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ScienceDirect
Food Chemistry 104 (2007) 409–420
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Monitoring the molecular changes by front face fluorescence spectroscopy throughout ripening of a semi-hard cheese

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Received 12 December 2005; received in revised form 16 July 2006; accepted 13 September 2006

Abstract

Twenty four semi-hard cheeses produced during autumn ($n = 12$) and summer ($n = 12$) periods were manufactured and ripened at an industrial scale. Tryptophan and vitamin A fluorescence spectra were scanned on the 24 cheeses at 2, 30 and 60 days of ripening. Principal component analysis (PCA) and factorial discriminant analysis (FDA) were applied on the spectral data sets. The first five principal components (PCs) of the PCA extracted from each data set (tryptophan or vitamin A) of cheeses produced during autumn or summer period were pooled into a single matrix and analysed by FDA. Regarding cheeses produced during the autumn period, the percentage of samples correctly classified was 95.8% and 86.1% for the calibration and validation samples, respectively. Similar results were obtained from cheeses produced during the summer period. Finally, concatenation technique was applied to the tryptophan and vitamin A spectra recorded on cheeses independently of their production seasons. Correct classification was observed for 87.5% and 80.6% for the calibration and validation samples, respectively. Although this statistical technique did not allow 100% correct classification for all groups, the results obtained were promising considering the significant effect of the season on the cheese characteristics.

Keywords: Semi-hard cheese; Ripening; Tryptophan; Vitamin A; Concatenation

1. Introduction

Texture of cheese is one of the important characteristics that determines its identity and quality (Creamer & Olson, 1982). An accurate balance of the steps involved in cheese elaboration is necessary to ensure appropriate final texture attributes. Physico-chemical transformations that occurred during the ripening stage such as changes in moisture, salt, lipid and protein contents affect textural characteristics of cheeses. Thus, understanding the protein and fat structures, and the interaction between cheese components during and after manufacture, can provide useful information in determining what constitutes a quality product (Mazerolles et al., 2001).

Dairy products are systems of several phases just like almost all kinds of food. The structural changes occurring during the production of cheeses are of great importance to the final characteristics of the product, since structure stability, water and fat binding, and texture characteristics are influenced directly by these changes, while a characteristic such as aroma may be influenced indirectly because of association of aroma components with structure elements. The recent developments within spectroscopic techniques has made it possible to get information from the spectra and then relate that information to the composition of the product, its rheological properties, conditions during manufacturing, use of special ingredients, etc.

Front face fluorescence spectroscopy could form the basis for such techniques due to the speed, ease of use

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doi:10.1016/j.foodchem.2006.09.020

and non-invasiveness. This technique provides information on the presence of fluorescent molecules such as tyrosine, phenylalanine and tryptophan residues in proteins, and their environment in biological samples (Lakowicz, 1983). The fluorescence properties of aromatic amino acids have also been used to study protein structure and protein interactions in cheeses during ripening (Dufour et al., 2000; Herbert et al., 2000) and during the retail process (Karoui & Dufour, 2003, 2006; Karoui, Laguet, & Dufour, 2003). In addition, this technique has been used for the evaluation of oxidative changes in processed cheese during storage (Christensen, Povlsen, & Sørensen, 2003), yogurt (Miquel Becker, Christensen, Frederiksen, & Haugaard, 2003), cream cheese and sour cream (Wold, Jørgensen, & Lundby, 2002). The emission of tryptophan residues are highly sensitive to their local environment, and, thus, is often used as an indicator group for protein conformational changes (Lakowicz, 1983). Using vitamin A as an intrinsic fluorescent probe, fluorescence spectra can also provide information on the physical state of triglycerides and protein-lipid interactions that occurred during ripening (Dufour et al., 2000).

