regarding the influence of fat type. The presence of PUMDG in ice cream formulations led to an increased agglomeration of fat globules as compared to SMDG. However, with this last emulsifier, the effect of the fat nature poorly affected the droplet size. It seemed that SMDG developed more interactions with fat, regardless the composition, than PUMDG, leading to less influence of the fat type on ice cream droplet size characteristics. On the other hand, the rheological data and melting time appeared to illustrate the influence of the fat nature. In particular, a high linear correlation was noticed between the storage modulus measured at 20 °C and the melting time (Fig. 6). However, since the melting time was not simply correlated to the fat globule diameters, other parameters such as globule interactions and/or fat crystallization, may also influence the melting behaviour of ice cream mixes. Oscillatory rheology helped in the interpretation of the complex behaviour of ice cream upon heating. Besides measuring the viscoelastic parameters of ice cream, it allowed the different components and their role in each temperature domain to be distinguished. Variation of the storage modulus as a function of temperature was indeed a pertinent parameter to follow the ice network melting, the fat globule aggregation state and destabilization, and the structural arrangement of proteins. It can be concluded that tan $\delta$ was a good indicator of loss of network structure in ice cream. However, the fact that ice creams based on refined coconut oil exhibited particular behaviour that could not be simply interpreted in terms of composition (acyl chain length and degree of unsaturation) suggested that specific interactions occurred between fat and MDG and influenced the functionality of the emulsifier. Thus, information at a molecular level is now required to improve the knowledge of the interactions occurring between the different components.

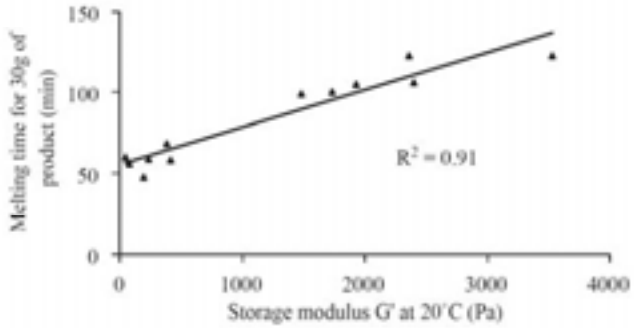


Fig. 6. Correlation between storage modulus at 20 °C and melting time of 30 g of product for the different ice cream formulations.

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## III.4. C. Granger, J.P. Da Costa, J. Toutain, P. Barey, M. Cansell (2005e). Mapping of ice cream formulation using front-face fluorescence spectroscopy. International Dairy Journal, in press.[5]

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# Mapping of ice cream formulation using front-face fluorescence spectroscopy

C. Granger[a,b], J.P. Da Costa[c], J. Toutain[d], P. Barey[b], M. Cansell[a,*]

[a]*Laboratoire Milieux Dispersés Alimentaires, ISTAB, Avenue des Facultés, F-33405 Talence cedex, France*
[b]*Degussa Food Ingredients, Business Line Texturant Systems France SAS, 32, Place ronde, F-92035 Paris La Défense, France*
[c]*ENITA de Bordeaux, BP 201, F-33175 Gradignan cedex, France*
[d]*TREFLE UMR 5508, Université Bordeaux 1, ENSAM, ENSCPB, 16, avenue Pey-Berland, F-33607 Pessac cedex, France*

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**Abstract**

Front-face fluorescence spectroscopy was used to characterize 16 ice cream formulations (four fats, two mono-/diglyceride mixtures, and two protein mixtures). Tryptophan fluorescence spectra were recorded directly from the ice cream samples. The discriminant ability of the data was investigated by principal component analysis (PCA) and partial least square (PLS) discriminant analysis. The similarity maps defined by principal components 1 and 2 allowed clear discrimination between the protein mixtures. The spectral pattern associated with protein PLS discrimination provided characteristic wavelengths that were the most suitable for separating the spectra. Whereas PCA allowed some clustering with respect to fat, in the case of the protein mixtures no discrimination was found as a function of emulsifier type. Classical methods (fat globule size distribution and melting time) were also used for ice cream characterization. Some formulations exhibited specific behaviours that made the understanding of their physical and molecular properties difficult when analysing only the physicochemical characteristics of the ingredients.


*Keywords:* Ice cream; Protein; Fat; Structure; Front-face fluorescence

## 1. Introduction

Ice cream is a complex colloidal system comprising ice crystals, air bubbles, partially coalesced or aggregated fat globules and a cryo-concentrated aqueous phase (Goff, 2002). The formation and the stabilization of the different microstructures involve all of the ice cream ingredients. Partitioning of the molecules, i.e., the proteins, the low molecular weight emulsifiers and the fat (free or partially-coalesced), occurs between the different interfaces and the cryo-concentrated phase. At a molecular level, interactions between protein and emulsifier at the fat globule interface, and between protein and fat at the air interface, may take place. An understanding of the various interactions and the resulting colloidal properties would allow numerous industrial situations to be dealt with, such as manufacturing and textural defects, ingredient assessment and new product development.

Among the few techniques that enable the monitoring, at a molecular level, of food samples, fluorescence spectroscopy is being used more and more as a consequence of the development of a front-face device that allows the characterization of powdered, turbid, emulsified and concentrated samples (Dufour, Devaux, Fortier, & Heymann, 2001; Herbert et al., 2000). Besides the sensitivity of this analytical method, the fluorescent properties of the tryptophan residues allow analysis of proteins. The fluorescence spectra of tryptophan residues have been used to monitor the structural modifications of proteins in food systems during wheat gluten treatment (Genot, Tonneti, Montenay-Garestier,

*Corresponding author. Tel.: +33 5 40 00 34 53; fax: +33 5 40 00 24 72.
*E-mail address:* m.cansell@istab.u-bordeaux1.fr (M. Cansell).

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Marion, & Drapon, 1992), milk heating and coagulation (Herbert, Riaublanc, Bouchet, Gallant, & Dufour, 1999), frankfurter processing (Allais, Viaud, Pierre, & Dufour, 2004) and soft cheese ripening (Dufour et al., 2001).

The aim of the present study was to investigate the influence of different fat/protein/emulsifier formulations on ice cream properties. Four types of fats, originating from vegetable and animal sources, and two protein mixtures, i.e., skim milk powder (SMP) and functional dairy proteins (FDP), were tested. The low molecular weight emulsifiers used consisted of two mixtures of mono- and diglycerides (MDG), i.e., either saturated (SMDG) or partially unsaturated (PUMDG). The ice cream structure was characterized by classical methods, e.g., fat globule size distribution and melting time, while front-face fluorescence was used to characterize the formulations on a molecular basis.

## 2. Materials and methods

### 2.1. Materials

Three vegetable fats (hydrogenated coconut oil, refined coconut oil and refined palm oil) were obtained from SIO (Bougival, France). Anhydrous milk fat was purchased from France Beurre (Quimper, France). SMP (35%, w/w, proteins; Coopérative d'Isigny Saint-Mère, Isigny Sur Mer, France) and FDP, prepared by the supplier using a partial heat denaturation of the protein (20%, w/w, proteins; Bel Industries, Vincennes, France) were the protein sources. Relative to the total protein content, SMP contained 80% (w/w) casein and 20% (w/w) whey proteins, while FDP contained 40% (w/w) casein and 60% (w/w) whey protein (Granger, Barey, Veschambre, & Cansell, 2005). Sucrose and corn syrup solids (40 DE, 80 Brix solid content) were purchased from Eurosucre (Paris, France) and Cerestar (France), respectively. Stabilisers (guar gum and locust bean gum) and emulsifiers, i.e., SMDG and PUMDG (60%, w/w, alpha monoglycerides) were donated by Degussa Food Ingredients (Baupte, France). PUMDG contained oleic (18:1) and linoleic (18:2) acids (20% and 3%, w/w, respectively, of total fatty acids). SMDG comprised only palmitic (16:0) and stearic (18:0) acids (56% and 42%, w/w, respectively, of total fatty acids).

### 2.2. Ice cream preparation

Sixteen different ice cream mixes were prepared based on the ingredients and proportions presented in Table 1. All ingredients except the fat and the corn syrup were dry blended, mixed with water and immediately blended (65 °C for 15 min) with the melted fat and corn syrup portion. Mixes were homogenized at 70 °C, at 170/30 bar (Manton Gaulin Lab 60, double-piston two-stage homogenizer, APV France, Evreux, France), pasteurized at 85 °C for 30 s (APV Junior, APV France, Evreux, France), cooled and immediately stored at 4 °C in a water bath and aged overnight at 4 °C under stirring. Batches of ice cream mix (20 L) were frozen (overrun: 100%, outlet temperature: −5 °C) in a continuous freezer (WCB ice cream CS 100, Waukesha Cherry Burrell, Milan, Italy). Ice creams were hardened at −40 °C and stored at −25 °C. For each recipe, three different batches were produced.

Table 1
Ingredients and their proportions used in the formulation of the different ice cream mixes

| Ingredient | Proportion (%, w/w) | Type |
|---|---|---|
| Fat[a] | 8 | Hydrogenated coconut oil<br>Refined coconut oil<br>Refined palm oil<br>Anhydrous milk fat |
| Emulsifier[a] | 0.3 | Saturated mono- and diglycerides<br>Partially unsaturated mono- and diglycerides |
| Protein source[a,b] | 10 | Skim milk powder<br>Functional dairy proteins |
| Sugars | 12 | Sucrose |
| | 6 | Corn syrup solids |
| Stabilisers | 0.2 | Guar gum and locust bean gum mixture |
| Water | 63.5 | |

[a]Each mix formulation contained only one type of fat, protein and emulsifier.
[b]Skim milk powder and functional dairy proteins contained 35% and 20% (w/w) protein, respectively.

### 2.3. Fat globule size analysis

Fat globule size distribution in ice creams was measured by integrated light scattering using a Mastersizer S (Malvern Instruments SA, Orsay, France). Ice cream samples were diluted, in the measurement cell, with water at 13 °C. Measurements were performed, at room temperature, using ultrasonication to ensure the absence of air bubbles. Fat globule mean diameter (evaluated by the volume-weighted average diameter, $d_{4,3}$) was recorded and the cumulative percentage of fat aggregates with diameters greater than 2.0 μm ($d > 2.0$ μm) was calculated. Three samples from each batch were analysed.

### 2.4. Melting test

All the samples were stored at −25 °C before carrying out the melting test. Ice cream samples (270 ± 5 g) were placed on a mesh grid (mesh size 1 cm × 1 cm) and allowed to stand at 29 °C at constant humidity. The

weight of the material passing through the screen was recorded as a function of time. The time necessary to melt 30 g ($T_{30}$) of product was read.

### 2.5. Fluorescence spectroscopy

Fluorescence measurements were performed using a Fluorolog®-3 spectrofluorimeter (Jobin Yvon, Horiba, Longjumeau, France) equipped with a thermostatically controlled front-face accessory. The incidence angle of the excitation radiation was set at 22.5° to ensure that reflected light, scattered radiation and depolarization phenomena were minimized. Ice cream samples (1 cm × 1 cm × 3 cm taken from the middle of the ice cream sample) were placed into a quartz cell. Fluorescence spectra were recorded at 1 °C with excitation and emission slits set at 0.4 and 1 mm, respectively. The emission spectra of tryptophan residues (300–360 nm) were recorded at an excitation wavelength of 290 nm with a charge-coupled device (CCD) multi-channel detector (270 M imaging spectrograph, Jobin Yvon) that allowed instant emission spectrum recording. Each fluorescent emission spectrum was accumulated from three measurements to minimize remaining scattering effects. Three samples were taken from each batch (three different batches for each recipe) and analysed for fluorescence. In total, fluorescence data represented 144 tryptophan spectra collected from the 16 formulations of ice cream.

### 2.6. Mathematical data analysis

#### 2.6.1. Analysis of fluorescence spectra

Emission fluorescence spectra were analysed between 310 and 350 nm in order to reduce the Raman diffusion effect at the beginning of the spectra and to work in the best response domain of the CCD. Each individual emission fluorescence spectrum was smoothed (unpublished results) and normalized to reduce the area under the spectrum to a value of 1 (Bertrand & Scotter, 1992). Two statistical treatments were applied to the fluorescence spectra: principal component analysis (PCA) (Jollife, 1986) and partial least square (PLS) discriminant analysis. PCA and PLS discriminant analysis were run on The Unscrambler® v8.0 (Camo Process AS, Oslo, Norway).

In the present application, PCA was applied to the 144 tryptophan spectra. The variables were the fluorescence intensities corresponding to the 634 intervals measured between 310 and 350 nm. The 2-D mappings obtained by PCA allowed the investigation of the main differences in fluorescence between formulations. When one of the principal components (i.e., one of the mapping axes) allowed discrimination between formulations, a spectral pattern was plotted to obtain the contribution of each wavelength. The contribution is the correlation coefficient between the wavelength and the principal component.

In the case where PCA axes did not allow any discrimination, the ability to use the fluorescence data to distinguish between the different ice cream ingredients, i.e., proteins, fats and emulsifiers, was investigated by PLS discriminant analysis. PLS regression techniques (Esbensen, 2001; Martens & Naes, 1989; Tenenhaus, 1998) were initially designed in the context of model production and prediction of quantitative variables when strong relationships exist between endogenous variables. For this reason, PLS regression was readily adapted to fluorescence spectra since strong correlations existed between consecutive emission wavelengths. PLS discriminant analysis (Tenenhaus, 1998) is an adaptation of PLS regression to the case of categorical variables. In our case, the dummy variables described the categorization of the formulations of protein (SMP or FDP), fat (hydrogenated coconut oil, refined coconut oil, refined palm oil or anhydrous milk fat) and emulsifier (SMDG or PUMDG). PLS discriminant analysis was used to derive discriminant functions, which are the linear combinations of the endogenous variables (i.e., the wavelength responses) that best model the dummy variables. As a result of PLS discriminant analysis, spectral patterns were obtained. In contrast to the spectral patterns obtained by PCA, the PLS-derived spectral patterns plot the regression coefficients of the designed discriminant functions. These spectral patterns showed the emission wavelengths that best discriminated each protein mixture and each protein mixture in association with fat or emulsifier. To carry out PLS discriminant analysis, the emission fluorescence spectra were split into a calibration set and a validation set. Of the three fluorescence spectra recorded corresponding to three different samples of each ice cream batch, two were assigned to the calibration set and the third was assigned to the validation set. The calibration set was used to derive the PLS discriminant functions of all protein, fat and emulsifier categories. The validation set was used to produce classification matrices that allowed verification of the relevance of the models for the discrimination of the different ingredients.

