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Characterisation of soft cheese by front face fluorescence spectroscopy coupled with chemometric tools: Effect of the manufacturing process and sampling zone

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Abstract

Fifteen traditional and stabilised retail soft cheeses (M1, M2 and M3), for which the manufacturing processes were different, were studied using front face fluorescence spectroscopy. Tryptophan, riboflavin and vitamin A fluorescence spectra were recorded at room temperature in two sampling zones (external (E) and central (C)) of the investigated cheeses. The 15 cheeses were discriminated using their spectra by applying principal component analysis (PCA) and common components and specific weights analysis (CCSWA). Using the PCA, the best result was obtained from the vitamin A fluorescence spectra. CCSWA was then applied to the three spectral data sets. Results showed that the CCSWA methodology allowed use of all the spectroscopic information given by the three intrinsic probes in a very efficient way. Fluorescence spectroscopy could provide useful fingerprints, allowing the identification of cheeses according to their manufacturing processes and sampling zones. The spectral patterns allowed information on the protein structure, protein–protein and protein–fat globule interactions and the degree of degradation of riboflavin to be derived at the molecular level.

Keywords: Soft cheese; Structure; Fluorescence; Identification

1. Introduction

Cheese structure is heterogeneous, with several of its constituents present as a solid matrix (paracasein), some as a liquid phase, and others such as fat, either solid or liquid, according to cheese technology and temperature (Pierre, Michel, Le Gräet, & Berrier, 1999). During the ripening, the caseins are broken down by proteolysis (Verdini, Zorilla, & Rubiolo, 2004). Several proteolytic agents are involved, such as in the hydrolysis of casein to large peptides, mainly by the coagulant and some indigenous milk enzymes (e.g., plasmin and Cathepsin D); the hydrolysis of large peptides to small peptides is caused by microbial proteinases and then the hydrolysis of small peptides to amino acids by microbial peptidases. The chemical and physical changes occurring during ripening cause the body of the freshly made cheese to lose its firm, tough and curdy texture and to become soft (Karoui & Dufour, 2003).

There is a wide range of soft cheeses around the world, mainly produced by differences in milk composition and key cheese manufacturing processes, that include the use of different starter cultures and adjuncts (Coker, Crawford, Johnston, Singh, & Creamer, 2005). The pH and curd structure, salt content, degree and mode of fat and protein breakdown during ripening, and ripening conditions affect textural characteristics of cheese (Law, 1984; Lawrence, Creamer, & Gilles, 1987; Richardson & Creamer, 1973). In cheeses ripened with the aid of moulds, such as Camembert and Brie, there is a characteristic mode of fat and

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protein breakdown, which affects both the flavour and the texture of these cheese varieties (Gripon, 1992; Karahadian & Lindsay, 1987). Indeed, the utilisation of lactic acid by *Penicillium* sp. induces the release of ammonia by its proteolytic activities (Noomen, 1977). The formation of NH_3 results from the deaminating action of surface microorganisms (Gripon, 1992; Hassouna, Mankaï, Manaï, Baccar, & Guizani, 1999; Noomen, 1983). Hence, the pH at the surface of such cheese varieties was approximatively around 7 whereas, at the centre, it was around 5.5 (Verdini et al., 2004). The clear distinction appearing between the pH of the surface and the pH of the inner part of soft cheese varieties, during ripening, has been attributed to the oxidation of lactic acid by Fox, Law, McSweeney, and Wallace (1999). As a consequence of the high surface pH, differences in the softness of soft cheeses between the centre and the surface have been reported (Karoui & Dufour, 2003; Verdini et al., 2004). This phenomenon results in a diffusion of soluble calcium and phosphate from the centre to the surface of the cheese, inducing the precipitation of calcium phosphate at the surface (Fox et al., 1999; Le Gräet & Brulé, 1988; Metche & Fanni, 1978). Salt content influences the rate of casein hydrolysis during cheese ripening, and the extension of this degradation process has an important role in the development of the flavour and texture.

The texture of cheeses can be determined by rheological measurements, such as uniaxial compression (Buffa, Trujillo, Pavia, & Guamis, 2001; Hennequin & Hardy, 1995; Pavia, Trujillo, Guamis, & Ferragut, 2000; Truong, Daubert, Drake, & Baxter, 2002; Volikakis, Biliaderis, Vamvakas, & Zerfiridis, 2004), penetrometry (Hennequin & Hardy, 1995; Lee & Klostermeyer, 2001), dynamic oscillatory rheology (Gunasekaran & Ak, 2000; Karoui & Dufour, 2003; Lee & Klostermeyer, 2001; Tunick et al., 1990), and texture profile analysis (Truong et al., 2002; Volikakis et al., 2004). Despite the usefulness of these techniques, a major drawback is that they are tedious and destructive, time-consuming, and require highly skilled operators. The use of non-destructive techniques to determine the quality of cheeses is increasing in response to consumer demands for cheese quality. The requirement to improve quality control and quality assurance influences the industry in general, as well as growers and processors of dairy products. Taking this into account, the development of new methods is of a great importance. Front face fluorescence spectroscopy is fast, relatively low-cost and provides, a great deal of information with only one test. It is considered to be sensitive, non-destructive, environmentally friendly and non-invasive, which makes this method suitable for on-line or at-line process control. In recent years, front face fluorescence spectroscopy has become popular for process monitoring in many industries, such as dairy products, in part because of the relative simplicity of the equipment required to record a spectrum. Fluorescence spectroscopy offers several inherent advantages for the characterisation of molecular interactions and reactions. First, it is 100–1000 times more sensitive than other spectrophotometric techniques (Strasburg & Ludescher, 1995). Second, fluorescent compounds are extremely sensitive to their environment (Dufour & Riaublanc, 1997). This environmental sensitivity allows characterisation of conformational changes, such as those attributable to the thermal, solvent or surface denaturation of proteins, as well as the interactions of proteins with other components. It has been shown that front face fluorescence spectroscopy can discriminate milk samples subjected to heat treatment from those subjected to homogenisation (Dufour & Riaublanc, 1997), and monitor structural changes in milk coagulation (Dufour, Lopez, Riaublanc, & Mouhous Riou, 1998; Herbert, 1999; Herbert, Riaublanc, Bouchet, Gallant, & Dufour, 1999) and cheese manufacture (Herbert et al., 2000; Mazerolles et al., 2001; Mazerolles, Devaux, Dufour, Qannari, & Courcoux, 2002). In addition, this technique has been used for the determination of lactulose and furosine in milks (Kulmyrzaev & Dufour, 2002) and to determine the effect of both light and modified atmosphere packaging on the oxidation of dairy products during storage (Christensen, Povlsen, & Sørensen, 2003; Miquel Becker, Christensen, Frederiksen, & Haugaard, 2003; Mortensen, Sørensen, Danielsen, & Stapelfeldt, 2003; Wold, Jørgensen, & Lundby, 2002). Indeed, Wold et al. (2002) have reported that fluorescence properties of riboflavin can be used to measure the degree of degradation of this molecule in dairy products. Finally, front face spectroscopy has been used to determine the geographic origin of PDO Gruyère and L'Etivaz PDO cheeses (Dufour, Karoui, & Bosset, 2003; Karoui et al., 2004a) and of Emmental cheeses from different European geographic origins, manufactured during winter and summer times (Karoui, Bosset, Mazerolles, Kulmyrzaev, & Dufour, 2005; Karoui, Dufour et al., 2005; Karoui et al., 2004b).