Few studies have been published concerning the monitoring of cheese texture at the molecular level (protein-protein, protein-water and protein-lipid interactions) that occur throughout ripening. Herbert et al. (2000) have monitored the changes at the molecular level of different varieties of soft cheeses manufactured at the industrial scale at two ripening stages (young and old) by using front face fluorescence spectroscopy. The eight investigated soft cheeses were discriminated according to their manufacturing processes and their ripening stages (young and old) by using tryptophan and vitamin A fluorescence spectra. Considering semi-hard cheeses, 16 cheeses varying in moisture (42.1–49.8%), protein (20.2–25.9%) and fat (23.7–31.1%) contents have been monitored at 1, 21, 51 and 81 days of ripening by using both front face fluorescence and mid infrared spectroscopies (Dufour, Devaux, Fortier, & Herbert, 2001; Mazerolles, Devaux, Dufour, Qannari, & Courcoux, 2002; Mazerolles et al., 2001). The researchers reported that cheeses with the highest moisture content and the lowest pH, calcium and nitrogen contents can be discriminated from those exhibiting the lowest moisture content and the highest pH, calcium and nitrogen contents regardless of the ripening stage (1, 21, 51 or 81 days). From these results, the authors concluded that two cheeses showing such differences at 1 day old follow parallel paths during the whole ripening stage. However, in their studies the investigated cheeses were manufactured at a pilot-scale dairy plant (Poligny, France) and ripened under controlled conditions. In addition, the above investigated cheeses were produced from milks which were collected from a limited number of farmers. So, it would be interesting to validate the relevance of the obtained results on representative cheese samples made from bulk milk produced during autumn and summer periods and manufactured at an industrial level. Indeed, it is well known that the production season of milk has an influence on the cheese quality (Fedele, Rubino, Claps, Sepe, & Morone, 2005; Fernández-García, Carbonell, Calzada, & Nuñez, 2006).

Because tryptophan and vitamin A fluorescence spectra scanned on cheese samples may consist of broad overlapping bands originating from various other substances present in cheese (Karoui et al., 2003), or are influenced by various quenching phenomena from the matrix, they cannot be interpreted using univariate statistical methods. Additionally, the tryptophan and vitamin A fluorescence spectra contain more data points for analysis than the number of cheese samples in the data sets. Therefore to extract relevant information from the spectral data, chemometric tools are more appropriate and useful. Typically, multivariate statistical methods such as principal component analysis (PCA) and factorial discriminant analysis (FDA) are used to relate variations in the spectra to the structure at the molecular level of cheeses (Herbert et al., 2000, 1999). As explained herein above, cheese is a complex system which presents a set of different properties (chemical, microbiological, etc.) that are likely to interact. This complexity has led researchers to collect several data tables on the same cheese samples in order to achieve an accurate and, hopefully, exhaustive characterisation of these samples. For exploring all these data sets throughout ripening, it is common to perform a multidimensional analysis on each data table and thereafter sum up the conclusions thus obtained as has been done by Herbert et al. (2000) and Mazerolles et al. (2001). Although the use of PCA and FDA on each spectral data set (tryptophan or vitamin A) can lead to interesting results, they cannot use all the information contained in the spectral data sets. This can be done by using the concatenation technique.

The objective of the current study was to evaluate the feasibility of using front face fluorescence spectroscopy as a non destructive technique to characterise cheeses at the molecular level throughout ripening independently of their production season.

2. Materials and methods

2.1. Cheese samples

Twenty four different Raclette cheeses (semi-hard cheeses) were produced during summer and autumn periods from one manufacturer. The Raclette cheeses (7 kg each) were ripened at the dairy plant at 15 °C and 85% relative humidity. Samples (25 mm diameter, 8 cm long) were taken using a core sampler. They were sampled in the middle of the cheese height and at 20 mm from the rind for the physico-chemical and fluorescence measurements. The fluorescence analyses were performed at 2, 30 and 60 days of ripening, whereas the physico-chemical analyses were only carried out at 60 days of ripening.

2.2. Physico-chemical analyses

The determination of pH, dry matter (DM), fat, total nitrogen (TN) and water-soluble nitrogen (WSN) was carried out as described by Bouton, Guyot, Dasen, and Grappin (1994).