#### 2.6.2. Analysis of variance

Analysis of variance (ANOVA), using Fisher's least significant difference (LSD) procedures (Stat-Graphics Plus software, Manugistics, Rockville, MD, USA), was performed on $d_{4,3}$, $d > 2.0\,\mu m$, and melting time ($T_{30}$) to discriminate among the 16 formulations.

## 3. Results and discussion

### 3.1. Influence of formulation on ice cream physical characteristics

Table 2 presents the results of ANOVA performed on a data set comprising three physical variables

characterizing the ice cream products. The two variables corresponding to the fat globule distribution were only loosely correlated. However, they were not strongly correlated with melting time.

The protein factor did not significantly influence the fat globule diameter. In contrast, for a given fat, SMDG led to lower diameters of fat globules in ice cream compared with PUMDG. Similar results showing the influence of MDG type on ice cream fat globule size have been previously reported (Barfod, Krog, Larsen, & Buchheim, 1991; Granger, Leger, Barey, Langendorff, & Cansell, 2005). The influence of fat type was only revealed for hydrogenated coconut oil that led to significantly lower $d_{4,3}$ values compared with the other fats. This result was previously interpreted in terms of specific interactions occurring between this fat and SMDG (Granger, Leger et al., 2005). Considering the emulsifier/fat interaction, the relative increase in $d_{4,3}$ values corresponding to the replacement of SMDG by PUMDG was similar for hydrogenated coconut oil and refined palm oil (about 26%), on the one hand, and for anhydrous milk fat and refined coconut oil (about 90%), on the other hand. This suggested that different molecular interactions would occur between these two sets of fats and the emulsifiers.

Fat droplet destabilization can be evaluated by the determination of the cumulative percentage of particles above a specific value (Bolliger, Goff, & Tharp, 2000). Based on previous studies on emulsified systems (Granger, Barey, Combe, Veschambre, & Cansell, 2003) and ice creams (Granger, Barey, Renouf, Langendorff, & Cansell, 2004), the value of 2 μm was taken as the limit between isolated fat globules and aggregated/coalesced particles. The $d>2\,\mu m$ was influenced by the main factors and all the interactions. High $d>2\,\mu m$ observed for PUMDG-based formulations corresponded to an increased aggregation/coalescence phenomenon of fat droplets occurring upon whipping/freezing (Bolliger et al., 2000; Gelin, Poyen, Courthaudon, Le Meste, & Lorient, 1994; Goff, 1997; Relkin, Sourdet, & Fosseux, 2003). When considering a specific fat/emulsifier couple, $d_{4,3}$ and $d>2\,\mu m$ followed a similar pattern. This suggested that this interaction significantly influenced the destabilization phenomenon in ice cream. The protein mixture influenced the $d>2\,\mu m$. This significant effect was attributed to the specific formulation based on refined coconut oil, PUMDG and FDP that developed higher fat destabilization. For the other formulations, the protein mixture used did not have a significant effect on $d>2\,\mu m$. This agreed with the fact that $d_{4,3}$ and $d>2\,\mu m$ were not strongly correlated.

Melting times of ice cream were independent of the protein mixture used in the formulation but were significantly influenced by the types of emulsifier and fat, as well as by the different interactions between the

Table 2
Analysis of variance showing the influence of ice cream ingredients on the physical properties of ice creams

| Component/component blend[a] | $d_{4,3}$ (μm)[b] | $d>2\,\mu m$ (%)[c] | $T_{30}$ (min)[d] |
|---|---|---|---|
| *Emulsifier* | | | |
| LS[e] | *** | *** | *** |
| SMDG | 1.8[a] | 17[a] | 52[a] |
| PUMDG | 2.8[b] | 35[b] | 101[b] |
| *Fat* | | | |
| LS | *** | *** | *** |
| HCO | 1.7[a] | 18[a] | 86[a] |
| RCO | 2.6[b] | 28[b] | 92[b] |
| RPO | 2.4[b] | 28[b] | 71[c] |
| AMF | 2.5[b] | 29[b] | 55[d] |
| *Protein* | | | |
| LS | NS | *** | NS |
| SMP | 2.2 | 25[a] | 75 |
| FDP | 2.4 | 27[b] | 77 |
| *Emulsifier/fat* | | | |
| LS | *** | *** | *** |
| HCO/SMDG | 1.5 | 15 | 59 |
| HCO/PUMDG | 1.9 | 22 | 112 |
| RCO/SMDG | 1.8 | 14 | 50 |
| RCO/PUMDG | 3.4 | 43 | 135 |
| RPO/SMDG | 2.1 | 22 | 56 |
| RPO/PUMDG | 2.7 | 35 | 86 |
| AMF/SMDG | 1.8 | 16 | 42 |
| AMF/PUMDG | 3.2 | 42 | 69 |
| *Emulsifier/protein* | | | |
| LS | *** | *** | *** |
| SMP/SMDG | 1.8 | 16 | 53 |
| SMP/PUMDG | 2.6 | 33 | 98 |
| FDP/SMDG | 1.8 | 17 | 50 |
| FDP/PUMDG | 3 | 37 | 103 |
| *Fat/protein* | | | |
| LS | *** | *** | *** |
| HCO/SMP | 1.6 | 16 | 78 |
| HCO/FDP | 1.8 | 20 | 93 |
| RCO/SMP | 2.3 | 24 | 85 |
| RCO/FDP | 2.9 | 33 | 99 |
| RPO/SMP | 2.4 | 28 | 76 |
| RPO/FDP | 2.4 | 28 | 66 |
| AMF/SMP | 2.5 | 29 | 62 |
| AMF/FDP | 2.4 | 28 | 49 |
| *Fat/protein/emulsifier* | | | |
| LS | *** | *** | *** |

[a]Abbreviations are as follows: SMDG, saturated mono- and diglycerides; PUMDG, partially unsaturated mono- and diglycerides; HCO, hydrogenated coconut oil; RCO, refined coconut oil; RPO, refined palm oil; AMF, anhydrous milk fat; SMP, skim milk powder; FDP, functional dairy proteins.

[b]Volume-weighted average diameter.

[c]Percentage of fat globules with diameter greater than 2 μm.

[d]Time necessary to melt 30 g of ice cream.

[e]LS, level of significance of the corresponding factor (emulsifier, fat or protein and their interactions); ***, factor significantly different; NS, factor not significantly different ($P>0.05$). Different letters in the same column (within each main effect) indicate values are significantly different ($P<0.05$).

three ingredients. The lower and higher melting times were obtained for the formulations based on anhydrous milk fat and refined coconut oil, respectively. The presence of unsaturated fatty acids in MDG structure led to a drastic increase in melting times compared with those obtained using the saturated emulsifier. This was even more pronounced with the two coconut oils, suggesting that the resulting ice creams were more textured than those obtained with the two other fats. The formulation involving PUMDG, FDP and refined coconut oil was characterized by a high melting time value (152 min) compared with those obtained for the other formulations. This specific ingredient selection was mainly responsible for the significance of the ingredient factors. Since fat aggregates contribute greatly to the structure of ice cream by forming a three-dimensional network (Goff, 1997), long melting times could be associated with increased fat globule destabilization. However, melting time values were not well correlated with fat globule diameters (Granger, Leger et al., 2005). This suggested that other parameters, such as mix formulation, globule interactions and/or fat crystallization, may also influence the melting behaviour of ice cream. This observation was strengthened by the fact that the four fats discriminated from each other with respect to the melting times, whereas only hydrogenated coconut oil was discriminated from the other formulations when the fat globule size distribution was considered. Thus, ice cream analysis, using fat globule size distribution and melting time, revealed different facets of the molecular interactions existing between emulsifier, fat and protein.

### *3.2. Discrimination between ice creams using tryptophan fluorescence spectra*

Fluorescent properties of fluorophores are known to be very sensitive to changes in their environment (Lakowicz, 1983; Marangoni, 1992). Although studies on milk (Dufour & Riaublanc, 1997; Herbert et al., 1999; Lopez & Dufour, 2001) and cheeses (Dufour et al., 2001; Herbert et al., 2000) showed that fluorescence measurements could reveal different protein structures, to the best of our knowledge no study has been performed on ice creams. Rheological studies (Granger, Leger et al., 2005) and thermal analysis (Granger, Schöppe, Leger, Barey, & Cansell, 2005) showed that ice cream structure was dependent on the formulation, i.e., fat type, emulsifier and protein. On a molecular basis, fluorescence could also be a pertinent method to indicate the different interactions occurring in this type of dairy product. Fig. 1 presents typical tryptophan emission fluorescence spectra of two different ice cream formulations. Although fluorescence spectra appeared to be very similar, some differences were detected in the maximum fluorescence intensity and/or the wavelength at which this maximum occurred.

Fig. 2 shows the mapping defined by principal components 1 and 2 of the PCA performed on fluorescence spectra of ice creams. Components 1 and 2 represented 83.2% and 15.2% of the total variance, respectively. This mapping separated the ice creams according to the protein mixture, so that two distinct clusters containing FDP- and SMP-based formulations were distinguished. This discrimination could be attributed, at least in part, to the organization of the molecules at the interface and/or in the cryo-concentrated aqueous phase. Indeed, rheological studies suggested that different microstructures were developed as a function of the protein type. These microstuctures could also reveal different interactions with the other components present in the formulation (Granger et al., 2004).

Since neither the first nor the second principal components allowed the total discrimination of the protein mixtures, PLS discriminant analysis was performed on the spectral data. The discriminant function was designed on the calibration set and then exercised both on the calibration and the validation sets. Good

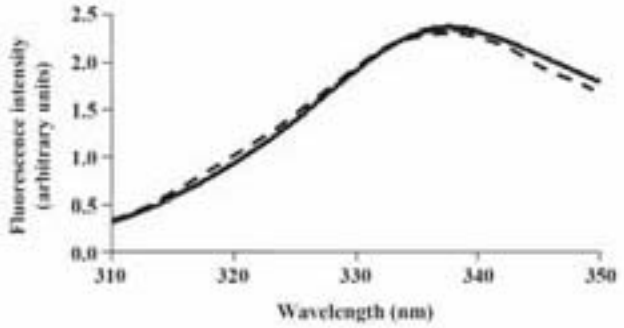


Fig. 1. Normalized tryptophan emission fluorescence spectra of ice creams based on saturated mono- and diglycerides and anhydrous milk fat and SMP (—) or hydrogenated coconut oil and FDP (- - -).

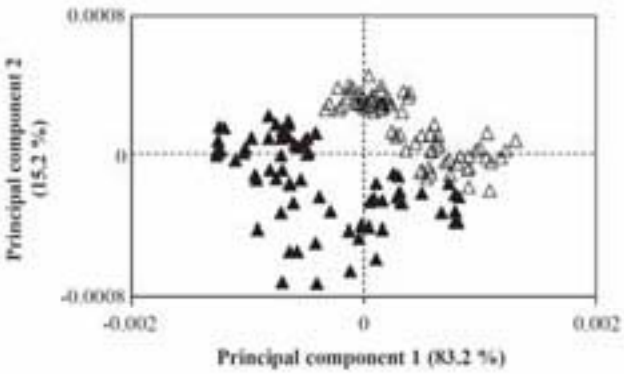


Fig. 2. Similarity map for the principal components 1 and 2 of the PCA performed on the tryptophan emission fluorescence spectra of the 16 ice cream formulations based on SMP (△) and FDP (▲).

classification rates were observed for the protein factor on both sets (94.7% and 95.7%, respectively). It was concluded that tryptophan fluorescence enabled efficient discrimination between SMP and FDP. Fig. 3 presents the spectral pattern associated with the protein discriminant function obtained by the mentioned PLS discriminant analysis. This spectral pattern showed the wavelengths that best discriminated between the two proteins. The spectral patterns can be used to derive structural information at a molecular level (Dufour et al., 2001). The most discriminant wavelengths were 313 and 333 nm, in agreement with values found in emulsified systems (Granger, Barey, Toutain, & Cansell, 2005; Lopez & Dufour, 2001; Rampon, Lethuaut, Mouhous-Riou, & Genot, 2001), corresponding to tryptophan residues in hydrophobic and hydrophilic environments, respectively. Moreover, FDP mixture emitted more at wavelengths around 333 nm, while SMP was characterized by emissions at wavelengths around 313 nm. This could be interpreted in terms of differences in protein conformation, protein behaviour at the interfaces and network formation. In particular, this latter assumption was based on the different structural arrangement observed, for these two protein mixtures, by dynamic rheology (Granger et al., 2004). Our results suggested that, compared with SMP, FDP should exhibit a more disordered structure due to the partial denaturation leading to more interactions with the aqueous medium.

To amplify the differences observed with the other factors, i.e., fat and emulsifier, PCA was then applied separately to the two data subsets corresponding to the protein mixtures SMP and FDP (Figs. 4(a) and (b), respectively). No distinction was found between the two emulsifier types used in ice cream formulations. In contrast, for both SMP- and FDP-based formulations, two clusters could be identified on PCA similarity maps as a function of the fat type used. In the case of SMP-based formulations (Fig. 4(a)), the first cluster comprised ice creams based on hydrogenated coconut oil and refined palm oil, the second comprised ice creams based on anhydrous milk fat and refined coconut oil. These two clusters were positioned on the left and right of the graphic, respectively. Thus, the first principal component allowed some discrimination of the proteins in association with the fats. Similar results were obtained with FDP-based formulations (Fig. 4(b)). In addition, it could be noticed that a slightly better discrimination between the fats was possible in each cluster. The discrimination of the two clusters could not be explained simply by the chain length and by the degree of unsaturation of the fatty acids. Indeed, the two coconut oils only differed by 8% in terms of unsaturated fatty acids, yet the corresponding ice creams were efficiently discriminated. Hydrogenated coconut oil (saturated) and refined palm oil (unsaturated) gave very similar fluorescence responses; consequently the formulations overlapped.

To indicate which wavelengths were involved in the discrimination of the different fat type formulations, the spectral patterns associated with the first factorial axes of the PCA were plotted for SMP- and FDP-based

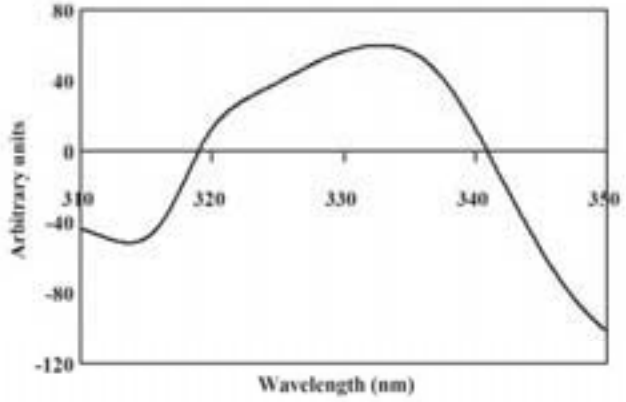


Fig. 3. Spectral pattern corresponding to the discriminant function obtained by PLS discriminant analysis performed on tryptophan emission fluorescence spectra recorded on the 16 ice cream formulations.