Most of the above studies regarding the potentiality of front face fluorescence spectroscopy to investigate the molecular changes in cheeses have so far been performed on semi-hard and hard cheeses. Few papers have been published on soft cheeses (Herbert et al., 2000; Kulmyrzaev et al., 2005). These studies have determined the texture at the molecular level and established a correlation between textural properties and several physicochemical parameters (pH, moisture content, fat content, extent of proteolysis), without considering the effect of the sampling zone. Verdini and Rubiolo (2002) have investigated the effect of the sampling zone during the ripening of Port Salut Argentino cheese, by using destructive techniques, such as compression and stress relaxation tests. Recently, Karoui and Dufour (2003) have investigated the changes, at the molecular level, of three different ripened soft cheese samples, cut between the surface and the centre, using front face fluorescence spectroscopy. However, in this study, no more than one cheese has been investigated for each cheese variety. In addition, only the tryptophan and vitamin A fluorescence spectra have been recorded on these cheese varieties. So, it would be interesting to validate the relevance of this technique to differentiate between the surface and the

centre by using a large number of soft cheeses. In this study, front face fluorescence spectroscopy, combined with multivariate statistical methods, was used to investigate changes at the molecular level of both the external (E) and central (C) zones of 15 ripened soft cheeses. In order to extract all the information contained in the fluorescence spectra, common components and specific weight analyses (CCSWA) were applied to the tryptophan, vitamin A and riboflavin spectral data sets.

2. Materials and methods

2.1. Cheese samples

Fifteen different soft cheeses, two traditional soft cheese varieties (M1, $n = 5$, and M2, $n = 5$) and one stabilised soft cheese variety (M3, $n = 5$) were purchased from a local Belgian supermarket. These cheeses were manufactured using different mesophilic starter cultures and they were of the white-mould acid-curd type (Herbert et al., 2000) and came from different batches. For each cheese, cheese samples (2 cm × 1 cm × 0.5 cm) were cut from two different cheese zones, the central zone and external zone, as described by Karoui and Dufour (2003).

2.2. Physico-chemical analyses

The determination of pH, dry matter, fat content, total nitrogen and water-soluble nitrogen were as previously described by Bouton, Guyot, Dasen, and Grappin (1994). These parameters were determined on the surface layer after removing the primary surface of mould growth which formed a thin, gray-white and felt-like rind, and on the centre part for the 15 commercial cheeses. All the analyses were done in duplicate.

2.3. Fluorescence spectroscopy

Fluorescence spectra were recorded using a Fluorolog-2 spectrofluorimeter (Spex-Jobin Yvon, Horiba, The Netherlands), mounted with a variable angle front-surface accessory. The incidence angle of the excitation radiation was set at 52° to ensure that reflected light, scattered radiation, and depolarization phenomena were minimized. The emission spectra of the tryptophan residues (305–450 nm) and riboflavin (400–640 nm) were recorded with the excitation wavelengths set at 290 and 380 nm, respectively. The excitation spectra of the vitamin A (280–350 nm) were recorded with the emission wavelength set at 410 nm. All spectra were corrected for instrumental distortions in excitation using a rhodamine cell as a reference channel. For each cheese sample, three spectra were recorded.

2.4. Mathematical analysis of data

In order to reduce scattering effects and to compare the samples, fluorescence spectra were normalised by reducing the area under each spectrum to a value of 1 according to Bertrand and Scotter (1992). Mainly, the shift of the peak maximum and the peak width changes in the spectra were considered, following this normalisation. The principal component analysis (PCA) was applied to the normalised spectra to investigate differences between the samples (Karoui et al., 2005). The PCA transforms the original variables into new axes called principal components (PCs), which are orthogonal, so that the data sets presented on these axes are uncorrelated with each other. Therefore, PCA expresses, as much as possible, the total variation in the data set in only a few PCs and each successively derived PC expresses decreasing amounts of the variance. This statistical multivariate treatment was earlier used to observe similarities among different soft cheeses (Herbert et al., 2000; Karoui & Dufour, 2003), reducing the dimension to two or three PCs, while keeping most of the original information found in the data.

In a second step, CCSWA was applied to the whole data sets. The objective of this technique is to describe several data sets observed for the same samples. CCSWA takes into account the maximum inertia (total variance) of the data sets (tryptophan (n, v_1)), riboflavin (n, v_2) and vitamin A fluorescence spectra (n, v_3), where n is the number of samples ($n = 15$ soft cheeses × 2 parts (external and central) × 3 repetitions for each cheese = 90 spectra), and v_1, v_2 and v_3 are the number of data points (variables) in the tryptophan, riboflavin and vitamin A fluorescence spectra. CCSWA consists of determining a common space of representation for all the data sets. Each table (tryptophan, riboflavin and vitamin A fluorescence spectral data) has a specific weight associated with each dimension for this common space. An important difference between the values of the specific weights for a given dimension would express the fact that this dimension reveals a physical phenomenon, which is visible by one probe and not by the others. CCSWA deals with analysis of co-inertia that is the total variance in data sets. CCSWA also enables the overall data collected to be described and takes into account the relationships between the different data sets (Karoui, Dufour et al., 2005; Kulmyrzaev et al., 2005; Mazerolles et al., 2002). Similarity maps of the samples can be drawn by projection on the planes defined by each couple of the $q_1, q_2, \ldots, q_n$ dimensions. Orthogonal spectral patterns related to the q_i dimension can be calculated. PCA and CCSWA were carried out using MATLAB software.

