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**MAPPING OF ICE CREAM FORMULATION USING FRONT-FACE  
FLUORESCENCE SPECTROSCOPY**

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Running title: Mapping of ice cream by fluorescence spectroscopy

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## **Abstract**

Front-face fluorescence spectroscopy was used to characterize 16 ice cream formulations (four fats, two mono- di-glyceride 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 which were the most suitable for separating the spectra. Whereas PCA allowed some fat clustering, no discrimination was found, for each protein mixture, as a function of the emulsifier type. Besides front-face fluorescence spectroscopy, classical methods (fat globule size distribution and melting time) were used for ice cream characterization. Some formulations exhibited specific behaviours that make the understanding of their physical and molecular properties difficult when using only the physicochemical characteristics of the ingredients.

**Key words:** 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 the ice cream ingredients. A partitioning of the molecules, i.e., the proteins, the low molecular weight emulsifiers and the fat (free or partially-coalesced), between the different interfaces and the cryo-concentrated phase occurs. 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 dealing with numerous industrial situations, such as manufacturing and textural defects, ingredient assessment, research and 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 through the development of front-face device that allows the characterization of powdered, turbid, emulsified and concentrated samples (Herbert, Mouhous-Riou, Devaux, Riaublanc, Bouchet, Gallant & Dufour, 2000; Dufour, Devaux, Fortier & Heymann, 2001). Besides the sensitivity of this analytical method, it takes advantage of the presence of proteins through fluorescence properties of tryptophanyl residues that they contain. Tryptophanyl residues fluorescence spectra have been used to monitor the structural modifications of proteins in food systems during wheat gluten treatment (Genot, Tonneti, Montenay-Garestier, Marion & Dracon, 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 the formulation on ice cream properties using a combination of different fat/emulsifier/protein mixtures. Four types of fats originating from vegetable and animal sources and two protein mixtures (skim milk proteins and functional dairy proteins) were tested. The low molecular weight emulsifiers used consisted of two mixtures of mono- and di-glycerides (MDG) 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. Front-face fluorescence was used to illustrate the molecular identity of the formulations.

## **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). Skim milk powder (SMP, 35 wt.% proteins) (Coopérative d'Isigny Saint-Mère, Isigny Sur Mer, France) and functional dairy proteins (FDP, 20 wt.% proteins) (Bel Industries, Vincennes, France) were the protein sources. The functionalization of the FDP consisted in a partial protein denaturation by heat treatment performed by the supplier. Relative to the total protein content, SMP contained 80 wt.% of caseins and 20 wt.% of whey proteins (Granger, Barey, Veschambre & Cansell, 2005a). In the case of FDP, caseins and whey proteins represented 40 and 60 wt.%, respectively (Granger et al., 2005a). Sucrose and corn syrup solids (40 DE, 80 Brix solid content) were purchased from Eurosucre (Paris, France) and Cerestar (France), respectively. Stabiliser (guar gum and locust bean gum) and emulsifiers, i.e., SMDG and PUMDG (60 % alpha monoglycerides) were donated by Degussa Food Ingredients (Baupte, France). PUMDG contained oleic acid (18:1; 20

wt.% of total fatty acids) and linoleic acid (18:2; 3 wt.% of total fatty acids). SMDG comprised only palmitic acid (16:0; 56 wt.% of total fatty acids) and stearic acid (18:0; 42 wt.% of total fatty acids).

## *2.2. Ice cream preparation*

Sixteen different ice cream mixes were prepared based on the compounds and associated proportions presented in Table 1. All ingredients except the fat and the corn syrup were dry blended, mixed with water and immediately blended at 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, Denmark). Ice creams were hardened at -40°C and stored at -25°C. For each recipe, three different batches were produced.

## *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 with water at 13°C, directly in the measurement cell. 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  $\mu\text{m}$  ( $d > 2.0 \mu\text{m}$ ) was calculated. Three samples, for each batch, were analysed.