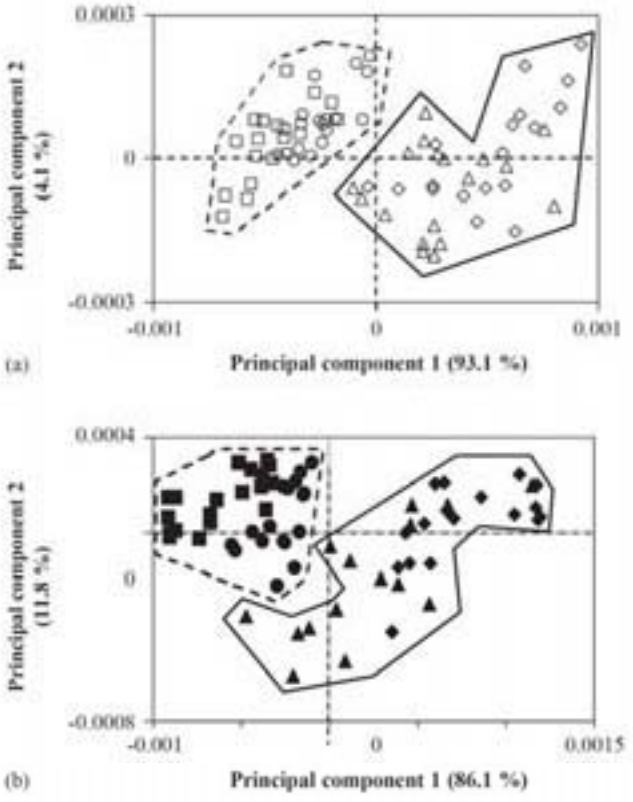


Fig. 4. Similarity maps for the principal components 1 and 2 of the PCA performed on the tryptophan emission fluorescence spectra of ice creams. PCA performed on formulations based on (a) SMP and (b) FDP. Hydrogenated coconut oil (○, ●), refined coconut oil (△, ▲), refined palm oil (□, ■) and anhydrous milk fat (◇, ◆).

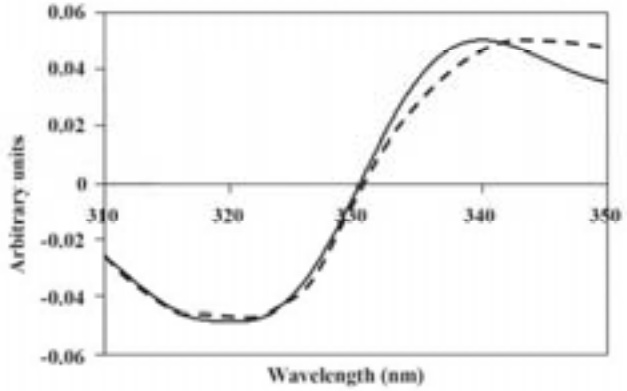


Fig. 5. Spectral patterns corresponding to the principal component 1 for PCA performed on tryptophan emission fluorescence spectra recorded on SMP-based formulations (—) or on FDP-based formulations (- - -).

formulations (Fig. 5). For both protein mixtures, negative and positive spectral peaks appeared around 320 and 340 nm, respectively. According to the spectral patterns and PCA mappings, it can be deduced that 320 nm characterized the protein mixtures associated with hydrogenated coconut oil and refined palm oil, whereas 340 nm was more relevant to the proteins with anhydrous milk fat and refined coconut oil. These wavelengths could be interpreted in terms of protein–fat interactions. SMP and FDP associated with hydrogenated coconut oil and refined palm oil would interact more closely than with anhydrous milk fat and refined coconut oil. Moreover, the small difference in unsaturated fatty acid composition for refined coconut oil and hydrogenated coconut oil could promote specific fat behaviour, i.e., amount, organization and/or polymorphic form of the crystallized matter that may lead to peculiar protein–fat associations.

## 4. Conclusions

The results obtained with the physical characterization of ice cream indicated the influence of the emulsifier, and to a lesser extent the influence of the fat type. In contrast, the fluorescence data allowed the identification, at a molecular level, of different protein structures and different interactions that may occur between protein and fat. In particular, the spectral patterns associated with the first axis of PCA indicated that the wavelengths involved in the discrimination of the different fat type formulations were different from those found for protein discrimination. This prevented correlation of the methods of characterization used in this work. All these results showed that specific interactions occurred between fat and surfactants, i.e., protein and emulsifier, so that the consideration of the chain length and the degree of unsaturation of the fatty acids of the fat phase could not be used to predict physical or molecular characteristics of the ice creams in a simple manner. These interactions should lead to the formation of different networks, i.e., partially agglomerated fat globules, air phase, ice crystals and protein and polymer networks. On the whole, multiple analytical methods were required to access the fine molecular structure, textural properties and interactions between ingredients in ice cream.

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**III.5.**

### Sensory analysis

The way in which the consumer perceives a product depends on a number of factors. Visual aspect and aroma are perhaps the paramount factors, but the role of texture must not be underestimated. In the case of ice cream, four textural descriptors, *i.e.*, coldness, airiness, firmness and unctuousness, were evaluated using sensory profile. The statistical analysis of the results was performed thanks to the collaboration with J.-P. Da Costa (ENITA de Bordeaux).

Figure 6, representing the correlation circle on PC 1 and 2, shows a projection of the initial variables in the factor space. Components 1 and 2 represented 30 % and 17 % of the total variance, respectively. Because the variables were far from the center and not close to each other, they were meaningful and not correlated. The correlation circle indicated that PC 1 was linked to unctuousness and coldness and PC 2 to airiness. Figure 7 shows the mapping defined by principal components 1 and 2 of PCA performed on sensory analysis of ice creams. This mapping separated the ice creams according to the protein mixture, so that two distinct clusters containing FDP- and SMP-based formulations were distinguished. Ice creams could not be discriminated with the fat or the emulsifier types. The discrimination with the protein mixture seemed to be independent of the product firmness. FDP-based products developed more airiness and coldness than SMP-ones. Moreover, the replacement of SMP by FDP led to poorly unctuous products. Some formulations exhibited specific behaviors so that they could not be classified in a group. This was the case for: i) the formulation based on refined coconut oil, FDP and partially unsaturated MDG that did not match the behavior of the other formulations containing this type of protein mixture; ii) the formulation based on SMP, partially unsaturated MDG and hydrogenated coconut oil that developed higher unctuousness than the other formulations.

## III.6. Conclusions

Ice creams were characterized by different methods. Each method was relevant of the impact of one or more ingredients on the property tested as summarized in Table 18. It is clear from this table that correlation between methods could not be expected when one method is influenced by the emulsifier while the other is more sensitive to the fat, for example. However, for a specific fat, *i.e.*, **refined coconut oil,** it was found that fat agglomeration, melting times and storage modulus at 20°C were positively correlated (Figures 8a and b) . This would imply that

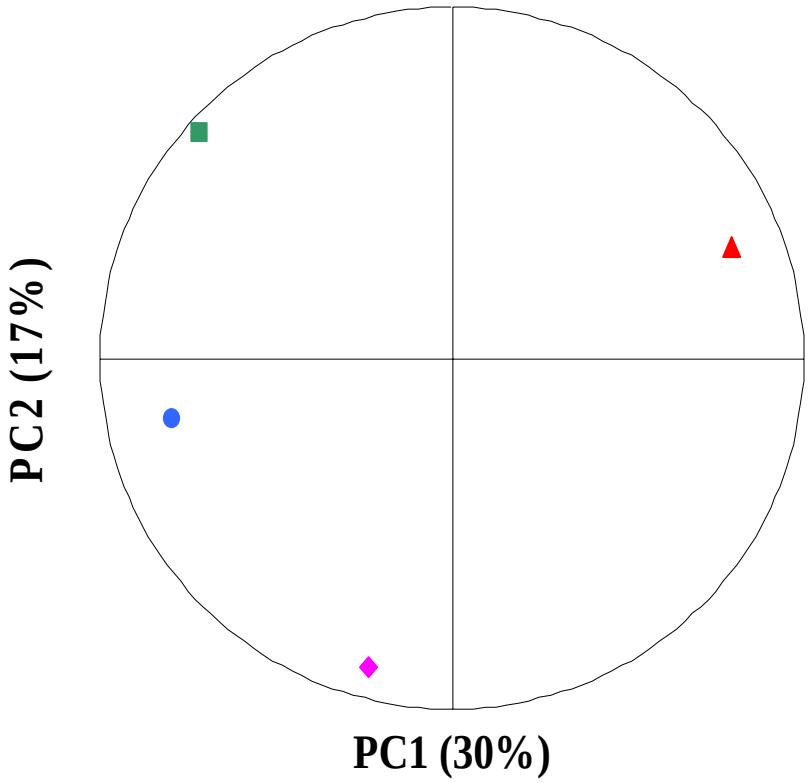


**Figure 6: Plot of descriptive attributes for the principal components 1 and 2 analyzed by principal component analysis performed of sensory analysis of ice creams. Unctuousness (▲), firmness (■), coldness (●) and airiness (♦).**

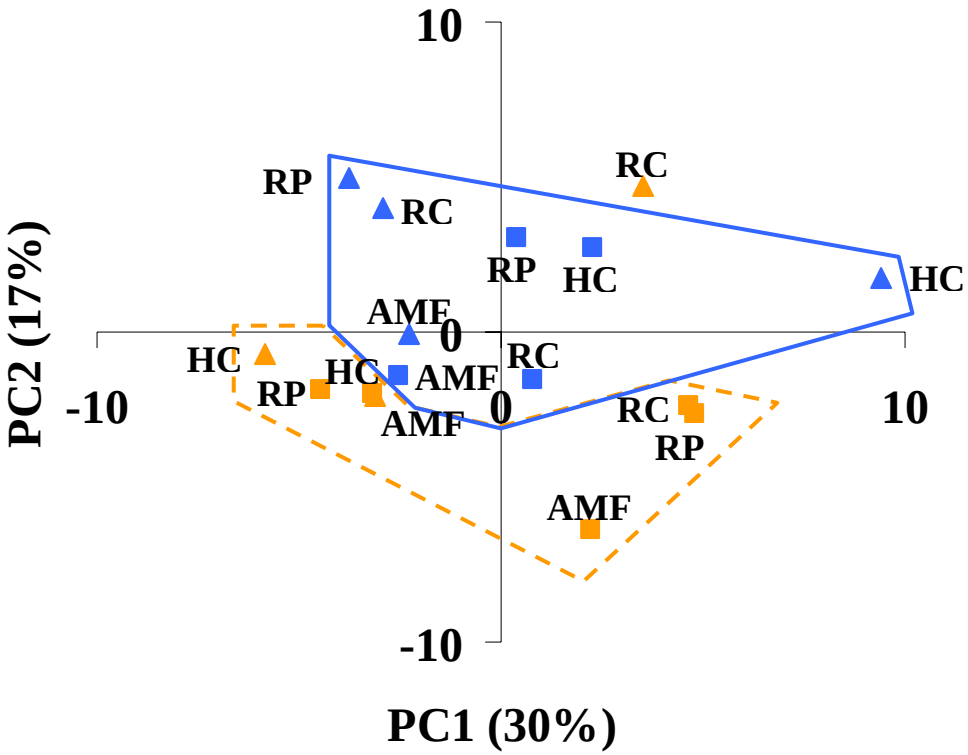


**Figure 7: Similarity map for the principal components 1 and 2 of the principal component analysis performed on sensory analysis of ice creams. Saturated mono- and diglyceride mixture (■, ■), partially unsaturated mono- and diglyceride mixture (▲, ▲), skim milk powder (■, ▲), functional dairy protein (■, ▲). RP: Refined palm oil; RC: Refined coconut oil; HC: Hydrogenated coconut oil; AMH: Anhydrous milk fat.**

**Table 18: Summary of the impact of an ingredient type on ice cream characterization obtained by a specific method.**

| Ingredient | Fat globule distribution | Melting time | Rheology | DSC | Front-face fluorescence | Sensory analysis |
|---|---|---|---|---|---|---|
| Emulsifier | ✕ (1) | ✕ | ✕ | | | |
| Protein mixture | | | ✕ | | ✕ | ✕ |
| Fat | ✕ | ✕ | ✕ | ✕ | ✕ | |

(1) significant influence of the ingredient.

DSC: differential scanning calorimetry.