3. Results and discussion

3.1. Physico-chemical characterisation of ripened soft cheeses

Fat, dry matter, fat in dry matter, pH, total nitrogen (TN) and water-soluble nitrogen (WSN), corresponding to both central (C) and external (E) zones of the investigated cheeses, are shown in Table 1. Differences in the composition and levels of proteolysis between the cheeses

Table 1
Physicochemical composition at the surface and at the centre of the three soft cheeses, M1, M2 and M3 [a,b]

Compositional parameter	Surface			Centre		
	Cheeses ($n = 15$)					
	M1	M2	M3	M1	M2	M3
pH	6.63[d]	7.36[f]	7.06[e]	6.29[c]	7.18[e]	6.59[d]
Fat (g 100 g^{-1})	23.7[d]	22.43[c]	33.53[e]	24.37[d]	22.78[c]	34.63[f]
Dry matter(g 100 g^{-1})	45.57[d]	44.23[c]	48.92[f]	46.76[e]	44.67[c]	50.21[g]
Fat in dry matter (g 100 g^{-1})	52[d]	50.72[c]	68.55[e]	52.12[d]	51.0[cd]	68.97[e]
TN (g 100 g^{-1})	3.33[e]	3.3[e]	2.49[c]	3.35[e]	3.31[e]	2.62[d]
(WSN/TN) × 100	90.6[c]	95.9[d]	91.1[c]	90.4[c]	95.3[d]	90.5[c]

[a] *Abbreviations*: TN, total nitrogen; WSN, water-soluble nitrogen; proteolysis index = 100 × (WSN/TN).
[b] Analysis of variance ($P < 0.05$).
[c,d,e,f,g] Mean values within a row sharing a common superscript do not differ significantly ($P > 0.05$); values presented are mean values of n samples.

suggested differences in milk composition, manufacturing process, and in the sampling zones: E and C. Analysis of composition and proteolysis data indicated that the samples differed mainly with respect to the level of proteolysis, as measured by the level of WSN for each of the investigated cheeses. Indeed, the WSN level amounted to 90.6 g 100 g^{-1}, 95.9 g 100 g^{-1} and 91.1 g 100 g^{-1} of the total nitrogen at the surface for the M1, M2 and M3, respectively, whereas, it was 90.4 g 100 g^{-1}, 95.3 g 100 g^{-1} and 90.5 g 100 g^{-1} at the centre (Table 1). For a given cheese variety, no significant difference ($P < 0.05$) was found between the C and E zones. In addition, no differences were found between CM1, CM3, EM1 and EM3 cheeses. These results are in agreement with previous findings reporting that maturation indices of Port Salut Argentino cheeses (determined as the percentage of WSN of the TN) increased throughout ripening, but there was no significant difference between the E and C zones (Verdini et al., 2004). The M2 cheeses had the highest maturation index at the two sampling zones. Indeed, this cheese variety showed visual softening at the C and E zones(compared to the two other cheese varieties). Indeed, a significant difference was observed between M2 cheeses and M1 and M3 cheeses at the E and C zones.

For each cheese, pH in zone E was significantly higher than in zone C. The M2 cheeses had the highest values of pH at both the C and E zones. This could be attributed to the oxidation of lactic acid. Indeed, following the depletion of lactic acid, NH_3 is produced at the surface, inducing the increase of the pH at the external layers of cheeses. This could be ascribed to the deamination of free amino acid that occurred during ripening, leading to the production of ammonia and α-keto acids in the investigated cheese varieties, as reported by McSweeney and Sousa (2000).

Concerning dry matter, significant difference at a level of 5% was found between M1, M2 and M3 cheeses. Indeed, M2 cheeses had the lowest dry matter content at both the C and E zones, while M3 cheeses had the highest amount. For cheeses M1 and M3, the dry matter content in zone E was significantly lower than in zone C, whereas M2 cheeses did not present significant difference between the C and E zones. The results obtained for M1 and M3 cheeses are in agreement with those of Karoui and Dufour (2003) who reported that the outer part of Camembert cheese had higher water content than the centre during ripening, despite surface evaporation.

Considering fat and fat in dry matter contents, significant difference ($P < 0.05$) was found between M1, M2 and M3 cheeses. The M2 cheeses had the lowest level contents of fat and fat in dry matter in zones C and E, while M3 cheeses had the highest ones (Table 1). For a given cheese, no significant difference was found between the C and E zones.

3.2. Fluorescence properties of tryptophan, riboflavin and vitamin A in the fat globules

The recorded fluorescence spectra give information regarding molecules containing conjugated double bounds. Aromatic amino acids and nucleic acids, tryptophan residues of proteins, vitamin A and riboflavin, in particular, are the best known fluorescent molecules in dairy products (Herbert et al., 2000; Karoui, Martin, & Dufour, 2005; Karoui & Dufour, 2003; Mazerolles et al., 2001, 2002). So a spectrum recorded on a cheese sample following excitation at 290 and 380 nm and emission at 410 nm included information on several fluorophores and may be considered as a characteristic fingerprint which allows the sample to be identified (Karoui, Martin et al., 2005; Karoui & Dufour, 2003).