#### *2.4. Melting test*

All the samples were stored at  $-25^{\circ}\text{C}$  before carrying out the melting test. Ice cream samples ( $270 \pm 5 \text{ g}$ ) were placed on a mesh grid (mesh size  $1\text{cm} \times 1\text{cm}$ ) and allowed to stand at  $29^{\circ}\text{C}$ , 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, France) equipped with a thermostatically controlled front-face accessory. The incidence angle of the excitation radiation was set at  $22.5^{\circ}$  to ensure that reflected light, scattered radiation and depolarization phenomena were minimized. Ice cream samples ( $1 \text{ cm} \times 1 \text{ cm} \times 3 \text{ cm}$  taken from the middle of the ice cream sample) were placed into a quartz cell. Fluorescence spectra were recorded at  $1^{\circ}\text{C}$  with excitation and emission slits set at 0.4 and 1 nm, respectively. The emission spectra of tryptophanyl residues (300 - 360 nm) were recorded at an excitation wavelength of 290 nm with a charge-coupled device (CCD) multi-channel detector (270M imaging spectrograph, Jobin Yvon, Horiba, Longjumeau, France) that allowed instant emission spectra recording. Each fluorescent emission spectrum resulted from the accumulation of three measurements in order to reduce the remaining scattering effects. Three samples were taken in each batch (three different batches for each recipe)

and analyzed for fluorescence. On the whole, fluorescence data represented 144 tryptophanyl residues spectra collected from the 16 formulations of ice creams.

## *2.6. Mathematical data analysis*

### *2.6.1 Analysis of fluorescence spectra*

Emission fluorescence spectra were analyzed 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 (Toutain, TREFLE UMR 5508, Université Bordeaux 1, ENSAM, ENSCPB, 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) (Jolliffe, 1986) and partial least square (PLS) discriminant analysis. PCA and PLS discriminant analysis were run on The Unscrambler<sup>®</sup> v8.0 (Camo Process AS, Oslo, Norway).

In the present application, PCA was applied to the 144 tryptophanyl residues spectra. The variables were the fluorescence intensities corresponding to the 635 wavelengths between 310 and 350 nm. The 2-D mappings obtained by PCA allowed the investigation of the main differences between formulations in terms of fluorescence emission. When one of the principal components (i.e., one of the mapping axes) allowed discrimination between formulations, a spectral pattern of the axis plotted the contribution of each wavelength to the axis. 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 of 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 (Martens & Naes, 1989; Tenenhaus, 1998; Esbensen, 2001) have been 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 well-adapted to the case of 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 categorical variables were the dummy variables describing the categorization of the formulations: 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 consisted of finding models for the dummy variables. These models, called discriminant functions, 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 with the spectral patterns obtained by PCA, here the spectral patterns plot the regression coefficients of the designed discriminant functions. These spectral patterns showed the emission wavelengths that best discriminated each protein, each fat or each emulsifier. In order to carry out PLS discriminant analysis, the emission fluorescence spectra were split into a calibration set and a validation set. For each ice cream batch, from the three fluorescence spectra recorded corresponding to 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 which allow 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  $d_{4,3}$ ,  $d > 2.0 \mu\text{m}$ , and melting times ( $T_{30}$ ) 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 were already reported (Barfod, Krog, Larsen & Buchheim, 1991; Granger, Leger, Barey, Langendorff & Cansell, 2005b). 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 et al., 2005b). 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 palm oil (about 90 %), on the other. 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 % 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  $\mu\text{m}$  was taken as the limit between isolated fat globules and aggregated/coalesced particles. The  $d > 2 \mu\text{m}$  was influenced by the main factors and all the interactions. High  $d > 2 \mu\text{m}$  observed for PUMDG-based formulations corresponded to an increased aggregation/coalescence phenomenon of fat droplets occurring upon whipping/freezing (Gelin, Poyen, Courthaudon, Le Meste & Lorient, 1994; Goff, 1997; Bolliger et al., 2000; Relkin, Sourdet & Fosseux, 2003). When considering a specific fat \* emulsifier couple,  $d_{4,3}$  and  $d > 2 \mu\text{m}$  followed the similar pattern. This suggested that this interaction influenced significantly the destabilization phenomenon in ice cream. The protein mixture influenced the  $d > 2 \mu\text{m}$ . This significant effect was attributed to the specific formulation based on refined coconut oil, PUMDG and FDP which developed higher fat destabilization. For the other formulations, the protein mixture used did not have a significant effect on  $d > 2 \mu\text{m}$ . This agreed with the fact that  $d_{4,3}$  and  $d > 2 \mu\text{m}$  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 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 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 that obtained for the other formulations. This specific ingredient selection was mainly responsible of the significance of the ingredient factors. Since fat aggregates contributed greatly to the structure of ice cream by forming a three dimensional network (Goff, 1997), long melting times could be associated with an increased fat globule destabilization. However, melting time values were not well correlated with fat globule diameters (Granger et al., 2005b). 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 considering 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 cream using tryptophanyl residues 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 (Herbert et al., 2000; Dufour et al., 2001) 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 et al., 2005b) and thermal analysis (Granger, Schöppe, Leger, Barey & Cansell, 2005) showed that ice cream structure was dependent on the formulation, i.e. fat nature, emulsifier and protein. On a molecular basis, fluorescence could also be a pertinent method to point out the different interactions occurring in this type of dairy product. Fig. 1 presents typical tryptophanyl residues emission fluorescence