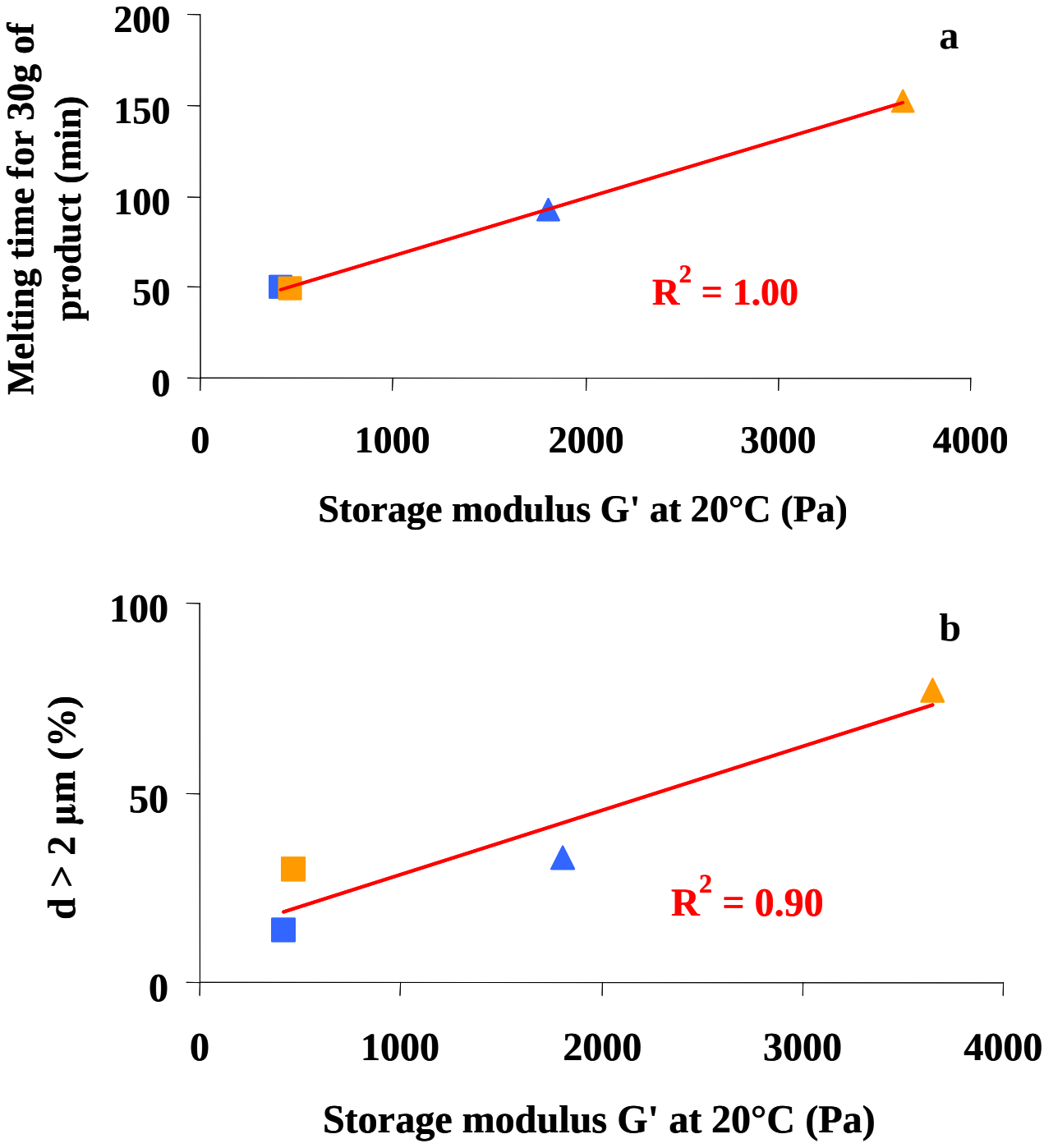


**Figure 8: Correlation between storage modulus (G') at 20°C and a: the percentage of particle > 2 µm in ice cream and b: time to melt 30 g of product (min) for refined coconut oil-based formulations. Saturated mono- and diglyceride mixture (■, ■), partially unsaturated mono- and diglyceride mixture (▲, ▲), skim milk powder (■,▲), functional dairy protein (■, ▲).**

ice cream structuring was mainly due to fat globule aggregation size relevant of the presence of emulsifiers and proteins. When **three different fats** (hydrogenated and refined coconut oils and refined palm oil) were considered, the correlation between the storage modulus measured at 20°C and the melting time was still found ($r^2 = 0.91$) (**Granger *et al.*, 2005d**, § C.III.3.). However, the correlation between the melting time and the fat globule distribution was lost ($r^2 = 0.42$) (**Granger *et al.*, 2005d**, § C.III.3.). Since the melting time was not simply correlated to the fat globule diameters, this suggested that other parameters, such as globule interactions and/or fat crystallization, may also influence the melting behavior of ice cream mixes. Similar results were found when the values relative to anhydrous milk fat-based samples were added. In particular, the correlation between the melting time and the storage modulus was maintained ($r^2 = 0.86$) (Figure 9), since no correlation was found between fat agglomeration, melting times and storage modulus at 20°C. Even if the rheological and fluorescence studied suggested that different microstructures were developed as a function of the protein mixture (§ C.III.2. and **Granger *et al.*, 2005e**, 0.) no correlation were found between these two methods. .

**Similarity in the behavior of ice cream mix and ice cream product** was found in rheological behavior as, in all cases, FDP-based formulations led to higher elastic modulus values than SMP-based ones. In particular the mixes based on hydrogenated and refined coconut oil and partially unsaturated MDG that were characterized by high viscosity values after ageing corresponded to ice creams that exhibited the higher G' values .

However, **differences in the behavior of ice cream mix and ice cream product** were also pointed out in: i) the absence of significant effect of the protein mixture used in ice cream formulation on the particle size distribution of ice cream, although, before the freezing stage, in mixes, FDP led to high fat globule flocculation (§ C.II.2.). Moreover, the influence of the emulsifier and fat types on **particle size distribution** was revealed in the case of ice cream product whereas these ingredients poorly influenced the globule size parameter in the case of mix; ii) thermal properties, as the differences observed between the emulsified fat and bulk **fat melting** profiles were quite small in the case of ice cream product, although emulsification in mix led to the modification of the main crystallization peak and to an additional exotherm.

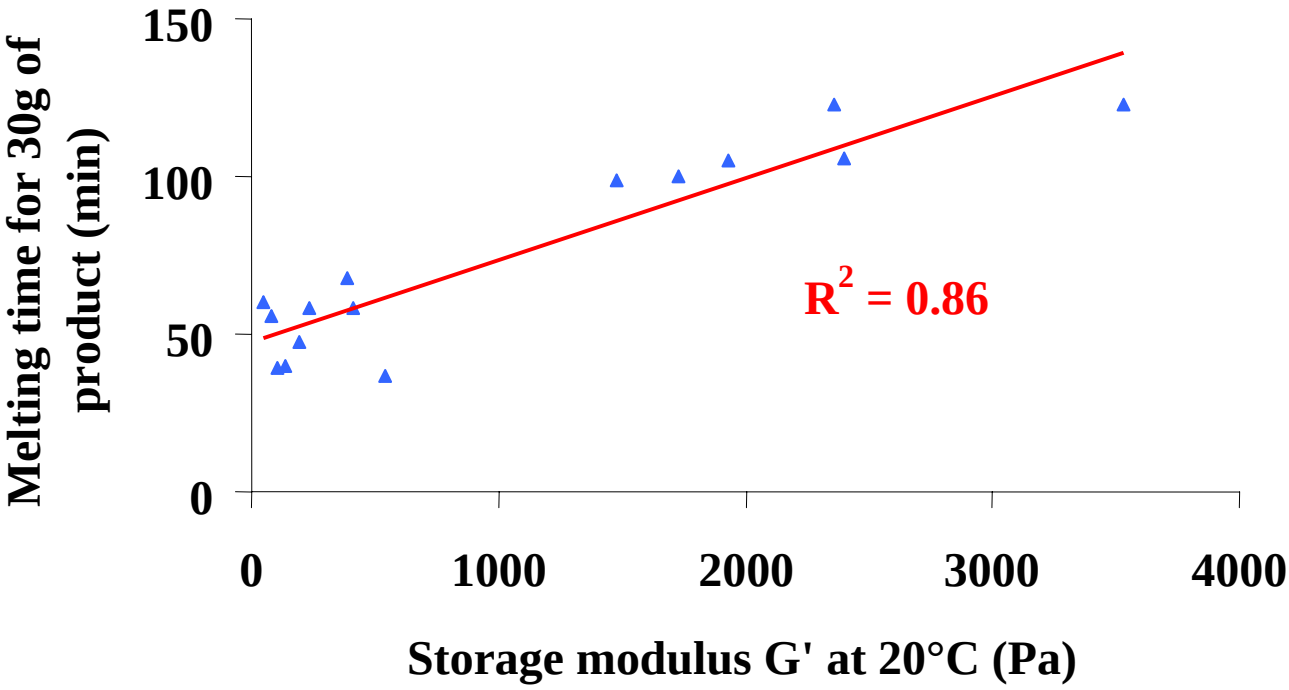


**Figure 9: Correlation between storage modulus (G') at 20°C and melting time of 30 g of product for the different ice cream formulations based on skim milk powder, saturated or partially unsaturated mono- and diglyceride mixtures, and hydrogenated coconut oil, refined coconut oil, refined palm oil or anhydrous milk fat.**

# D. CONCLUSIONS AND PERSPECTIVES

When an ice cream formulation is depicted, it appears as a complex mixture of ingredients. Each ingredient plays an important role during the manufacture of ice cream and in the textural and organoleptic properties of the final product. The selection of the ingredients must take into account several requirements such as technological and economical constraints, marketing demand for new product design, consumer taste, and more and more nutritional purpose. In the face of the development of the ingredient market, the choice of the appropriate ingredients that match these requirements is an important challenge. Thus, it is worth understanding the roles of the various ingredients during the ice cream manufacture and in the final product properties. We have focused our attention on the impact of the fat nature since this parameter is certainly the less investigated, although the role of fat in the development of the ice cream characteristics and in particular its sensory properties is well known.

First, our work agreed with the competitive adsorption / desorption phenomenon occurring between proteins and low molecular weight emulsifiers especially during the ageing period, already demonstrated by others. However, the systematic analysis of the different ice cream formulations allowed some tendencies to be brought out: i) protein denaturation by heat treatment that would allow the formation of a protein network around the fat droplets prevented protein desorption during ageing; ii) the unsaturation degree of the MDG enhanced the protein desorption phenomenon. However, these results had to be modulated by the nature of the fat used for the initial oil-in-water emulsion. This strongly suggested that interactions between the tensioactive molecules and fat are taking place leading to specific behavior of the oil-in-water emulsions after the homogenization step and during ageing.

Second, the influence of the formulation was also revealed on the final product especially when the melting time or the sensory perception were investigated. However, when all the formulations were considered, no obvious tendency concerning the influence of the unsaturation degree of the emulsifier or the chain length and the unsaturation degree of the fat could be stated. Indeed, some formulations revealed specific association between ingredients. The random behavior of the resulting ice cream prevented the prediction of the ice cream properties just by considering its formulation. On the whole, only a mapping of the different ice creams as a function of their formulation could be obtained. As already mentioned, it is worth noting that the characteristics of the ice creams, melting time or textural properties, could not be completely predicted by using the physicochemical characterizations of the oil-in-water emulsion or of the mix. For example, the protein desorption phenomenon observed in emulsions that is assumed to be essential for fat agglomeration to occur during the freezing step, was not highly correlated to the percentage of fat agglomerates in ice cream products. In other words, although the

understanding of oil / water interface created in the first step of ice cream processing is of major importance to manage the manufacture of a good quality ice cream product (no protein desorption or high fat coalescence before the freezing step lead to defective products), the emulsion or the mix characteristics are not sufficient to entirely describe the final product properties.

Finally, during this study, numerous methods were used to characterize the different systems, *i.e.*, emulsion, ice cream mix and ice cream. We have searched correlation between the different results obtained and considered that a correlation exists between two methods when the regression coefficient was higher than 0.8. By selecting specific formulations, some correlations could be found. However, when all the formulations were considered correlations may be lost. The lack of correlation between most of the methods used leads to several comments: i) to be correlated, methods have to reveal the influence of, at least, one same component of the formulation. For example, DSC and sensory analysis results could not be correlated since they are influenced by the fat nature and the protein type, respectively; ii) it is important to cross various methods of characterization in order to get a widespread vision of the ice cream properties; iii) some methods were appropriate to probe one system but could not be accurate to characterize another system. This was the case for front-face fluorescence. In the emulsion sytem, fluorescence measurement allowed discriminating and quantifying the proteins in two distinct environment, *i.e.*, in the aqueous phase and adsorbed at the fat globule surface and thus could be correlated with the spectrophotometric method. In contrast, in ice cream product, the presence of numerous interfaces prevented the accurate quantification of the proteins loaded at the different interfaces and dispersed in the cryo-concentrated phase. In this case, although fluorescence data allowed a mapping of the different products, this technique could no be correlated with another one.

In this work, we have clearly demonstrated that fat, emulsifier and protein interacted differently as a function of their physicochemical characteristics. It would be of great interest to further investigate these interactions. This could go through, the use of new techniques. Applications of atomic force microscopy (AFM) and scanning near-field optical microscopy (SNOM) to study protein-surfactant interactions at air / water and oil / water interfaces has revealed new unexpected generic models for surfactant-induced destabilization of protein-stabilized foams and emulsions. AFM confirmed the existence of protein networks at the interface. Moreover, the mechanism by which surfactant displaces protein was also illustrated by this method (Morris, 2004). As ice cream contains a mixture of proteins and emulsifiers at the

interfaces, there is a need of identify the structure of mixed films and to study the behavior of this structure upon decreasing temperature. In this case, it is necessary to label different proteins in order to determine their location at the interface. Preliminary studies on BSA-Tween 20 films at air / water interfaces have shown that it is possible to label BSA with fluorescent tags without significantly altering the displacement process. The small size of the surfactant domains during the early stages of displacement requires the use of SNOM to visualize mixed protein / surfactant films (Gunning, *et al.*, 2001). Moreover, dynamic interfacial tension measurements could be also of great interest to provide information on the adsorbed layers at the interface. The behavior of proteins and low molecular weight emulsifiers at continuously expended or compressed interfaces can be used to determine the type of interactions between adsorbed molecules, and the effect of protein adsorption on interfacial rheological properties (shear and dilational) (Bos and van Vliet, 2001).

We have focused this work on the oil / water interface. However, the control of the formation and the stabilization of air bubbles is also crucial for a good quality ice cream. Here again, the ingredient selection should influence the air phase structure and stability. Few techniques enable the monitoring of the air phase in food samples. We have started the air phase analysis through the study of ice creams differing in the emulsifier nature (saturated or partially unsaturated MDG), the protein mixture (SMP or FDP), and/or the fat type (anhydrous milk fat, refined palm oil, hydrogenated or refined coconut oils). Ice creams were analyzed just after being manufactured and after being submitted to heat chocks to simulate one year ageing. Air bubble size was visualized under an optical microscope placed in a refrigerated chamber. Air phase structure was evaluated based on the ability of an aerated ice cream to change in volume as a result of external pressure change. This analysis developed in collaboration with J. Renoir (Degussa Texturant Systems, Baupte) was performed via a pressure/depressure response method. This technique allowed the measurement of gas channeling in ice cream samples. Such channeling was expected to be involved in the shrinkage behavior of ice cream upon storage and so to be implicated in the final product quality. The first results suggested that the shrinkage phenomenon was correlated neither to the air cell initial size nor to the structural stability as evaluated by fat globule size distribution and melting behavior. Moreover, the shrinkage capacity seemed to be dependent on the ice cream formulation. Air phase analysis is still under investigation.