Tryptophan residues in protein were excited at 290 nm and the emission spectra were recorded between 305 and 450 nm. In fact, the emission fluorescence region (305–450 nm) considered in this study allowed study of the fluorescence of the tryptophan (maximum emission at about 345 nm), as well as the fluorescent Maillard-reaction products (maximum emission at 440 nm). As fluorescent Maillard-reaction products are bound to the proteins, fluorescence transfer may occur between tryptophanyl residues in proteins and those molecules exhibiting a maximum excitation wavelength at about 340 nm (Kulmyrzaev & Dufour, 2002). Normalised emission fluorescence spectra of M1 and M3 cheeses exhibited a maximum at about 350 nm for the C zone and 355 nm for the E zone (Fig. 1). In addition, the width of fluorescence spectra was larger for the E than for the C cheese samples. This

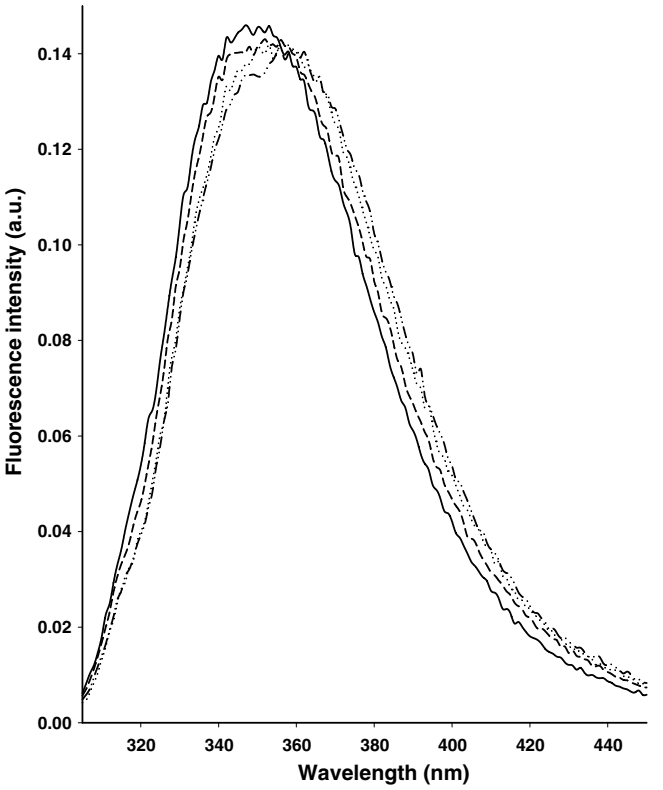

Fig. 1. Normalised fluorescence spectra of tryptophan recorded following excitation at 290 nm on central M1 zone (—), external M1 zone (. . .), central M3 zone (– – –) and external M3 zone (–··–··–) cheeses.

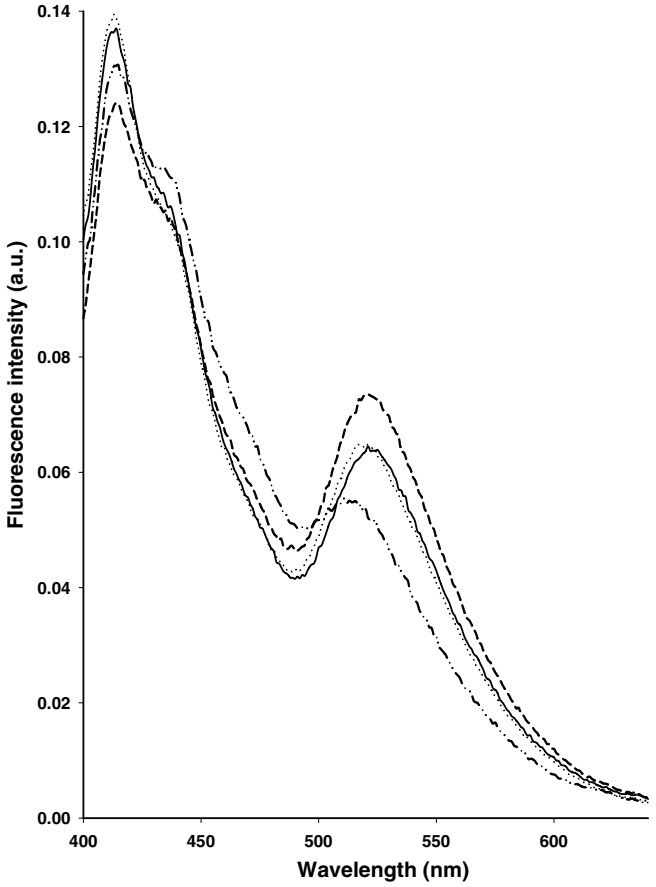

Fig. 2. Normalised fluorescence spectra of riboflavin recorded following excitation at 380 nm on central M1 zone (—), external M1 zone (. . .), central M3 zone (– – –) and external M3 zone (–··–··–) cheeses.

indicates a higher diversity for the environment of tryptophan residues between the sampling zones of the investigated soft cheeses. An explanation may arise from the partial proteolysis of casein during ripening, resulting in an increase of tryptophan exposure to solvent for the E cheese samples, as has been reported by Karoui and Dufour (2003) and as is shown in Table 1. Sampling zones significantly alter fluorescence properties of cheeses which are clearly illustrated by the emission spectra obtained. Indeed, the intensities of the fluorescence emission at 350 and 440 nm were higher for the C and E cheese samples, respectively (Fig. 1).

Emission spectra recorded after excitation at 380 nm of the investigated cheeses showed also some differences according to the sampling zones (Fig. 2).

Fig. 2 shows that there are three spectral regions of particular interest: the broad peak at about 522 nm is due to the riboflavin, as has previously been suggested by Miquel Becker et al. (2003), Wold et al. (2002), and Wold et al. (2005). The second region between 405 and 480 nm showed some changes according to the sampling zone; the fluorophores responsible for these special bands are not known. This region typically shows fluorescence from stable oxidation products formed by aldehydes and amino acids (Kikugawa & Beppu, 1987). In the same region, lumichrome, a photo breakdown product from riboflavin, exhibits fluorescence in the 444–479 nm region (Fox et al., 1999). Finally, β-carotene can also absorb around 400–500 nm. β-Carotene can also undergo photodegradation (Hansen & Skibsted, 2000), which may influence the fluorescence spectra. The last region is from 600 to 640 nm, characteristic of porphyrin and chlorin compounds, as has been reported by Wold et al. (2005).

Considering the vitamin A fluorescence spectra, the shape of the spectra showed two maxima located at 305 and 322 nm and a shoulder at 295 nm (Fig. 3). The shapes of the spectra change with the sampling zone. In addition, as the shapes of the spectra showed large differences between the E and C cheese samples, they may be considered as fingerprints for the identification of both the sampling zones and cheese varieties. Recently, it has been reported that the shapes of the vitamin A excitation spectrum is correlated with the physical state of the triglycerides in the fat globules (Dufour et al., 1998; Karoui & Dufour, 2003). Their researches have shown that the fluorescent properties of this fluorophore are very sensitive to the changes in the solvent viscosity. Finally, it has been suggested that the changes in the shape of vitamin A spectra may also result from fluorescence transfer between tryptophan residues of proteins and vitamin A located in the fat globule membrane (Kulmyrzaev et al., 2005).