spectra of two different ice cream formulations. Although fluorescence spectra appeared to be very close, 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 partly, 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 microstructures could also reveal different interactions with the other components present in the formulation (Granger et al., 2004).

Since neither the first principal component nor the second one 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 on the validation set. Good classification rates were observed for the protein factor, on both the calibration and validation sets (94.7% and 95.7%, respectively). It was concluded that tryptophanyl residues 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 shows 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 (Rampon, Lethuaut,

Mouhous-Riou & Genot, 2001; Lopez et al., 2001), corresponding to tryptophanyl residues in hydrophobic and hydrophilic environments, respectively. Moreover, FDP mixture emitted more at wavelengths around 333 nm, while SMP was characterized by wavelengths around 313 nm. This could be interpreted in terms of differences in protein conformation, behaviour at the interfaces, and network formation. In particular, this latter assumption is based on the different structural arrangement observed, for these two types of proteins, 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.

In order 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 (Fig. 4a 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. 4a), the first cluster comprised the ice creams based on hydrogenated coconut oil and refined palm oil, the second one consisted of those based on anhydrous milk fat and refined coconut oil. These two clusters were positioned on the left and on the 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. 4b). 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 % of unsaturated fatty acids and the corresponding ice creams were efficiently discriminated. Hydrogenated coconut oil, a

**Commentaire [j1]** : Between the fats?

saturated oil, and refined palm oil, an unsaturated one, led to close fluorescence responses, so that the formulations overlapped.

In order to point out 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 formulations (Fig. 5a and b). For both protein mixtures, negative and positive spectral peaks appeared around 320 and 340 nm, respectively. According to the spectral patterns and to the PCA mappings, it can be deduced that the wavelength at 320 nm characterized the protein mixtures associated with hydrogenated coconut oil and refined palm oil whereas the wavelength at 340 nm was more relevant to the proteins with anhydrous milk fat and refined coconut oil. These wavelengths could be interpreted in terms of peculiar 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. It is worth noting that refined coconut oil differed from hydrogenated coconut oil by only 8 % of unsaturated fatty acids. This small difference in composition could induce significant differences in the fat behaviour, i.e., amount, organization and/or polymorphic form of the crystallized matter, that may lead to peculiar protein-fat associations.

#### **4. Conclusion**

The results obtained with the physical characterization of ice cream pointed out 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 have occurred between protein and fat. In particular, the spectral patterns associated with the first axes 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. This prevented the possibility to correlate 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 simply predict physical or molecular characteristics of the ice creams. 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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Figures