# E. CONCLUSIONS ET PERSPECTIVES

La crème glacée est constituée d'un mélange complexe d'ingrédients. Chacun d'eux joue un rôle important dans la fabrication du produit et conditionne, en partie, ses propriétés texturales et organoleptiques. Le choix des ingrédients doit prendre en compte plusieurs critères tels que les contraintes technologiques et économiques, la demande marketing pour la conception de nouveaux produits, le goût du consommateur, et de plus en plus l'aspect nutritionnel du produit. Face au développement du marché des crèmes glacées, la sélection des ingrédients appropriés répondant à ces multiples conditions constitue un défi important. C'est dans ce contexte que nous avons étudié l'influence de divers ingrédients sur la mise en place des structures de la crème glacée au cours de la fabrication et sur les propriétés du produit fini. Nous avons porté une attention particulière à la matière grasse car l'impact de la nature de cet ingrédient est certainement un des moins étudiés, bien que son importance dans le développement des caractéristiques des crèmes glacées et, en particulier, des propriétés sensorielles soit reconnu.

Dans un premier temps, un phénomène compétitif d'adsorption / désorption aux interfaces a été mis en évidence, dans les émulsions, entre les protéines et les émulsifiants de faible poids moléculaire, notamment pendant la période de maturation. L'analyse systématique des différentes formulations a permis de dégager les tendances suivantes : i) la dénaturation partielle des protéines par traitement thermique permettrait la formation d'un réseau protéique autour des gouttelettes d'huile limitant la désorption protéique pendant la maturation ; ii) le degré d'insaturation des MDG augmente le phénomène de désorption protéique. Cependant, ces résultats doivent être modulés en fonction de la nature de la matière grasse utilisée pour formuler l'émulsion huile-dans-eau. Ceci suggère fortement que des interactions entre les molécules tensioactives et la matière grasse existent et conditionnent un comportement spécifique des émulsions après l'étape d'homogénéisation et pendant la maturation.

Dans un deuxième temps, une étude a porté sur l'influence de la formulation sur les propriétés des crèmes glacées. Quand toutes les formulations sont prises en considération, aucune tendance nette tant au niveau de l'influence du degré d'insaturation de l'émulsifiant ou de la longueur de la chaîne grasse et du degré d'insaturation de la matière grasse n'a pu être mise en évidence. En effet, quelques formulations se sont distinguées et ont révélé une association spécifique entre ingrédients. La réponse aléatoire des crèmes glacées à une variation de formulation empêche la prévision des propriétés des produits finis en considérant uniquement les ingrédients présents. De ce fait, seule une cartographie des différentes crèmes glacées en fonction de leur formulation a pu être établie. De plus, il est intéressant de noter que les caractéristiques des crèmes glacées (temps de fonte ou propriétés organoleptiques …) ne sont pas prévisibles sur l'unique base des caractéristiques physico-chimiques de l'émulsion huile-dans-eau ou du mix. Par

exemple, le phénomène de désorption protéique observé dans les émulsions, considéré comme essentiel pour que l'agglomération de la matière grasse se produise pendant l'étape de foisonnement / congélation, n'a pas été entièrement corrélé avec le pourcentage de globules gras agglomérés dans les crèmes glacées.

Au cours de cette étude, de nombreuses méthodes ont été employées pour caractériser les différents systèmes : émulsion, mix et crème glacée. Nous avons recherché les corrélations entre les différents résultats obtenus en considérant que deux méthodes étaient corrélées quand le coefficient de régression linéaire était supérieur à 0,8. En choisissant certaines formulations, quelques corrélations ont été trouvées. Cependant, quand toutes les formulations sont prises en compte, certaines corrélations sont perdues. Le manque de corrélation entre la plupart des méthodes employées entraîne plusieurs commentaires : i) pour être corrélées, les méthodes doivent mettre en évidence l'influence d'au moins un même composant de la formulation. Par exemple, les résultats de DSC et d'analyse sensorielle ne peuvent pas être corrélés puisqu'ils sont influencés, respectivement, par la nature de la matière grasse et par le type de protéine ; ii) il est important de croiser diverses méthodes de caractérisation afin d'obtenir une vision globule des propriétés des crèmes glacées ; iii) certaines méthodes appropriées pour suivre un système ne le sont pas pour caractériser un autre système. C'est le cas de la fluorescence. Dans le cas des émulsions, la mesure de fluorescence a permis de distinguer et de quantifier la présence de protéines situées dans deux environnements distincts (dans la phase aqueuse et adsorbées à la surface des globules gras). Cette technique a pu être corrélée à une méthode de dosage spectrophotométrique. En revanche, dans les crèmes glacées, la présence de nombreuses interfaces a vraisemblablement empêché la quantification précise des protéines adsorbées aux différentes interfaces et dispersées dans la phase cryo-concentrée. Dans ce cas, bien que les données de fluorescence aient permis d'obtenir une cartographie des différents produits, cette technique n'a pas pu être corrélée avec une autre.

Dans ce travail, nous avons clairement démontré que la matière grasse, les émulsifiants et les protéines interagissaient différemment en fonction de leurs caractéristiques physico-chimiques. Il serait donc intéressant d'étudier plus en détail ces interactions. Ceci pourrait se faire par l'utilisation de nouvelles techniques. Les applications de la microscopie à force atomique (AFM) et de la microscopie optique en champ proche (SNOM) pour étudier les interactions protéine / émulsifiant aux interfaces air / eau et huile / eau ont abouti à l'élaboration de nouveaux modèles génériques pour décrire la déstabilisation protéique induite par les émulsifiants dans les mousses et émulsions. L'AFM a confirmé l'existence de réseaux de protéines aux interfaces. De plus, le mécanisme par lequel les émulsifiants déplacent les protéines a été également illustré par

cette méthode (Morris, 2004). Comme la crème glacée contient un mélange de protéines et d'émulsifiants aux interfaces, il est utile d'identifier la structure des films mixtes et d'étudier le comportement de cette structure lors de la diminution de température. Dans ce cas, il est nécessaire de marquer les différentes molécules afin de déterminer leur emplacement à l'interface. Les études préliminaires sur des films mixtes composés de BSA-Tween 20 aux interfaces air / eau ont prouvé qu'il était possible de marquer la BSA avec des molécules fluorescentes sans changer de manière significative le processus de déplacement. La petite taille des domaines formés par les émulsifiants dans les premiers temps du déplacement protéique exige l'utilisation de la méthode SNOM afin de visualiser ces films mixtes (Gunning, *et al.*, 2001). Les mesures dynamiques de tension interfaciale peuvent être également d'un grand intérêt pour fournir des informations sur les couches adsorbées à l'interface. Le comportement des protéines et des émulsifiants aux interfaces expansées ou comprimées sans interruption, peut permettre de déterminer le type d'interactions entre les molécules adsorbées, et l'effet de l'adsorption protéique sur les propriétés rhéologiques interfaciales (cisaillement et dilatation) (Bos and van Vliet, 2001).

Nous avons concentré nos études sur l'interface huile / eau. Cependant, le contrôle de la formation et de la stabilisation des bulles d'air est également un élément crucial pour obtenir une crème glacée de bonne qualité. Ici encore, le choix des ingrédients devrait influencer la structure et la stabilité de la phase aérée. Peu de techniques permettent l'observation de cette phase dans les échantillons alimentaires. Nous avons commencé l'analyse de la phase aérée par l'étude de crèmes glacées formulées à partir de différents MDG (saturé ou partiellement insaturé), de différents mélanges protéiques (SMP ou FDP), et/ou de différentes matières grasses (beurre concentré, huiles de coprah hydrogénée ou raffinée et huile de palme raffinée). Les crèmes glacées ont été analysées juste après fabrication et après avoir été soumises à des chocs thermiques pour simuler un vieillissement d'un an de stockage dans des conditions normales. La taille des bulles d'air a été observée sous un microscope optique placé dans une chambre frigorifiée. La structure de la phase aérée a été évaluée à partir de la capacité des bulles d'air à changer de volume en fonction des variations de pression externe. Cette analyse a été développée en collaboration avec J. Renoir (Degussa Texturant Systems, Baupte). Cette technique permet de mesurer le phénomène de cavernage (coalescence partielle des bulles d'air) présent dans les échantillons de crèmes glacées. Ce phénomène semble être corrélé à la rétractation du produit au cours du stockage et ainsi est impliqué dans la qualité du produit fini. Les premiers résultats suggèrent que la rétractation n'est corrélée ni à la taille d'initiale des bulles d'air ni aux caractéristiques structurales du produit (évaluées par la distribution granulométrique des globules gras ou le comportement à la fonte). En revanche, la capacité de rétractation semble dépendre des

ingrédients présents dans la formulation des crèmes glacées. Une étude plus approfondie de la phase aérée est actuellement en cours.

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# APPENDIXES

**Appendix 1:** Calculation of the protein concentration at the surface of the fat globules

**Emulsion = aqueous phase + oil phase**

1-Composition of the emulsion:

817g of water
100g of protein mix
} 917g of aqueous phase $\xrightarrow{d = 1}$ 917ml

80g of fat
3g of MDG
} 83g of fat phase $\xrightarrow{d = 0,914}$ 75,86ml

2-Total protein dosage

X mg of protein / ml of emulsion

$$\frac{\text{X mg de protein / ml of emulsion} \times \text{volume emulsion}}{\text{volume of aqueous phase}} = \text{x mg of protein / ml of aqueous phase}$$

= x mg of protein / mg of aqueous phase
= x % de initial total protein

2-Composition of the emulsion after centrifugation:

y mg of protein / ml of aqueous phase = y mg of protein / mg of aqueous phase
y % of protein in the aqueous phase after treatment

If it is considered that the volume (or mass) of the aqueous phase was equal in the initial emulsion and after centrifugation, then, the quantity of protein in the fat phase is the difference between the 2 in percent.

The percentage of adsorbed protein at the fat globule surface is:

$100 - (y/x) \rightarrow$ **Z% of protein in the fat phase** (with x et y in mg/ml)

**Appendix 2:** C. Granger, P. Barey, N. Combe, P. Veschambre, M. Cansell (2003). Influence of the fat characteristics on the physicochemical behavior of oil-in-water emulsions based on milk protein-glycerol esters mixtures. Colloids and Surfaces. B: Biointerfaces, 32, 353-363.[6]

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# Influence of the fat characteristics on the physicochemical behavior of oil-in-water emulsions based on milk proteins-glycerol esters mixtures

C. Granger [a,b], P. Barey [b], N. Combe [c], P. Veschambre [d], M. Cansell [a,*]

[a] *Laboratoire 'Milieux dispersés alimentaires: physico-chimie, formulation et vectorisation nutritionnelle', ISTAB, Avenue des Facultés, F-33405 Talence, Cedex, France*
[b] *Degussa Texturant Systems France SAS, Baupte, F-50500 Carentan, France*
[c] *Unité de Biochimie et Nutrition, ITERG, Avenue des Facultés, F-33405 Talence, Cedex, France*
[d] *Laboratoire 'Biosciences de l'Aliment', ISTAB, Avenue des Facultés, F-33405 Talence, Cedex, France*

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**Abstract**

Oil-in-water emulsions based on 10% milk protein preparation, 0.3% mono-di-glycerides (MDG) and 8% vegetable oil were prepared for models typifying ice cream formulations. Two MDG (saturated and partially unsaturated) and four fats (oleic oil, hydrogenated and refined coconut oils, refined palm oil) were chosen to investigate the interactions occurring between the oil phase, the MDG and the milk proteins. Influence of temperature (4 °C) and ageing (24 h at 4 °C) was also tested. The emulsions were characterized for protein desorption, particle size distribution and rheological properties. The dynamic surface activity of the milk proteins and the MDG at the oil-water interface was also determined. At 20 °C, emulsions were mostly stabilized by proteins although the protein load at the globule surface strongly depended on the emulsifier and the oil phase natures. A displacement of the proteins adsorbed at the oil droplet interface by the lipid surfactant was a consequence of the temperature decrease and/or ageing step, suggesting a disruption of the interfacial protein interactions. This disruption was more or less marked depending on the physicochemical characteristics of the surfactant and the oil used (amount of crystallized matter, fatty acid chain length and unsaturation degree). In parallel, the variation of the apparent viscosity of the various emulsions upon temperature was well correlated with the solid fat content. On the whole, the results obtained suggested that not only the surfactant molecules, i.e. emulsifiers and proteins, but also the fat used in the emulsion formulation participated in the development of the interface characteristics and rheological properties.


*Keywords:* Oil-in-water emulsion; Coconut oils; Palm oil; Milk proteins; Mono-di-glycerides; Interface characteristics

## 1. Introduction

Many food products appear in the form of oil-in-water emulsions where the oil phase is in a fluid

* Corresponding author. Tel.: +33-5-4000-3453; fax: +33-5-4000-2472.
*E-mail address:* m.cansell@istab.u-bordeaux1.fr (M. Cansell).

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state (milk, coffee whiteners, dressings) or in a (partially) crystalline state (ice cream mixes, whipping cream). Emulsions being thermodynamically unstable systems, oil droplet stabilization is usually achieved by the use of proteins acting as surfactants [1,2], along with low molecular weight emulsifiers [3–6]. In the case of ice creams that can be regarded as oil-in-water emulsions before the freezing state [7,8], mono-di-glyceride (MDG) mixtures are often associated with milk proteins that exhibit excellent emulsifying properties [2,7,9,10]. Although MDG are not required to aid emulsification, they play a major role in the destabilization of fat globules during freezing/aeration step [4]. To improve the quality of the emulsion and/or the final product, it is important to define the main factors affecting their physicochemical properties. Studies have pointed out the influence of the processing such as the parameters of homogenization [6,11] and the ageing time at low temperature [9]. The mixture composition also greatly determines the emulsion behavior upon emulsification, processing and storage, either in terms of the physical properties of the aqueous and fat phases or in terms of the presence of surface-active species at the oil droplet interface. As the presence of MDG in dairy-type emulsions can affect both the solid fat content [4,12] and the amount of proteins adsorbed at the oil-water interface [7,9,10,13], the protein-MDG-fat combination is a key factor in the development of strategies for controlling emulsion stability or instability. During the low temperature ageing step, the properties of the emulsion change. The lipid surfactant interfacial crystallization squeezes out proteins from the fat droplet surface [13]. It is well demonstrated that this displacement reduces the stability of the fat globules to partial coalescence that occurs during the freezing process [9,14]. On the whole, surface-active molecules modulate the interface characteristics by their ability to lower the interfacial tension that is related to the amount of adsorbed emulsifiers, by the thickness of the adsorbed layer and by the competitive adsorption between them and the various molecules present at the interface [2,5,15,16]. In addition, fat crystals in the oil droplets and at the interface influence the emulsion stability [17]. Although it is assumed that the oil characteristics used in the food industry may influence the adsorbed protein layer, little is known about the influence of the polarity of the oil phase, the crystallization and melting behaviors, or the presence of small impurities in the oil on the resulting interfacial parameters [5].