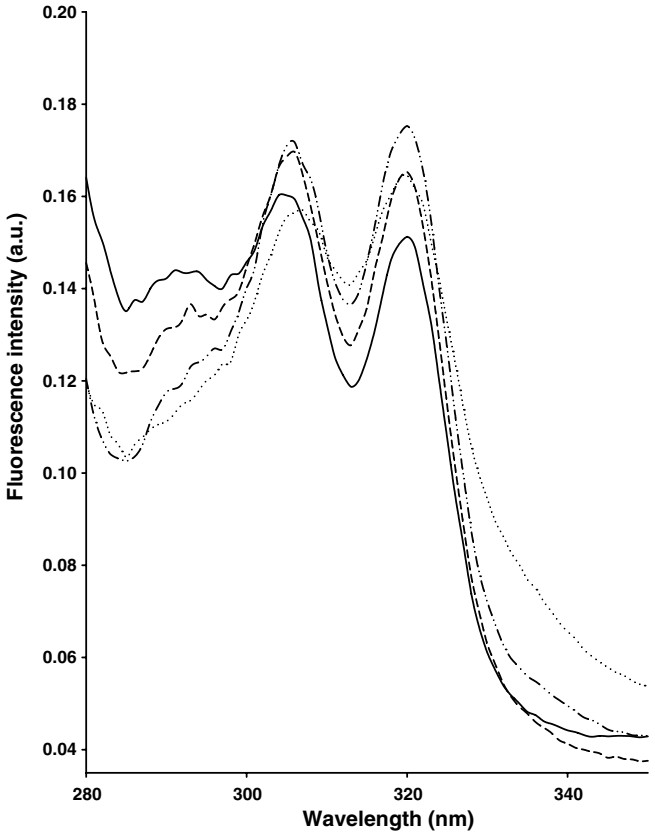

Fig. 3. Normalised fluorescence spectra of vitamin A recorded following emission at 410 nm on central M1 zone (—), external M1 zone (...), central M3 zone (– – –) and external M3 zone (–··–··–) cheeses.

3.3. Evaluation of the discriminant ability of tryptophan fluorescence spectra recorded on soft cheese

PCA was applied to the tryptophan fluorescence spectra recorded on the investigated cheeses to visualise the distribution of cheese samples according to their sampling zones and manufacturing processes. The map defined by the PC1 and PC3, which took into account 93.7% of the total variance, is shown in Fig. 4(a). Considering the PC1, accounting for 91.8% of the total variance, a good discrimination between the E and C zones was observed for M1 and M3 cheese samples. Indeed, E M1 and E M3 cheese samples had negative score values, while C M1 and C M3 cheese samples presented positive score values. Concerning the M2 cheeses, no discrimination was found between the two zones. Indeed, the E M2 and C M2 cheese samples presented one group. This could be explained by similar structures at the molecular level of the two investigated zones of this cheese variety. The obtained results confirmed those found with the physicochemical analysis since no difference in the water content was found between the E M2 and C M2 cheese samples, which was not the case for the M1 and M3 cheeses.

Spectral patterns associated with the PCs provide the characteristic wavelengths that may be used to discriminate between spectra. Spectral patterns are similar to spectra and may be used to derive structural information at the molecular level (Dufour, Subirade, Loupil, & Riaublanc, 1999). The spectral pattern 1 associated with the PC1 (Fig. 4(b)) presented a positive peak at 332 nm and a negative peak at 385 nm. It indicates a shift of the maximum emission of tryptophan residues to the higher wavelengths for E M1, E M3, CM2 and E M2 cheese samples. It was concluded that the environment of tryptophan residues was relatively more hydrophilic for E M1, E M3, E M2 and C M2 cheese samples. This phenomenon may be related to the partial proteolysis of caseins, resulting in an increase of tryptophan exposure to the solvent. Another explanation may arise from the differences, in the pH values and the water content between the E and C zones of the M1 and M3 cheeses, as shown in Table 1 (Herbert et al., 2000; Karoui & Dufour, 2003). Indeed, it is well known that lowering the water content increases the intermolecular links, while higher moisture content causes a swelling of the para-casein matrix and decreases the molecular interactions (Karoui & Dufour, 2003).

3.4. Evaluation of the discriminant ability of riboflavin fluorescence spectra recorded on soft cheese

In total, 90 riboflavin fluorescence spectra were collected for the 15 investigated soft cheeses and the PCA was applied to this data set. Compared with the results obtained from the tryptophan fluorescence spectra, a similar trend was observed for the riboflavin spectra. Indeed, the similarity map defined by the PCs 1 (76.9% of the total variance) and 3 (3.2% of the total variance) showed a discrimination between the E and C zones of M1 and M3 cheeses, while no discrimination was observed between the E and C zones of M2 cheeses (Fig. 5). Considering the PC1, E M3 cheese samples had mostly positive score values, while C M3 cheese samples had negative score values. The PC2 discriminate E M1 cheese samples located on the negative side from C M1 cheese samples located on the positive side.

The spectral pattern 1 associated with the PC1 presented a negative peak around 525 nm. This peak corresponded to riboflavin. Indeed, riboflavin is commonly present in dairy products and is known to initiate lipid oxidation (Wold et al., 2005). Thus, the peak at 525 nm could be due to a decrease in the level content of riboflavin for the E zone of the M3 cheese samples. Therefore, the quantity of riboflavin in the inner layers of M3 cheeses was higher than at the surface, in agreement with the findings of Marsh, Kajda, and Ryley (1994). This difference in the amount of riboflavin between the surface and inner layers could be explained by the fact that, in dairy plants, lighting is provided by various white fluorescent lamps with maximum radiant emission at 450–500 nm, a wavelength range most detrimental to riboflavin photodegradation (Sattar, deMan, & Alexander, 1977). The spectral pattern 1 also represented a positive peak at 412 nm. The spectral pattern 3 presented a positive peak at 435 nm and a negative peak at 508 nm. These peaks