2.3. Fluorescence spectroscopy

Fluorescence spectra were recorded using a Fluoro-Max-2 spectrofluorimeter (Spex-Jobin Yvon, Longjumeau, France). The incidence angle of the excitation radiation was set at 56° to ensure that reflected light, scattered radiation and depolarisation phenomena were minimised. Slices of 2 cm length, 1 cm height and 0.2 cm thickness were prepared from the samples. Spectra of cheese slices mounted between two quartz slides were recorded at 20 °C. The spectrofluorimeter was equipped with a thermostated cell and the temperature was controlled by a Haake temperature controller. The emission spectra of tryptophan residues (305–400 nm) were recorded with the excitation wavelengths set at 290 nm and the excitation spectra of vitamin A (250–350 nm) were scanned with the emission wavelength set at 410 nm. All spectra were corrected for instrumental distortions in excitation using a rhodamine cell in the reference channel. For each cheese, three spectra were recorded on three different slices.

2.4. Mathematical analysis of data

In order to reduce the scattering effects and to compare cheese 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 achieved following this normalisation.

The PCA was applied on the fluorescence spectra recorded on cheese samples in order to extract information from the data sets. The PCA transforms the original independent variables (wavelengths) onto new axes, or principal components (PCs). These PCs are orthogonal, so that the data set presented on these axes are uncorrelated with each other (Jolliffe, 1986). By plotting the PCs, one can view interrelationships between different variables, detect and interpret sample patterns, groupings, similarities or differences (Herbert et al., 2000, 1999). The spectral patterns associated with the PCs could be drawn. They reveal the characteristic wavelengths for which large differences are observed in the spectra. They are similar to spectra and can be used to derive structural information at the molecular level (Dufour, Subirade, Loupil, & Riaublanc, 1999). While similarity maps allow comparison of the spectra in such a way that two neighbouring points represent two similar spectra, the spectral patterns exhibit the absorption bands that explain the similarities observed on the maps.

In a second step, the FDA was performed on the first 5 PCs (which contain the whole information found in the tryptophan and vitamin A spectral data sets) resulting from the PCA applied to the fluorescence spectral data. The aim of this technique is to predict the membership of an individual to a qualitative group defined as a preliminary (Safar, Bertrand, Devaux, & Genot, 1994). A group was created for each ripening time, (i.e., 2, 30 and 60 days ripening). The method cannot be applied in a straightforward way to continuous spectra because of the high correlations occurring between the wavelengths. Advantages were found in the preliminary transformation of the data into their PCs.

FDA assesses new synthetic variables called ''discriminant factors'', which are not correlated and allowed the best separation of the qualitative groups (Herbert et al., 2000). The individual cheese samples can be reallocated within one of the three groups (2, 30 or 60 days ripening). For each cheese, the distance from the various centre of gravity of the groups is calculated. The cheese sample is assigned to the group where its distance between the centre of gravity is the shortest. Comparison of the assigned group to the real group is an indicator of the quality of the discrimination.

With cross-validation, the same samples are used both for model estimation and testing. A few samples are left out from the calibration data set and the model is calibrated on the remaining data points. Then the values for the left-out samples are predicted and the prediction residuals are computed. The process is repeated with another subset of the calibration set, and so on until every object has been left out once; then all prediction residuals are combined to compute the validation step. Several versions of the cross validation approach can be used: the original version of the method, full cross validation, leaves out only one sample at a time.

As the number of observations was quite small (24 cheese samples produced during summer and autumn periods), the FDA was validated by a segmented cross-validation. Segmented cross-validation leaves out a whole group of samples at a time. This technique has been successful applied for the discrimination between fresh and frozen-thawed fish (Karoui, Thomas, & Dufour, 2006). The PCA and FDA were performed using StatBoxPro (Grimmer Logiciels, Paris, France).

3. Results and discussion

3.1. Physicochemical characterisation of cheeses

The results for pH, DM, fat, fat in DM, TN and WSN of the investigated cheeses after 60 days of ripening are reported in Table 1. The differences in composition and levels of proteolysis between cheeses suggest differences in milk composition and biochemical changes during ripening.