The overall objective of the present work was to improve the understanding of the complex interactions occurring between MDG, milk proteins and different vegetable fats during the homogenization and ageing stages in order to get a better knowledge of the role of fats in the physicochemical characteristics of the prepared emulsions. The fats were chosen for their wide use in food industry. The amount of proteins adsorbed to the surface of fat globules and the fat particle size were measured on emulsions as a function of temperature and ageing. The emulsions were also characterized in terms of rheological properties. The results obtained on emulsions were correlated with interfacial tension measurements.

## 2. Experimental procedures

### 2.1. Materials

Vegetable fats, i.e. hydrogenated and refined coconut oils, refined palm oil (SIO, Bougival, France) and high oleic sunflower oil (Lesieur, France) were used for the preparation of oil-in-water emulsions. The physicochemical characteristics of these fats are presented in Table 1. The skimmed milk powder (SMP) (Coopérative d'Isigny Saint-Mère, Isigny sur Mer, France) contained 35 wt.% proteins among which caseins and whey proteins represented 75.1 and 24.9 wt.%, respectively. Two lipid emulsifiers consisting of mixtures of MDG were supplied by Degussa Texturant Systems (Baupte, France) and mainly differed in their fatty acid compositions (Table 2).

### 2.2. Emulsion preparation

Oil-in-water emulsions were composed as follows: vegetable fat 8 wt.%, SMP 10 wt.%, MDG 0.3 wt.% adjusted to 100% with water. Emulsions

Table 1
Physicochemical characteristics of the four vegetable fats used for the preparation of oil-in-water emulsions

| | Hydrogenated coconut oil | Refined coconut oil | Refined palm oil | Sun flower oil |
|---|---|---|---|---|
| *Solid fat content (%)* | | | | |
| Temperature (°C) | | | | |
| 5 | 91.6 | 86.4 | 54.5 | |
| 10 | 89.1 | 78.8 | 52.6 | |
| 15 | 78.0 | 64.5 | 45.9 | |
| 20 | 51.3 | 36.3 | 30.6 | |
| *Fatty acids (%)* | | | | |
| 12:0 | 45 | 45 | – | – |
| 14:0 | 19 | 19 | – | – |
| 16:0 | 10 | 11 | 44 | 5 |
| 18:0 | 13 | 3 | 5 | 4 |
| 18:1 | – | 8 | 38 | 43 |
| 18:2 | – | – | 10 | 46 |

Table 2
Physicochemical characteristics of the 2 mono- and di-glyceride (MDG) mixtures used for the preparation of oil-in-water emulsions

| | Saturated MDG | Partially unsaturated MDG |
|---|---|---|
| | 60% min monoglycerides | |
| *Solid fat content (%)* | | |
| Temperature (°C) | | |
| 15 | 96.0 | 91.9 |
| 25 | 95.7 | 88.0 |
| 35 | 95.5 | 75.9 |
| 45 | 94.1 | 43.9 |
| 55 | 72.9 | 8.2 |
| *Fatty acids (%)* | | |
| 16:0 | 56 | 49 |
| 18:0 | 42 | 26 |
| 18:1 | – | 20 |
| 18:2 | – | 3 |

were prepared using a two-step homogenizer (Manton Gaulin SA 1200 AB, The Netherlands) operating at a pressure of 17.5 MPa first stage, 3.0 MPa second stage, at 90 °C. Initially, two premixes, one containing the fat and the emulsifier, the other the aqueous protein solution, were prepared and preheated separately at 70 °C. Immediately, they were mixed, brought under continuous stirring to 90 °C and maintained at this temperature during 5 s. Then, the premix was immediately homogenized. After homogenization, the emulsions were first cooled down to 20 °C then to 4 °C and aged for 24 h at 4 °C under stirring. All analyses were performed at least in duplicate with a fresh emulsion prepared for each replicate.

### 2.3. Interfacial tension measurements

The interfacial tension ($\gamma$) of the different oil-water systems was measured using the drop-weight method [18]. The drop correction factor used was proportional to the ratio of the capillary radius ($r$) to the cube root of the drop volume ($r/V^{1/3}$) [19]. The interfacial tension was determined on lipid systems in the presence of 0.3% of saturated or partially unsaturated MDG and/or 10% of SMP. The protein solution was heated at 90 °C for 5 s in order to mimic the heat treatment used in the emulsion preparation. The influence of the temperature decreasing from 45 to 4 °C was evaluated as long as the lipid phase remained in a fluid state, i.e. using pure high oleic sunflower oil and a 80/20 (wt./wt.) mixture of sunflower oil and a vegetable fat (refined and hydrogenated coconut oils and refined palm oil). The interfacial tension at 45 °C was also determined for systems with various proportions of the vegetable fats added to the sunflower oil phase. In a typical experiment, the

aqueous phase that contained the proteins was placed in a syringe equipped with a steel capillary (capillary outer diameter 0.406 mm, flat tip) and injected into the oil phase containing the lipid emulsifier by moving the piston. The sample vessel was thermostated from an external bath. The system was kept under isothermal conditions at the required temperature for 20 min to 1 h depending on the working temperature. This time was taken as a compromise between the waiting time and the attainment of true equilibrium values. As the drop detachment was detected visually, we hypothesized that three drops were required to get significant statistical results. The measurements were performed at least in duplicate for each protein-emulsifier-oil phase combination.

### 2.4. Rheological studies

Viscosity was recorded on a Physica Modular Compact Rheometer 300 (Anton-Paar, Germany) with a cone (7.5 cm diameter, 0.992° angle) and plate geometry with smooth surfaces. Viscosity measurements were performed on emulsions at 20 and 4 °C after homogenization and on preparations at 4 °C after ageing. Samples were transferred to the rheometer plate set at the working temperature. Viscosity was recorded at a shear rate of 1 $s^{-1}$ and measured for 3 min.

### 2.5. Particle size analysis

Mean particle size diameter (as evaluated by the volume weighted average diameter $d_{4,3}$), specific surface area ($m^2$ $ml^{-1}$) and particle size distribution of the emulsions were determined by integrated light scattering using a Mastersizer 2000 (Malvern Instruments SA, Orsay, France). Emulsions were diluted in the sample chamber with water at ≈1:1000. The duplicate measurements were carried out at room temperature on the freshly made emulsions or after ageing (24 h at 4 °C). Some emulsions were left in the presence of a dissociating medium (sodium dodecyl sulfate (SDS), 1 wt.%) to disperse the droplet aggregates formed and so to discriminate between coalescence and flocculation processes in the emulsion [7]. These experiments allowed the calculating of the aggregation factor to be expressed as a ratio of the apparent and real mean droplet diameters [6].

### 2.6. Protein displacement from emulsion droplet surface

Freshly homogenized emulsions at 20 and 4 °C and aged preparations were centrifuged in order to separate the oil phase and aqueous phase (15,000 × $g$, 30 min, 20 or 4 °C, Suprafuge 22, Heraeus Sepatech) [9]. The subnatant aqueous phase was recovered by sucking off with a syringe and stored at −20 °C until protein analysis. The Bradford method [20] was used to quantify the total protein content of the aqueous phase. Bovine serum albumin was used as a standard. The percentage of fat-adsorbed proteins was calculated as the difference between percent of total proteins and percent of proteins in the aqueous phase after correction for the volume fraction of fat.

### 2.7. Fat crystallization by pulse NMR

The measurements were carried out on a pulse low-resolution NMR spectrometer (Minispec PC20, Bruker Spectrospin, Germany) operating at 20 MHz for protons. Analysis of solid fat content (SFC) in the raw vegetable fats and MGD was performed according to normalized methods ISO 8292. SFC was also determined on emulsions after homogenization at 20 and 4 °C and after ageing (24 h at 4 °C). SFC was analyzed by a standard program as described in Ref. [9].

### 2.8. Statistical analysis

Analyses of variance, Fisher's least significant difference (LSD) procedures, were made using Stat Graphic Plus software (Manugistics, MD).

## 3. Results and discussion

### 3.1. Parameters influencing the size characteristics of the oil-in-water emulsions

The influence of temperature and ageing on emulsions of various fat, and emulsifier composi-

tions was assessed by particle size analysis. The parameter $d_{4,3}$ for particle mean diameter is often chosen to follow changes in emulsion droplet sizes [21,22], since emulsion instability with respect to phase separation is more dependent on the volume than the surface of oil droplets. In the case of saturated MDG-based systems (Fig. 1a), whatever the fat oil used, emulsions exhibited a mean diameter around 0.5 μm. The calculation of the aggregation factor (1.3 for the emulsion based on refined palm oil and partially unsaturated MDG, for example) indicated that there was only little immediate aggregation in relation to sufficient amount of surfactants in the form of either protein or emulsifier to cover the entire oil-water interface created by the homogenizer procedure [1,14]. The temperature variations as well as the ageing process did not influence significantly the size parameters indicating that the emulsions were well stabilized. The size behavior of the emulsions prepared with the partially unsaturated MDG appeared to be more dependent on the oil nature (Fig. 1b). Indeed, emulsions based on hydrogenated coconut oil presented twice higher droplet mean diameters compared to that obtained with the others fats, independently of temperature. The result with hydrogenated coconut oil corresponded to a biphasic distribution with ≈5% of particles with diameters higher than 3 μm. Although in this last population some aggregated droplets were present (aggregation factor equal to 1.3 at 20 °C), the initial oil particle size after SDS treatment (mean diameter around 0.9 μm) was also higher than that found for the other formulations. Surprisingly, after ageing, the size distribution became narrow mainly due to the disappearance of the aggregation phenomenon (0.7% of particles with diameters higher than 3 μm). In contrast, 24-h storage at 4 °C favored an increase in the oil droplet size for the emulsion based on refined coconut oil. The complex emulsion behavior observed with the partially unsaturated MDG pointed out the influence of the oil phase and may be correlated to an important modification of the protein coverage at the interface (see below).

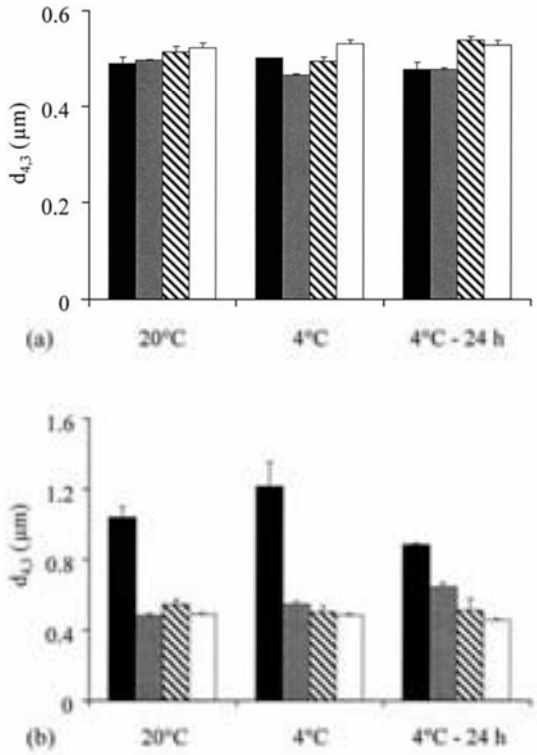


Fig. 1. Variation of the droplet mean diameter ($d_{4,3}$) as a function of temperature and time for different emulsion compositions: (a) based on saturated MDG; (b) based on partially unsaturated MDG and containing hydrogenated coconut oil (■); refined coconut oil (▨); refined palm oil (▩) and high oleic sunflower oil (□). Data are means ± S.D. of duplicate samples and at least two separate experiments.

### 3.2. Variations of the protein coverage of the droplet surface

The amount of proteins loaded onto the fat globule surface was quantified for the different emulsion formulations, after homogenization at 20 and 4 °C and after a 24-h ageing period (Fig. 2a, b). When the saturated MDG was considered (Fig. 2a), at 20 °C just after homogenization, the more unsaturated fatty acids in the oil phase, the less proteins were adsorbed. Thus, the protein load at the fat globule surface varied from 41% with the more saturated fat to 17% for the more unsaturated oil. As the droplet mean diameters for the four fat-based emulsion formulations were similar (Fig. 1a), the variations in the protein coverage corresponded indeed to different amounts of proteins at the interface. Therefore, the adsorbed proteins calculated from the specific surface area data varied from 7.3 to 3.3 mg $m^{-2}$ for the

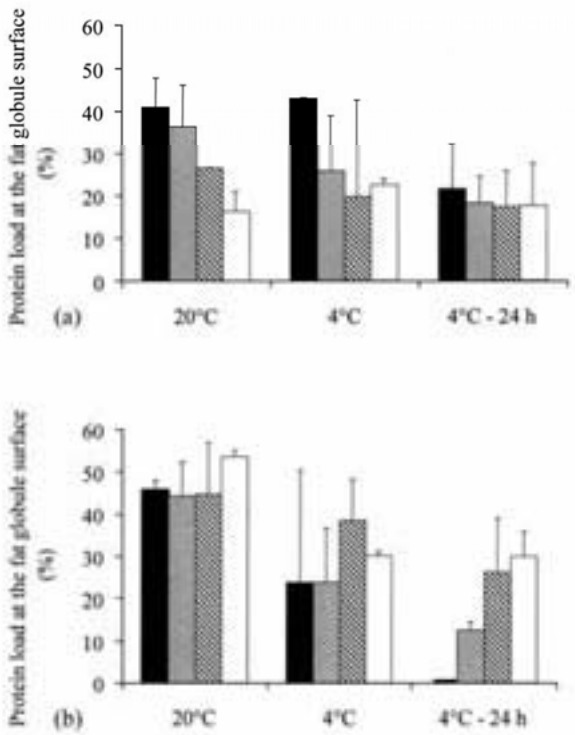


Fig. 2. Protein proportion adsorbed on fat globule surfaces (% of total protein) as a function of temperature and ageing time for different emulsion compositions: (a) based on saturated MDG; (b) based on partially unsaturated MDG and containing hydrogenated coconut oil (■); refined coconut oil (▩); refined palm oil (▨) and high oleic sunflower oil (□). Data are means ± S.D. of duplicate samples and at least two separate experiments.

hydrogenated coconut oil and the sunflower oil, respectively. This result suggested that specific interactions occurred between the fatty acids of the oil phase and that of the saturated MDG. Besides the influence of the oil nature on the droplet protein coverage at 20 °C (Fig. 2a), some protein desorption could have already occurred due to the presence of a large part of the saturated MDG in the crystallized stage (see Table 1 and results on the interfacial tension measurements). In contrast, with the partially unsaturated MDG (Fig. 2b), the protein droplet coverage appeared to be independent of the fat composition. Moreover, the protein load at the fat globule surface was higher for the partially unsaturated MDG compared with the saturated MDG. For a similar droplet size distribution (Fig. 1a, b), the amount of proteins adsorbed on the droplet interface could be compared. The protein coverage was actually dependent on the natures of lipid emulsifier and oil phase used, suggesting that the composition of the interfacial layer was established by competition between these two types of surfactants during homogenization. The saturated MDG and the partially unsaturated MDG influenced differently the interface protein composition during the homogenization step. Since at 20 °C, the two emulsifiers were not characterized by the same SFC (Table 2), the difference in behavior may account either for a difference in MDG crystal amount and/or structure at the interface, or for specific interactions between the fatty acid chains of the MDG and the lipid phase.