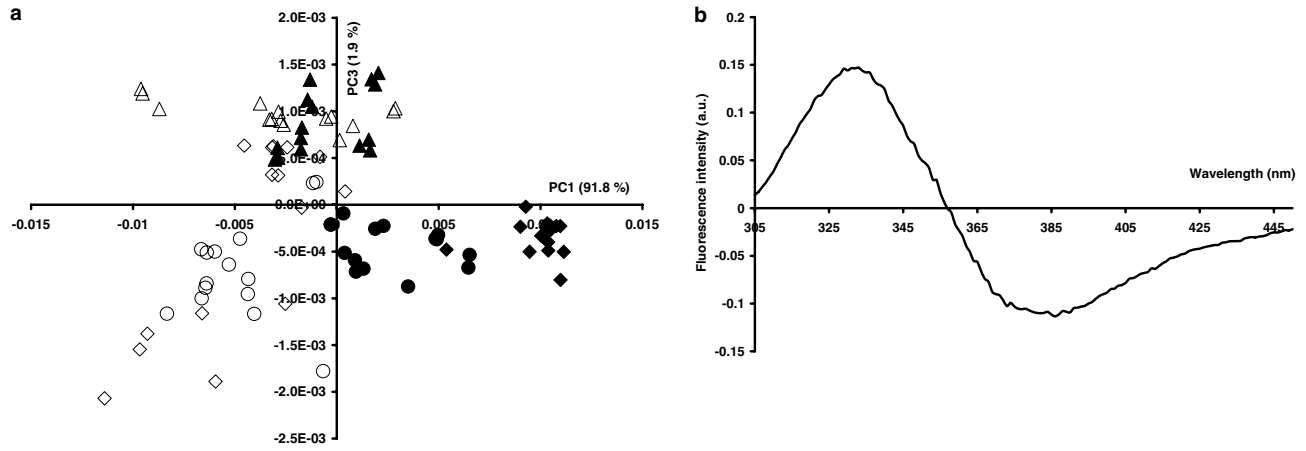

Fig. 4. (a) Principal component analysis similarity map determined by principal components 1 (PC1) and 3 (PC3) for the tryptophan fluorescence spectra of external M1 zone (◇), central M1 zone (◆), external M2 zone (△), central M2 zone (▲), external M3 zone (○) and central M3 zone (●) cheeses. (b) Spectral pattern corresponding to PC1 of tryptophan fluorescence spectra for M1, M2 and M3 cheeses cut at the external and central zones.

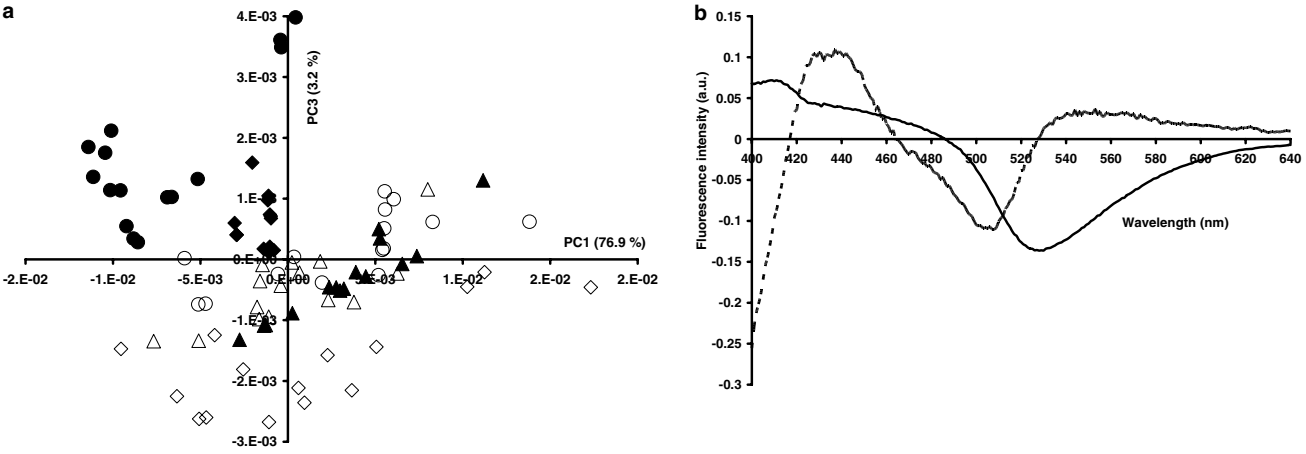

Fig. 5. (b) Principal component analysis similarity map determined by principal components 1 (PC1) and 3 (PC3) for the riboflavin fluorescence spectra of external M1 zone (◇), central M1 zone (◆), external M2 zone (△), central M2 zone (▲), external M3 zone (○) and central M3 zone (●) cheeses. (b) Spectral pattern corresponding to PC1 (—) and PC3 (---) of riboflavin fluorescence spectra for M1, M2 and M3 cheeses cut at the external and central zones.

could be attributed to the lumichrome and lumiflavin compounds. Indeed, it has been reported that these compounds present emission maxima in the ranges 444–479 and 516–522 nm (Wold et al., 2005). Thus, the peak at 508 nm could be ascribed to the oxidation of riboflavin. It was concluded that E M1 and E M3 cheeses were more oxidised than that the C M1 cheese samples.

3.5. Evaluation of the discriminant ability of vitamin A fluorescence spectra recorded on soft cheese

PCA was applied to the vitamin A fluorescence spectra recorded on the investigated cheeses cut at the E and C zones. The map defined by the PC1 and PC3 is shown in Fig. 6.

The results for the discrimination between the sampling zones and manufacturing processes were better with the vitamin A than with the tryptophan or riboflavin fluorescence spectra. Indeed, Fig. 6 shows that E M1, C M1, E M3, C M3 cheese samples were well separated. In addition, a trend to a good discrimination between the E M2 and C M2 cheese samples was observed on the map. It was concluded that the vitamin A fluorescence spectra could be considered as fingerprints, allowing a good identification of soft cheeses as a function of their sampling zones and cheese manufactures. This result was in agreement with the results of others (Herbert et al., 2000; Karoui & Dufour, 2003) who have found that vitamin A is a useful probe to discriminate soft cheeses as a function the manufacturing processes. Indeed, it was shown that a better classification of eight different marketed soft cheeses was obtained from the vitamin A fluorescence spectra (96% and 93% for the calibration and validation samples, respectively) than with the tryptophan fluorescence spectra (95% and 92%, for the calibration and validation samples, respectively) (Herbert et al., 2000).