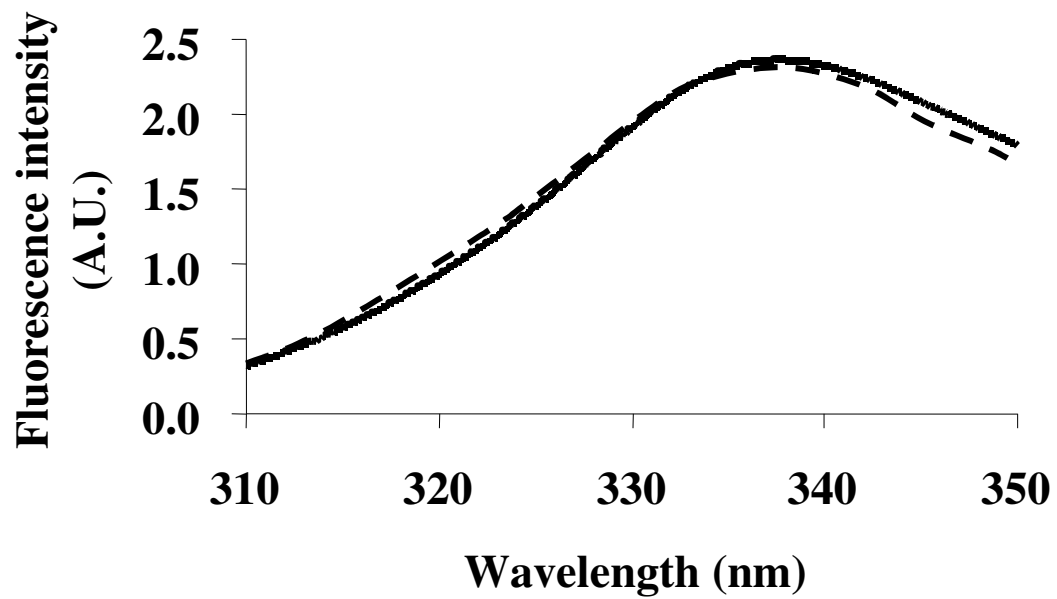


Fig. 1. Normalized tryptophanyl residues emission fluorescence spectra of ice creams based on saturated mono- and di-glycerides and anhydrous milk fat and skim milk powder ( — ) or hydrogenated coconut oil and functional dairy proteins ( ..... ). A.U.: arbitrary units.

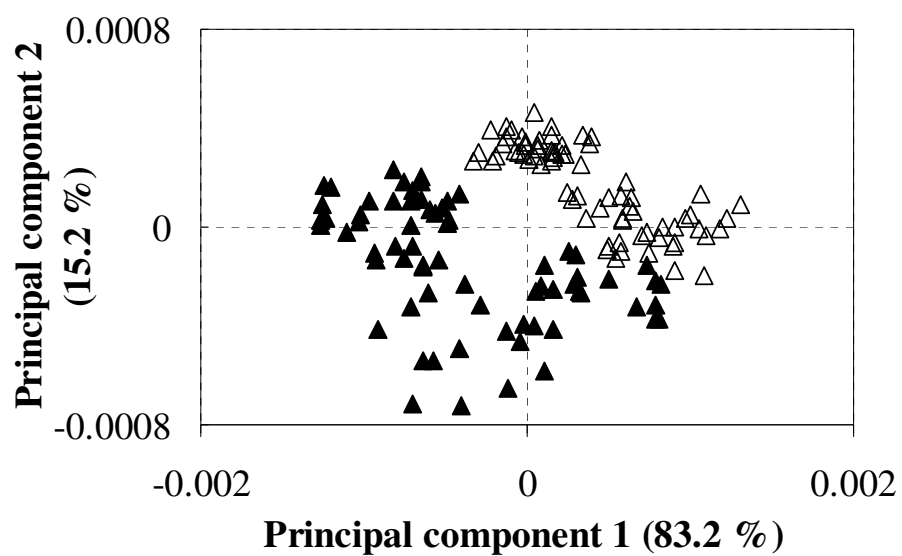


Fig. 2. Similarity map for the principal components 1 and 2 of the principal component analysis performed on the tryptophanyl residues emission fluorescence spectra of the 16 ice cream formulations based on skim milk powder ( $\triangle$ ) and functional dairy proteins ( $\blacktriangle$ ).