At 4 °C, SMP amount at the oil-water interface depended on the emulsifier used and on the fat oil type (Fig. 2a, b). It is well known that the amount of protein adsorbed at the surface of fat globules in an ice cream mix rapidly decreases during the first hour of the ageing period [9]. As the variation of the initial amount of adsorbed protein may not be only attributed to the emulsion formulation, the results are only discussed after the 24-h ageing period. When the saturated MDG was considered, similar protein loads were found whatever the lipid phase used. When the values at 20 and 4 °C were compared, the maximum SMP desorption was obtained with refined coconut oil (50%) (Fig. 2a). With the partially unsaturated MDG, protein coverage depended on the lipid phase and the protein desorption ranged from 42 to 100% for refined palm oil and hydrogenated coconut oil, respectively (Fig. 2b). Whatever the fat used, unsaturated MDG was better at displacing milk proteins from the droplet surface than the saturated emulsifier. This was especially the case in formulations based on hydrogenated coconut oil, i.e. 100 and 48% for the partially unsaturated MDG and the saturated MDG, respectively. This result agrees with a higher level of protein desorption reported in ice cream emulsions based on hardened coconut fat stabilized with glycerolmonooleate than in those containing glycerolmonostearate [9]. As the fatty acid length and the unsaturation degree of the fat increased, the influence of the emulsifier nature on protein desorption tended to vanish. Thus, in addition to the marked dependence of protein load at the fat globule surface on temperature, the adsorbed layer

composition was clearly sensitive to the nature of the lipid phase that induced particular protein-lipid and lipid-lipid interactions.

The emulsions containing the saturated MDG and refined palm oil were stabilized for their protein coverage as soon as the temperature reached 4 °C and no evolution of the protein load was observed as a function of time. This may result from the fact that saturated monoglycerides are known to be good initiators of fat crystallization [4,9] and the match in saturation and number of carbon atoms in the chains of the fatty acids of both the MDG and the oil phase. For the other emulsion formulations, the ageing period appeared necessary to get the maximal displacement of the proteins from the oil-water interface as already demonstrated [9]. This result, added to the fact that protein desorption was even more important when the unsaturated fatty acid content of the lipid phase was low, may account for lipid and/or emulsifier crystallization not being immediately complete at 4 °C. This incomplete fat crystallization at 4 °C after homogenization was shown by SFC measurements. For example, emulsions containing the partially unsaturated MDG and refined palm oil were characterized by a SFC of 2.6 and 6.2% before and after ageing, respectively. In the same way, it was shown that the addition of an emulsifier into the oil phase before emulsification modifies its crystallization process (crystallization temperature, crystal morphology) through specific interactions between the added emulsifier molecules and the crystallizing molecules in the emulsion droplets [12,17].

Although sunflower oil is not used in ice cream formulation, the experiments performed with this type of oil enable the identification of the roles of the crystallized emulsifier and of the lipid phase crystallization on the protein desorption process, since this type of oil remains liquid at 4 °C. No significant protein desorption was measured, whatever the emulsifier type and the ageing process (Fig. 2a, b). This suggested that, for this type of oil, the proteins loaded at the droplet interface were independent of the presence and/or the amount of crystallized emulsifier at the interface. These results imply that the interaction of both crystallized fat and crystallized emulsifier is necessary for effective protein desorption from the droplet surface.

### 3.3. Interfacial tension measurements

Interfacial tension analyses were performed to study the behavior of food emulsifiers at different oil-water interfaces. Although the competition for the interface may be biased in this type of experiments since the surface of the droplets formed was small with regard to that developed in emulsions, interfacial tension measurements allowed to point out the competition of the lipid emulsifiers and the proteins at the interface especially when temperature conditions varied [13]. However, to be accurate, interfacial tension experiments have to be performed with an oil phase characterized by a low solid fat content. Therefore, the influence of fat type, i.e. hydrogenated or refined coconut oils and refined palm oil, pure or mixed with oleic sunflower oil, on interfacial tension values, was investigated at 45 °C (Fig. 3). At the sunflower oil-water interface, an interfacial tension of ≈ 8.3 and 5.7 mN/m was measured, for the saturated and partially unsaturated MDG, respectively. These values

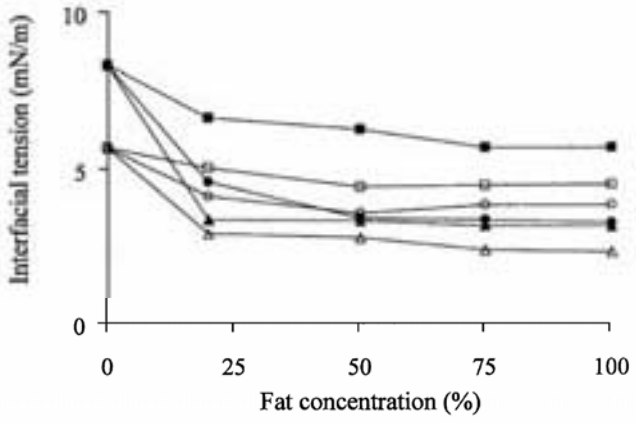


Fig. 3. Interfacial tension of high oleic sunflower oil—fat mixtures/water interfaces at 45 °C. High oleic sunflower oil—hydrogenated coconut oil mixtures with 0.3% saturated MDG (■) or 0.3% partially unsaturated MDG (□); high oleic sunflower oil-refined coconut oil mixtures with 0.3% saturated MDG (▲) or 0.3% partially unsaturated MDG (△); high oleic sunflower oil-refined palm oil mixtures with 0.3% saturated MDG (●) or 0.3% partially unsaturated MDG (○). All values represent mean ±10% and are typical of at least four different experiments.

were slightly lower than that reported in the literature [9,13,21]. This may result from differences in the method used for measuring the interfacial tensions, the MDG purity and/or the amount of emulsifier present.

When 100% fat systems were considered, saturated MDG was more active at the interface with refined palm oil and refined coconut oil than with hydrogenated coconut oil, as higher interfacial tensions were obtained. This influence of the fat nature was also found with the partially unsaturated MDG, but to a lesser extent. These results suggested that the interfacial tension value was influenced, at least partly, by both the chain length and the unsaturation degree of the oil phase fatty acids. The interactions between the oil and the lipid emulsifier would occur through the hydrophobic parts of the molecules. Thus, surface activity could be favored when the fatty acid length of the fat fitted that of the emulsifier, as in the case of refined palm oil (Table 1) and the saturated MDG (Table 2). In contrast, increasing the unsaturation degree of the oil phase fatty acids, like in sunflower oil, reduced the possibility of interaction since the *cis* double bound created a bend in the fatty acid structure that would enable the rest of the molecule to interact with the emulsifier. However, these hydrophobic interactions only explained partly the results obtained. For instance, whatever the MDG used, systems based on hydrogenated and refined coconut oils were characterized by significantly different surface tension values, although these two oils only differed by 8% of oleic acid (Table 2).

Whatever the oil and MDG types, upon fat addition to sunflower oil, surface tension values first started to decrease up to 20% of added fat and, then, remained at a quasi constant value. This suggested that, for a given fat-emulsifier couple, there was a defined ratio of these two lipids for which the interface was stabilized at the optimum. Beyond, or even before, this ratio, it seemed that no additional interactions at the oil-water interface were created so that the surface tension remained quasi constant. For hydrogenated coconut oil in the presence of partially unsaturated MDG, the fact that the surface tension was independent of the oil addition suggested that no specific interaction occurred between this particular oil-emulsifier couple.

The effect of temperature was assessed by measuring the interfacial tension in the presence of SMP and/or lipid emulsifiers, for systems in which 20% of the sunflower oil was replaced by one of the three fats studied. Results are presented in the case of refined palm oil (Fig. 4). The temperature was only decreased to 15 °C, since below this temperature the high solid fat content of the oil phase prevented accurate interfacial tension measurements. With the partially unsaturated MDG, the interfacial tension remained constant as a function of temperature. In contrast, when saturated MDG was used, the interfacial tension decreased when a specific temperature was reached. This latter result was similar to that reported in the literature for pure oleic oil, and was related to emulsifier crystallization [9,13]. SFC measurements on MDG showed that, at 15 °C, more than 90% of both emulsifiers were in the solid state (Table 2). Thus, the difference in interfacial activity found for the two lipid emulsifiers could not simply be explained by a critical amount of crystals at the interface but also by the crystal morphology and consequently their inter-

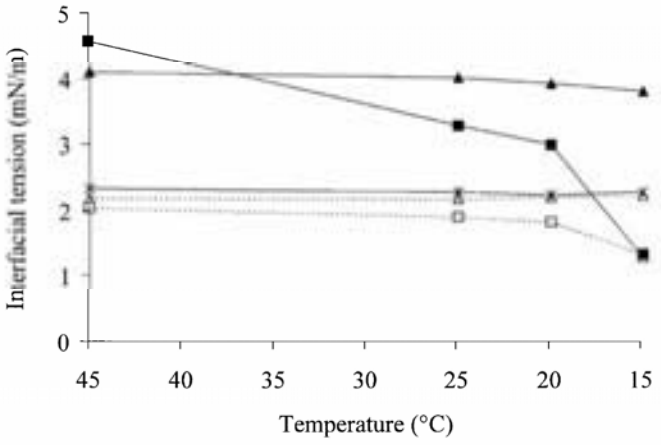


Fig. 4. Interfacial tension of high oleic sunflower oil/refined palm oil (80/20%)-water interfaces as a function of temperature in presence of 0.3% saturated MDG against distilled water (■); 0.3% partially unsaturated MDG against distilled water (▲); 0.3% saturated MDG against SMP (10%) aqueous solution (□, dashed line); 0.3% partially unsaturated MDG) against SMP (10%) aqueous solution (△, dashed line); 10% SMP solution (*). All values represent mean ±10% and are typical of at least four different experiments.

facial positioning. With only SMP in the aqueous phase, the interfacial tension remained at ≈ 2 mN/m, irrespective of the temperature (from 45 to 15 °C) (Fig. 4), in agreement with Ref. [13]. Results obtained with SMP mixed with emulsifiers showed that as long as a temperature of 20 °C was not reached, SMP imposed its interfacial tension. Upon cooling, the interfacial activity of the saturated MDG dominated. It is known that monoglycerides destabilize protein based-emulsions, i.e. the monoglycerides squeeze out proteins from the interface at or below the critical temperature for monoglyceride crystallization [13]. On the whole, the addition of 20% of refined palm oil to sunflower oil and the characteristics of the MDG used did not modify the general interfacial patterns compared to those reported in the literature [9,13].

### *3.4. Parameters influencing the rheological characteristics of the oil-in-water emulsions*

The apparent viscosity was measured on emulsions with various fat and emulsifier types, after homogenization (at 20 and 4 °C) and upon ageing (24 h at 4 °C). After the homogenization step, at 20 °C, emulsions exhibited low viscosities independent of their compositions (Fig. 5a, b). Decreasing the temperature from 20 to 4 °C induced an increase in the apparent viscosity in all emulsions. Upon ageing, all emulsions (except that based on oleic oil) exhibited an apparent viscosity of ≈ 1 Pa·s. Increase in the apparent viscosity may be due to different factors such as a variation in viscosity of the aqueous phase itself, a change in the volume fraction of solute, an increasing droplet concentration and/or a variation in the droplet size [21,23,24]. In particular, the increased protein content in the water phase induced by protein desorption in the presence of a low molecular weight emulsifier could result in depleted flocculation of the emulsion droplets and thus to an increase in the apparent viscosity [21]. The increase in viscosity observed (Fig. 5a, b) could not be attributed to a single factor. Indeed, no significant change in particle size diameter was observed when the temperature was varied (Fig. 1a, b) and different amounts of proteins were

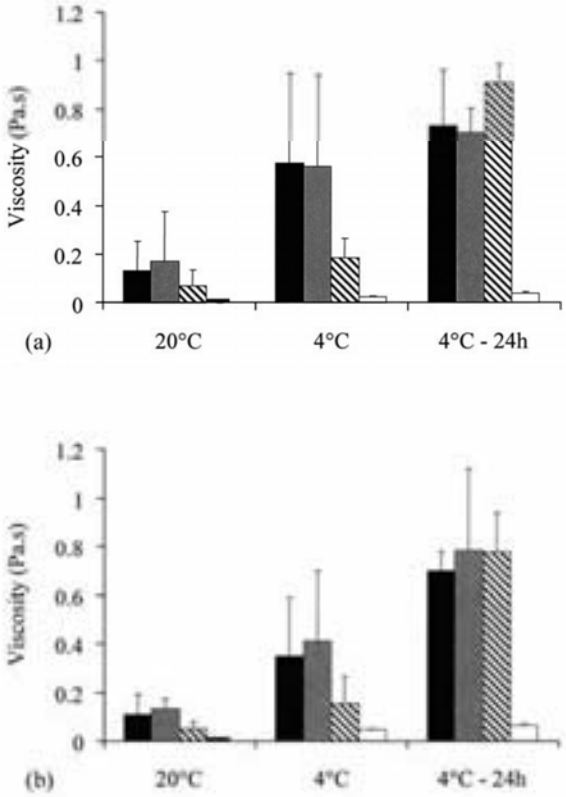


Fig. 5. Variation of the emulsion apparent viscosity as a function of temperature and time for different emulsion compositions: (a) based on saturated MDG; (b) based on partially unsaturated MDG and containing hydrogenated coconut oil (■); refined coconut oil (▨); refined palm oil (▧) and high oleic sunflower oil (□). Data are means ± S.D. of at least duplicate experiments.

found in the aqueous phase (Fig. 2a, b). Although 8% of solid fat may not be enough to induce changes in viscosity, the viscosity variations were well correlated to the solid fat content of the emulsions (results not shown). For oleic oil based-emulsions, the apparent viscosity slightly varied and no fat crystallization occurred. For refined palm oil based-emulsions, the apparent viscosity increased as a function of time whatever the lipid emulsifier type. This may be explained by incomplete fat crystallization at 4 °C after homogenization that was achieved during ageing as already mentioned. As a whole, apparent viscosity behaviors suggested that the obtained values were influenced, at least partly, by the fat crystallization and its process kinetics which were dependent on the unsaturated fatty acid content of the oil phase.