The difference observed in the vitamin A spectra is related to the changes of lipid structure, but the interpretation at the

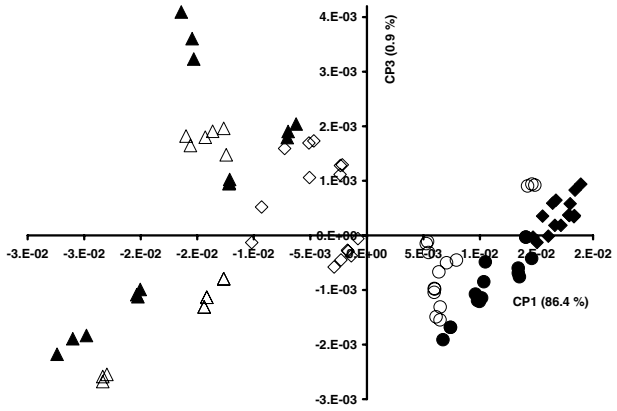

Fig. 6. Principal component analysis similarity map determined by principal components 1 (PC1) and 3 (PC3) for the vitamin A fluorescence spectra of external M1 zone (◇), central M1 zone (◆), external M2 zone (△), central M2 zone (▲), external M3 zone (○) and central M3 zone (●) cheeses.

molecular level is more difficult. Indeed, much less explanation is known about the relationship between the shape of the vitamin A spectra and the organisation of lipids than between the shape of the tryptophan spectra and protein structure. Nevertheless, it is well known that the fluorescent properties of this fluorophore are very sensitive to changes of solvent viscosity (Herbert et al., 2000; Karoui & Dufour, 2003; Karoui, Laguet, & Dufour, 2003). Recently, a good correlation between the shapes of the vitamin A fluorescence spectra and the viscosity of lipids in the fat globules has been found (Dufour et al., 1999).

The examination of the spectral pattern 1 (data not shown) showed an opposition between a positive peak at 295 nm and negative peaks at 313 and 332 nm. This spectral pattern has previously been obtained for data recorded during the ripening of semi-hard cheese (Mazerolles et al., 2001) and for different soft cheese varieties (Karoui & Dufour, 2003). From the two previous studies, it has been concluded that the shape of the spectral pattern of the vitamin A could be related to change in the physical state of the triglycerides during ripening of semi-hard cheeses (Mazerolles et al., 2001) and to the different interactions between the protein network and the fat globules originating from the different manufacturing and ripening processes (Karoui & Dufour, 2003).

3.6. Joint analysis by CCSWA of the three spectral data tables recorded on stabilised and traditional soft cheeses

The CCSWA method was applied to the three normalised data sets containing tryptophan, riboflavin and vitamin A fluorescence spectra data sets. The common components 1 (q_1) and 3 (q_3) gave similar weights: the first common component expressed 79.53% and 59.35% of the inertia of the fluorescence, corresponding to the tryptophan and vitamin A, respectively, and a relatively small part (10.5%) of the inertia of the riboflavin fluorescence data, (Table 2). The third common component expressed

Table 2
Weight diagram for the first three common components q_1, q_2 and q_3 (inertia percentage of each data set explained by the considered component) using common components and specific weights analysis: CCSWA[a]

	q_1	q_2	q_3
Riboflavin fluorescence spectra	10.50	76.35	1.57
Tryptophan fluorescence spectra	79.53	1.20	11.69
Vitamin A fluorescence spectra	59.35	0.26	33.91

[a] CCSWA was performed on the riboflavin, tryptophan, and vitamin A fluorescence spectra.

33.91% and 11.69% of the inertia of the vitamin A and tryptophan fluorescence data and a tiny part of the inertia (1.57%) of the riboflavin fluorescence spectra (Table 2). By contrast, the second common component (q_2) expressed 76.35% of the inertia of riboflavin fluorescence data and a small part of the inertia of the tryptophan and vitamin A data (1.20 and 0.26, respectively).

It can be concluded that the spectral data recorded using on the one hand tryptophan and vitamin A fluorescence spectra and, on the other hand, riboflavin fluorescence spectra were independent and that the common components 1 and 3 were related to different phenomena observed by each intrinsic probe.

It is well known that the shape of the fluorescence spectra of the vitamin A located in the core and the membrane of fat globules is sensitive to the physical state of the triglycerides and to the protein–fat globule interactions in the cheese matrix, while protein structure, protein–protein, and protein–fat globule interactions alter the shape of the tryptophan fluorescence spectra. According to the results obtained from the CCSWA, the spectra tables of tryptophan and vitamin A refer to the same phenomena. As the sole common phenomenon that can be observed from the vitamin A and tryptophan fluorescence spectra are the fat globule–protein interaction in the cheese matrix (see explanation above), it can be concluded that both spectral data sets allow us to describe the changes in the fat globule–protein interactions, in agreement with previous findings (Kulmyrzaev et al., 2005).

The plane defined by the common components 1 and 3 showed a clear discrimination between the cheese varieties and sampling zones (Fig. 7(a)). Considering q_1, C M1 and C M3 cheese samples had negative score values, while the other cheese samples (E M1, E M2, C M2, and E M3) had mostly positive score values. In addition, a good discrimination of E and C zones of M1 and M3 cheeses was observed according to the q_1. Again E M2 and C M2 cheese samples were not well discriminated, but they are well separated from the other cheese varieties. Indeed, considering the q_3, negative score values were observed for the E M2 and C M2 cheese samples, while the other cheese samples had mostly positive scores. Fig. 7 showed a good discrimination of stabilised cheeses (E M3, C M3) from traditional cheeses (EM 1, C M1, E M2, and C M2), independently of their sampling zones. These results were not obtained with the PCA performed on tryptophan, riboflavin or vitamin A fluorescence spectra.