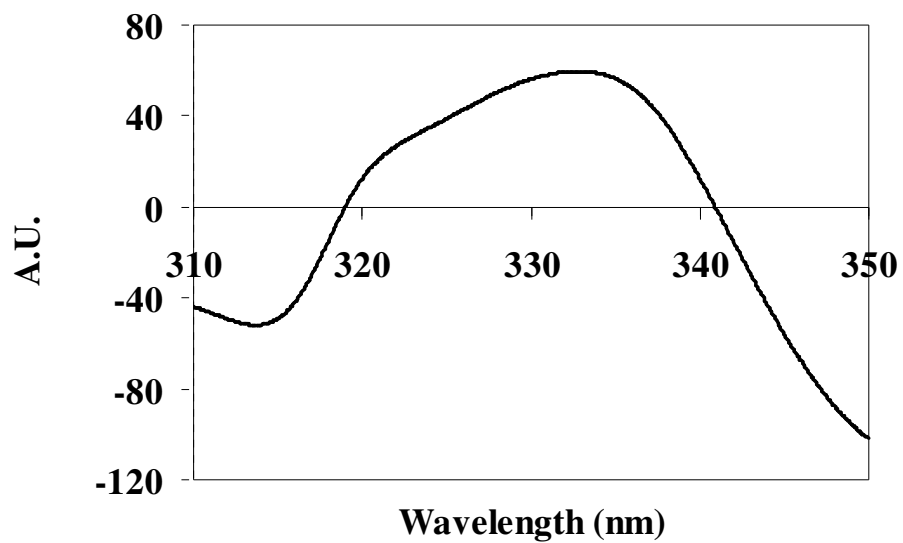


Fig. 3. Spectral pattern corresponding to the discriminant function obtained by partial least square discriminant analysis performed on tryptophanyl residues emission fluorescence spectra recorded on the 16 ice cream formulations. A.U.: arbitrary units.

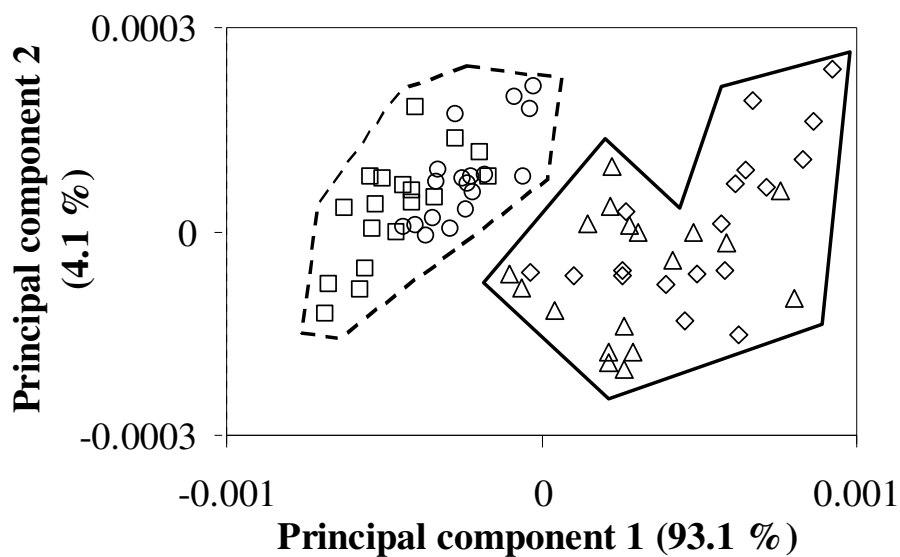
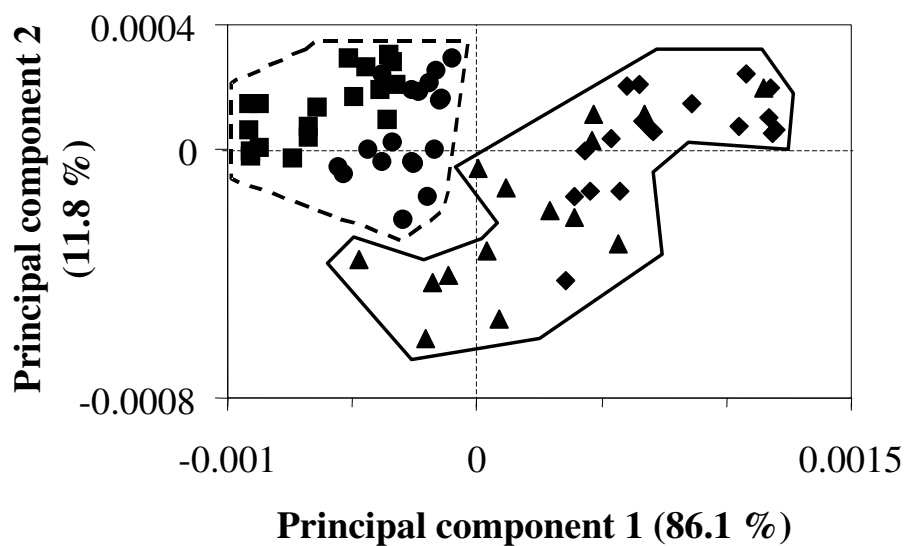