In conclusion, this study highlighted the role of the emulsion formulation, i.e. the fat type and the emulsifier nature, on the interface characteristics just after preparation and when submitted to process parameters such as cooling and ageing. The interactions between the oil phase and the protein and lipid emulsifiers use different mechanisms. On the one hand, in the case of low molecular weight molecules, such as mono-diglycerides, the interactions with the oil phase triacylglycerols occur through the fatty acid regions. The match in the hydrophobic parts of the molecules seemed to depend on both the chain length and the unsaturation degree of the two lipid types and their physical state (liquid or crystallized). The development of this 'fit' would partly determine the interface properties. On the other hand, it is well known that globular proteins, such as the caseins that are predominantly found in milk proteins, exhibit hydrophobic domains that may also interact with the oil phase. These interactions may be influenced by temperature since modifications of protein conformation are reported at low temperature. The influence of the fat type on the occurrence of these interactions is under investigation as a function of temperature.

Besides the specific interactions that may occur between the oil phase and the protein and lipid emulsifiers, the surfactants may also interact and compete at the interface. Milk proteins could first act in emulsion stabilization during the homogenization process by inhibiting the coalescence and/or aggregation processes, since they are more surface active than MDG. As temperature decreased, the role of the proteins in the emulsion stabilization decreased in relation to that of the MDG. The displacement of proteins from the interface suggested a marked disruption of the interfacial protein interactions due to the surfactant addition. This disruption may account for: (i) a reorganization of the interface due to the crystallization of the adsorbed MDG; (ii) the solid content of the lipid phase, (iii) the formation of protein-surfactant complexes through the hydrophobic binding sites; and/or (iv) protein conformation modifications at low temperature. Further investigations are being made to more accurately characterize the protein role in the interfacial properties. In particular, further research is needed to clarify the influence of each protein type in the milk protein mixture during the homogenization step and upon ageing.

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**Appendix 3:** C. Granger, V. Langendorff, N. Renouf, P. Barey, M. Cansell (2004). Impact of formulation on ice cream microstructures: an oscillation thermo-rheometry study. Journal of Dairy Science, 87, 810-812.[7]

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# *Short Communication*: Impact of Formulation on Ice Cream Microstructures: an Oscillation Thermo-Rheometry Study

**C. Granger,[1,2] V. Langendorff,[2] N. Renouf,[2] P. Barey,[2] and M. Cansell[1]**
[1]Laboratoire "Milieux dispersés alimentaires: physico-chimie, formulation et vectorisation nutritionnelle", ISTAB, Université Bordeaux 1, France
[2]Degussa Food Ingredients, Business Line Texturant Systems France SAS, France

## ABSTRACT

Oscillation thermo-rheometry was used to underline the existence of the different microstructures in ice cream. Varying ice cream formulations illustrated the impact of each ingredient, i.e., fat, proteins and lipid emulsifiers, and their interactions on the establishment of different networks.
(**Key words:** ice cream, rheology, lipid emulsifier - proteins - fat formulation)

Ice creams are described as polyphasic food systems including ice crystals, air bubbles, isolated and partially coalesced fat globules, and a cryo-concentrated aqueous phase containing sugars, proteins, and polysaccharides (Goff, 2002). All these components form a complex colloidal system that includes different microstructures. While several studies showed that rheometry correlates well with ice crystals (size and connectivity) and the air fraction volume (Goff et al., 1995; Wildmoser et al., 2003), this technique has not yet been used to characterize the fat globule and protein networks. The present work aims to correlate the rheological properties of ice creams and their different microstructures using various incomplete formulations.

Ice cream formulations contained 8% refined coconut oil (SIO, France), 10% skim milk powder (Coopérative d'Isigny Saint-Mère, France), 12% sucrose (Saint Louis, France), 6% corn syrup solids (Cerestar, France), 0.2% stabilizer (guar gum and locust bean gum, Degussa Food Ingredients, France) and 0.3% partially unsaturated mono-di-glycerides (Degussa Food Ingredients). Model ice creams were prepared without proteins or lipid emulsifiers that were replaced by sucrose. Homogenized mixes were prepared and aged at 4°C as described by Granger et al. (2003). Ice creams (overrun orders: 100%, outlet temperature −5°C) were made in a continuous freezer (Waukesha Cherry Burrell WCB CS 100) then, hardened at −40°C and stored at −25°C. Ice cream processing was carried out in duplicate. Mean particle size diameter (evaluated by the volume weighted average diameter $d_{4,3}$) and particle size distribution of oil droplets in mixes and final products were determined by integrated light scattering using a Mastersizer 2000 (Malvern Instruments SA). Samples were diluted in the sample chamber with water or dissociative medium, 1% SDS, at approximately 1:1000. The percentage of partial coalescence of fat was calculated on the basis of particles with diameters higher than 2 $\mu$m as the difference between the proportions measured in the presence of SDS in the initial mix and final product. Measurements on ice creams were performed using ultrasonication to ensure the absence of air bubbles. The rheological measurements were performed using a controlled stress rheometer (Physica MCR 300) fitted with streaked parallel plates (1 mm gap). Before being placed on to the rheometer, the products were thermostatically controlled for 2 h at −10°C. The samples were left for 15 min in the rheometer before the experiment. The storage modulus (G′), loss modulus (G″), and damping factor ($\tan\delta$ = G″/G′) were measured at 2 different deformation amplitudes, i.e., 0.05% between −10 and 5°C (0.2°C/min) and 0.1% between 5 and 60°C (0.2°C/min), at a frequency $\omega$ of 1 Hz. These amplitudes were chosen on the basis of the determination of the linear visco-elastic regime of ice cream by a deformation amplitude sweep test.

For the complete ice cream formulation, fat globule mean diameter evolved from 1.2 $\mu$m in the mix to 4.2 $\mu$m in the final product. Fat partial coalescence was found close to 3%. The withdrawal of milk proteins from the ice cream formulation led to an increase in the fat droplets in the mix ($d_{4,3}$ = 2.5 ± 0.4 $\mu$m), some fat destabilization during freezing (with 18% of particles partially coalesced) and a lower overrun (40% instead of 100% for the other formulations). In contrast, when the lipid emulsifier was removed from the complete

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Corresponding author: M. Cansell; e-mail: m.cansell@istab.u-bordeaux1.fr.

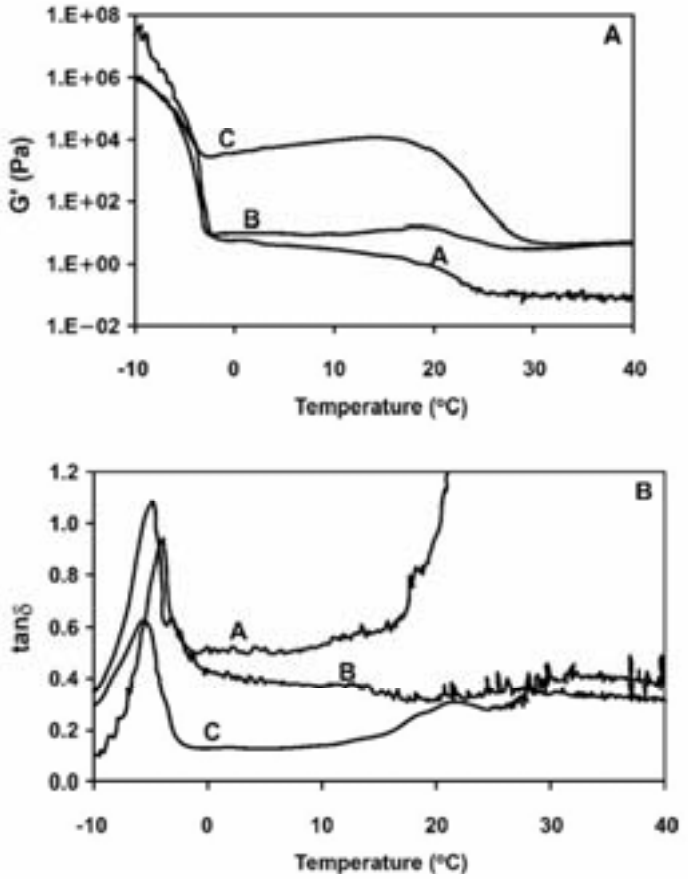


**Figure 1.** Rheological characteristics, storage modulus (G′) (a) and tan$\delta$(b), of different ice cream formulations as a function of the temperature. Incomplete ice cream formulations without proteins (curve A); without lipid emulsifier (curve B); and complete ice cream formulation (curve C). The curves are typical of at least 2 different preparations.

formulation, a strong steric stabilization of the fat globules was observed throughout the steps of the whole process ($d_{4,3}$ = 1.3 ± 0.1 $\mu$m for the mix and the final product).

The rheological behavior of the complete and the 2 incomplete formulations as a function of temperature showed a steep decrease in the storage modulus between –10 and –2°C (Figure 1a) associated with a peak in the damping factor (Figure 1b). In this temperature range, G′ variations were correlated with a loss of cooperative interactions between ice crystals associated with their melting (Goff et al., 1995; Wildmoser et al., 2003) and –2°C corresponded to the ice melting temperature of our system calculated according to Bradley and Smith (1983). Because the 3 samples behaved similarly in the negative temperature range, although the products exhibited differences in overrun, fat droplet mean diameters and degree of partial coalescence, it seemed that, in our experimental conditions, thermo-rheometry was not able to detect structural differences at these temperatures, most probably because the systems were overwhelmed by the presence of ice. Between –2°C and 17°C, G′ values and tan$\delta$ stabilized, at different values depending on the formulation (Figure 1a and b). In the case of the formulation containing only mono-diglycerides as emulsifier, lower overrun and poorly stabilized fat globules led to a weaker and less structured product (curve A, Figure 1a). The replacement of the low molecular weight emulsifier by milk proteins in the incomplete formulation only slightly modified G′ values and tan$\delta$ (curve B, Figure 1a and b), although fat globules were well stabilized against partial coalescence and a 100% overrun was obtained. When the rheological behavior of the complete (curve C, Figure 1a and b) and incomplete ice cream formulations (curves A and B, Figure 1a and b) were compared between –2 and 17°C, the magnitude of the storage modulus G′ clearly characterized a stiff and well structured product, suggesting that cooperative interactions between the different ingredients had taken place. This behavior could be related to the presence of a partially coalesced fat network. However, the influence of the melting of the locust bean gum cryo-gel, enhanced by phase separation from the proteins and/or the smaller air bubble size could not be excluded. Between 17 and 30°C, for all formulations, a decrease in the storage modulus was observed in relation to complete fat melting. However, a peak of tan$\delta$ was only present for the ice cream product. This corresponded to the disappearance of the fat globule network. In the case of the formulation containing only mono-di-glycerides as emulsifier, the loss modulus decreased more slowly than the storage modulus (tan$\delta$ higher than 1 at 20°C), resulting in a system that responded less elastically with increasing temperature (curve A, Figure 1b). In contrast, similar and lower (<1) tan$\delta$ values obtained from the incomplete formulation, based on proteins only (curve B, Figure 1b) and the ice cream product (curve C, Figure 1b), indicated the existence of a remaining microstructure. This could be attributed, at least partly, to the presence of proteins in association with stabilizers. Moreover, the fact that these 2 formulations reached similar G′ values above 30°C (curves B and C, Figure 1a) suggested that interactions between milk proteins and fat took place to ensure a structured product.

This study demonstrated that the rheological characteristics of ice creams were related to the existence of different microstructures (ice, dispersed air, partially aggregated fat phase and aqueous phase) that influenced the final texture either simultaneously or individually as a function of the temperature range considered.

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## Résumé

Les émulsifiants et les protéines participent à la création et à la stabilisation des différentes interfaces formées au cours de la fabrication des crèmes glacées qui fait évoluer le système d'une simple émulsion huile-dans-eau vers une mousse partiellement solide. La compréhension des mécanismes mis en jeu aux interfaces et la connaissance des propriétés des ingrédients sont nécessaires pour optimiser la fabrication et la stabilisation du produit fini. C'est dans ce contexte que différents systèmes, émulsion huile-dans-eau, mix de crème glacée et crèmes glacées ont été fabriqués à partir de quatre types de matières grasses différentes et stabilisés par deux types de mono- et diglycerides et deux sources protéiques. Des caractérisations multiples, telles que le profil thermique des matières grasses, le comportement rhéologique ou la réponse en fluorescence, appliquées à ces trois systèmes ont été utilisées. Il a été mis en évidence, dans les émulsions et dans les mixes, un phénomène compétitif aux interfaces entre les molécules tensioactives, principalement pendant la période de maturation. Ce phénomène est, en autre, influencé par le degré d'insaturation de l'émulsifiant et de la matière grasse. L'influence de la formulation a été également mise en évidence sur le produit fini, notamment en termes de comportement à la fonte et de perception organoleptique. Peu de corrélation entre les différentes techniques utilisées et entre les différents systèmes étudiés ont été observées suggérant la mise en place d'associations spécifiques entre ingrédients.



**Mots clés :** Crème glacée, matière grasse, émulsifiant, protéine, interface



## Abstract

The emulsifiers and the proteins take part in the creation and the stabilization of the various interfaces formed during the steps of ice cream processing which evolves from a simple oil-in-water emulsion to a partially solid foam. The aim of this study was to understand and control the mechanisms of action of the tensioactive molecules during the different steps of ice cream manufacture. In this context, various systems, oil-in-water emulsion, ice cream mix and ice cream, were manufactured based on four types of fat and stabilized by two types of mono- and diglyceride mixtures and two protein sources. Multiple of characterization, such as thermal behavior of fat, rheological parameters and fluorescence response, applied to these three systems were used. It was highlighted in the emulsions and mix systems that a competitive phenomenon at the oil-water interfaces occurred between emulsifiers and proteins especially during the ageing period. This phenomenon was influenced, among other things, by the degree of unsaturation of the emulsifier and of the fat. The influence of the formulation was also pointed out on the finished product in particular in term of melting behavior and organoleptic perception. Little correlation between the various techniques used and between the various systems studied was observed suggesting that specific associations between ingredients were taking place.



**Key words:** Ice cream, fat, emulsifier, protein, interface