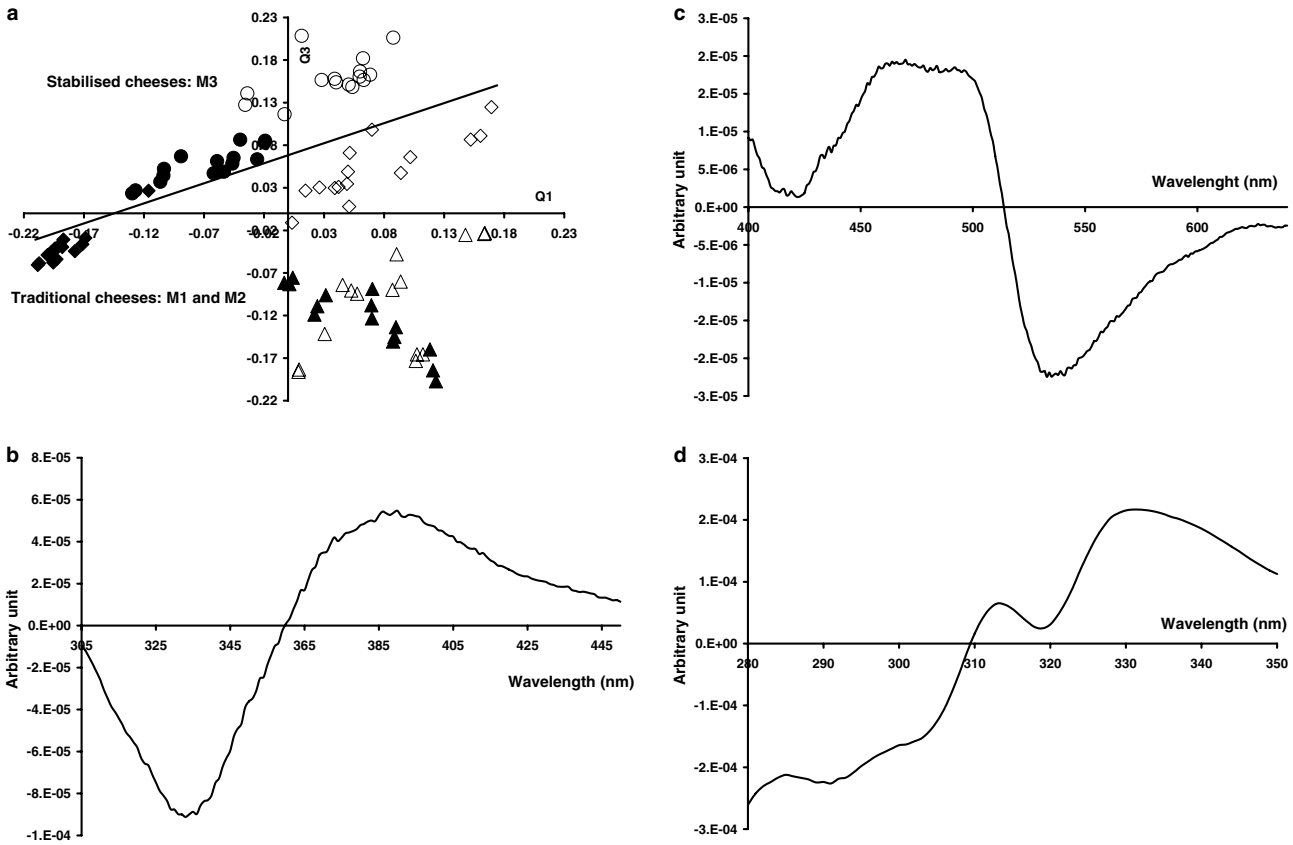

Fig. 7. (a) Common components and specific weights analysis (CCSWA) similarity map defined by the common components 1 (q_1) and 3 (q_3) of external M1 zone (◇), central M1 zone (◆), external M2 zone (△), central M2 zone (▲), external M3 zone (○) and central M3 zone (●) cheeses; spectral patterns of tryptophan (b), riboflavin (c) and vitamin A (d) associated with the common component 1 (q_1).

Examinations of the spectral patterns in tryptophan fluorescence region, riboflavin fluorescence region and vitamin A fluorescence region associated with the q_1 is shown in Figs. 7(b)–(d), respectively.

Fig. 7(b) showed an opposition between a negative peak located at 334 nm and a positive peak at 387 nm. This spectral pattern indicated that E M1, C M2, E M2 and E M3 cheese samples were in hydrophilic environments. Concerning the spectral pattern of the riboflavin data set, an opposition between two peaks located around 460 and 495 nm and the one located at 533 nm was found (Fig. 7(c)). This indicated that C M1 and C M3 cheese samples were less oxidised than the other cheese samples. It is well known that riboflavin is generally bound to proteins. Like other fluorophores, such as tryptophan and vitamin A, the fluorescence emission of riboflavin is highly sensitive to its local environment, and it can be used as an indicator for the oxidation in dairy products, as well as an indicator of protein conformation and interaction changes that occured in cheese. Thus, the changes that occurred in riboflavin fluorescence spectra could also be related to the difference in the pH values between the E and C zones of the investigated cheeses, as shown in Table 1. However, further research will be needed to validate this hypothesis. The spectral pattern of the vitamin A (Fig. 7(d)) was characterised by two positive peaks, at 313 and 330 nm, and a negative peak at 285 nm. A similar spectral pattern has been reported in a previous study (Kulmyrzaev et al., 2005).

From the obtained results, it appears that the approach using the three data sets allowed us to manage, in a very efficient way, all the spectroscopic information collected on the investigated soft cheeses. Each of the investigated probes provides information which can be used for recognising the cheese variety and sampling zone. The CCSWA method sums all of this information on two common components (q_1 and q_3) taking into account the relationship between the different data tables. These results, which were not obtained by the PCA performed separately on each of the tryptophan, riboflavin, and vitamin A fluorescence spectral data sets showed that the CCSWA methodology allowed use of all the spectroscopic information given by the three intrinsic probes in a very efficient way.

4. Conclusion

The results obtained in this study demonstrated that front face fluorescence spectroscopy, coupled with chemometric tools, has the potential to determine the structure at the molecular level of the external (E) and central (C) zones as well as the manufacturing process of three varieties of soft cheeses. This study demonstrated that a significant difference between the external and the central zones of M1

and M3 cheeses was found, while only a trend to a good discrimination between the external and the central zones of M2 cheeses was achieved using the common components and specific weight analysis (CCSWA) on the tryptophan, riboflavin and vitamin A fluorescence spectra. The results obtained showed that the methodology, consisting in coupling of different intrinsic probes, using appropriate chemometric methods (CCSWA), enables more relevant information to be obtained than when using the PCA.

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