Fig. 4. Similarity maps for the principal components 1 and 2 of the principal component analyses performed on the tryptophanyl residues emission fluorescence spectra of ice creams. (a) Principal component analysis performed on formulations based on skim milk powder. (b) Principal component analysis performed on formulations based on functional dairy proteins. Hydrogenated coconut oil (○, ●); Refined coconut oil (△, ▲); Refined palm oil (□, ■); Anhydrous milk fat (◇, ◆).



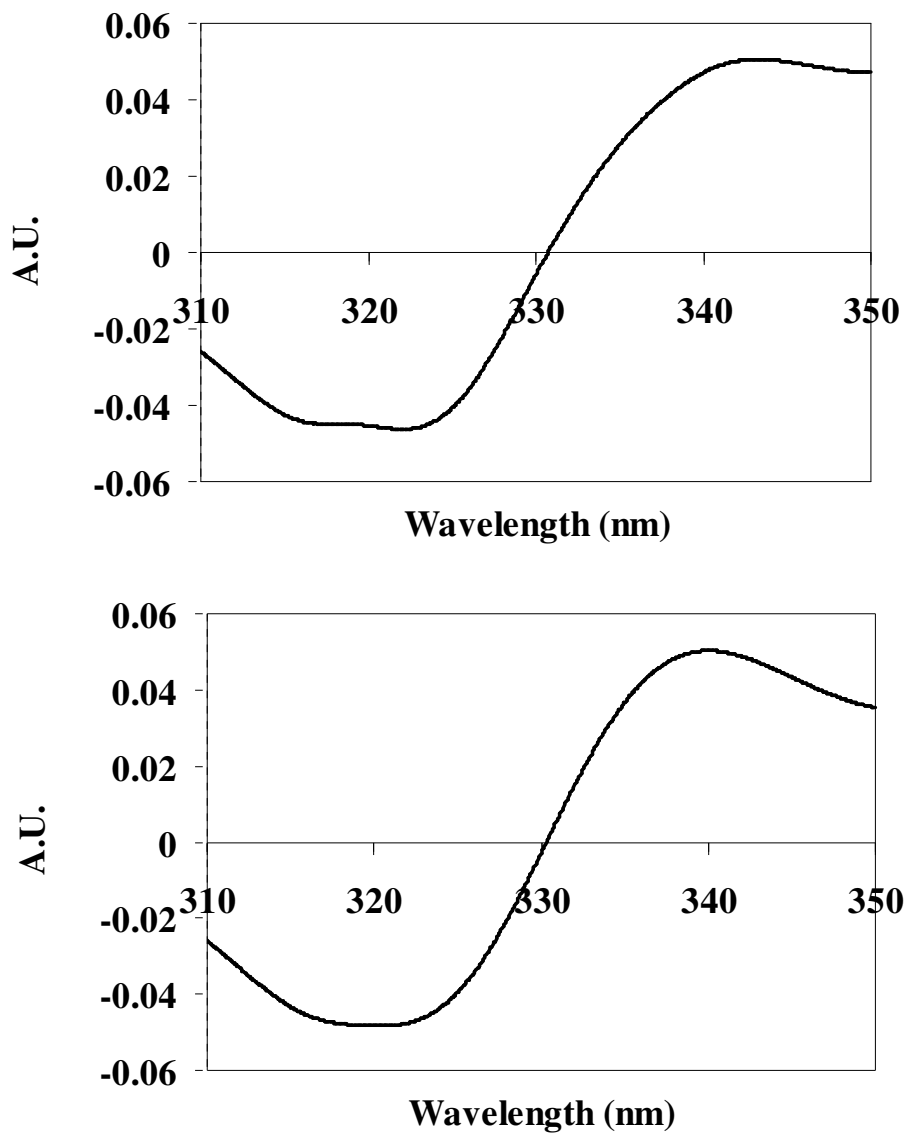


Fig. 5. Spectral patterns corresponding to (a) the principal component 1 for principal component analysis performed on tryptophanyl residues emission fluorescence spectra recorded on skim milk powder-based formulations, (b) the principal component 1 for principal component analysis performed on tryptophanyl residues emission fluorescence spectra recorded on functional dairy protein-based formulations. A.U.: arbitrary units.

Table 1

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

Compound	Proportion (wt.%)	Type
Fat <sup>1</sup>	8	Hydrogenated coconut oil
		Refined coconut oil
		Refined palm oil
		Anhydrous milk fat
Emulsifier <sup>1</sup>	0.3	Saturated mono- and di-glycerides
		Partially unsaturated mono- and di-glycerides
Protein source <sup>1, 2</sup>	10	Skim milk powder
		Functional dairy proteins
Sugars	12	Sucrose
	6	Corn syrup solids
Stabilizers	0.2	Guar gum and locust bean gum mixture
Water	63.5	

<sup>1</sup> Each mix formulation contained only one type of fat, of protein and of emulsifier; <sup>2</sup> Skim milk powder and functional dairy proteins contained 35 and 20 wt.% proteins, respectively.

Table 2

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

	d <sub>4,3</sub> (µm) <sup>1</sup>	d > 2 µm (%) <sup>2</sup>	T <sub>30</sub> (min) <sup>3</sup>
<i>Emulsifier</i>			
LS <sup>4</sup>	*** <sup>5</sup>	***	***
SMDG <sup>6</sup>	1.8 <sup>a</sup>	17 <sup>a</sup>	52 <sup>a</sup>
PUMDG <sup>7</sup>	2.8 <sup>b</sup>	35 <sup>b</sup>	101 <sup>b</sup>
<i>Fat</i>			
LS	***	***	***
HCO <sup>8</sup>	1.7 <sup>a</sup>	18 <sup>a</sup>	86 <sup>a</sup>
RCO <sup>9</sup>	2.6 <sup>b</sup>	28 <sup>b</sup>	92 <sup>b</sup>
RPO <sup>10</sup>	2.4 <sup>b</sup>	28 <sup>b</sup>	71 <sup>c</sup>
AMF <sup>11</sup>	2.5 <sup>b</sup>	29 <sup>b</sup>	55 <sup>d</sup>
<i>Protein</i>			
LS	NS <sup>12</sup>	***	NS
SMP <sup>13</sup>	2.2	25 <sup>a</sup>	75
FDP <sup>14</sup>	2.4	27 <sup>b</sup>	77
<i>Emulsifier * fat</i>			
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
<i>Emulsifier * protein</i>			
LS	***	***	***
SMP * SMDG	1.8	16	53
SMP * PUMDG	2.6	33	98
FDP * SMDG	1.8	17	50
FDP * PUMDG	3.0	37	103
<i>Fat * protein</i>			
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
<i>Fat * protein * emulsifier</i>			
LS	***	***	***

<sup>1</sup> Volume weighted average diameter; <sup>2</sup> Percentage of fat globules with diameters higher than 2  $\mu\text{m}$ ; <sup>3</sup> Time necessary to melt 30 g of ice cream; <sup>4</sup> LS: level of significance of the corresponding factor (emulsifier, fat or protein and their interactions) ( $P < 0.05$ ); <sup>5</sup> \*\*\*: significant factor ( $P < 0.05$ ) ; <sup>6</sup> SMDG: saturated mono- di-glycerides; <sup>7</sup> PUMDG: partially unsaturated mono- di-glycerides; <sup>8</sup> HCO: hydrogenated coconut oil; <sup>9</sup> RCO: refined coconut oil; <sup>10</sup> RPO: refined palm oil; <sup>11</sup> AMF: anhydrous milk fat; <sup>12</sup> NS: factor not significant ( $P > 0.05$ ); <sup>13</sup> SMP: skim milk powder; <sup>14</sup> FDP: functional dairy proteins.  
a-c: different letters in the same column (within each main effect) indicated significant difference ( $P < 0.05$ );