Table 1
Physico-chemical compositions of the 60 days-old semi-hard cheeses produced during summer and autumn periods

Raclette samples	pH	Dry matter g.100 g^{-1}	Fat g.100 g^{-1}	Fat in dry matter (%)	TN[a] g.100 g^{-1}	WSN[b] g.100 g^{-1}
Autumn production						
1	5.19	54.90	27.50	50.09	24.15	4.39
2	5.2	55.70	27.50	49.37	23.26	4.12
3	5.22	54.70	27.00	49.36	23.18	4.18
4	5.06	56.10	26.30	46.88	24.51	3.71
5	5.18	55.00	25.50	46.36	23.78	3.94
6	5.08	54.30	25.50	46.96	24.47	4.03
7	5.08	54.80	28.00	51.09	23.89	3.72
8	5.13	57.50	27.50	47.83	23.87	3.09
9	5.16	56.50	27.75	49.12	24.28	3.61
10	5.11	56.30	28.00	49.73	24.18	3.26
11	5.1	54.70	27.50	50.27	23.57	3.81
12	5.13	55.80	27.75	49.73	23.9	3.37
Summer production						
13	5.25	57.00	28.50	50.00	24.48	4.14
14	5.28	56.70	28.50	50.26	23.8	4.09
15	5.27	55.80	28.00	50.18	24.05	4.28
16	5.2	55.80	26.00	46.59	25.07	3.72
17	5.21	56.00	26.50	47.32	24.82	3.59
18	5.23	56.80	26.50	46.65	24.61	3.81
19	5.18	57.00	28.00	49.12	24.95	3.67
20	5.17	57.00	28.00	49.12	24.56	3.71
21	5.18	56.90	27.50	48.33	24.39	3.68
22	5.16	53.50	26.20	48.97	22.35	3.84
23	5.19	52.90	26.50	50.09	23.11	3.62
24	5.21	54.70	27.60	50.46	23.68	3.83

[a] Total nitrogen.
[b] Water soluble nitrogen.

Analysis of proteolysis data indicated that cheeses differed with respect to the levels of TN. The results of the ANOVA showed that Raclette cheeses manufactured during summer differed significantly ($P < 0.05$) in pH from those produced during the autumn period. Indeed, cheeses produced during summer time had the highest pH value (5.21), while those produced during autumn season had the lowest one (5.14). Additionally, cheeses manufactured during summer period had the highest TN, WSN, fat and fat in DM contents while those made in autumn period had the lowest ones.

3.2. Fluorescence properties of protein tryptophans and of vitamin A in the fat globules throughout ripening

Since there are some changes in the physico-chemical composition of cheeses throughout ripening, it was assumed that their structure at the molecular level and, as a consequence, the environment of the tryptophan and vitamin A of cheeses throughout ripening was different.

Concerning tryptophan emission spectra of cheeses made with milk produced in autumn time, maxima located at 338, 340 and 340 nm (Fig. 1) were observed for young (2 days old), semi-ripened (30 days) and ripened (60 days of ripening) cheeses, respectively. It indicated that the environment of tryptophan residues was relatively more hydrophilic for ripened cheeses. Similar results were obtained for cheeses made with milk produced during the summer period (data not shown).

The obtained spectra in Fig. 1 showed different shapes of spectra throughout ripening. Indeed, the width of fluorescence spectra was larger for cheeses of 30 and 60 days old than for those of 2 days old (young cheeses). This indicates a higher diversity for the environment of tryptophan residues in ripened cheeses. Tryptophan residues are very sensitive to their environment (Lakowicz, 1983). In more polar solvents/environments, this fluorophore in excited state will relax to a lower energy state. This means that the tryptophan emission will be shifted towards longer wavelengths (red-shift) in more polar environment as seen in Fig. 1 for ripened cheeses. An explanation may arise from the partial proteolysis of casein during ripening resulting in an increase of tryptophan exposure to solvent (Herbert et al., 2000; Mazerolles et al., 2002; Mazerolles et al., 2001). Indeed, it is well known that during cheese ripening, proteolysis generates partially hydrolysed caseins and peptides. In addition, it is suggested that native caseins and their degradation components induce changes in the molecular environment of tryptophan residues (Mazerolles et al., 2001) which explains the broadening of the tryptophan emission spectra between 2 and 60 days of cheese ripening.

Considering vitamin A fluorescence spectra, the shapes of the spectra of cheeses produced during autumn period throughout ripening are shown in Fig. 2.

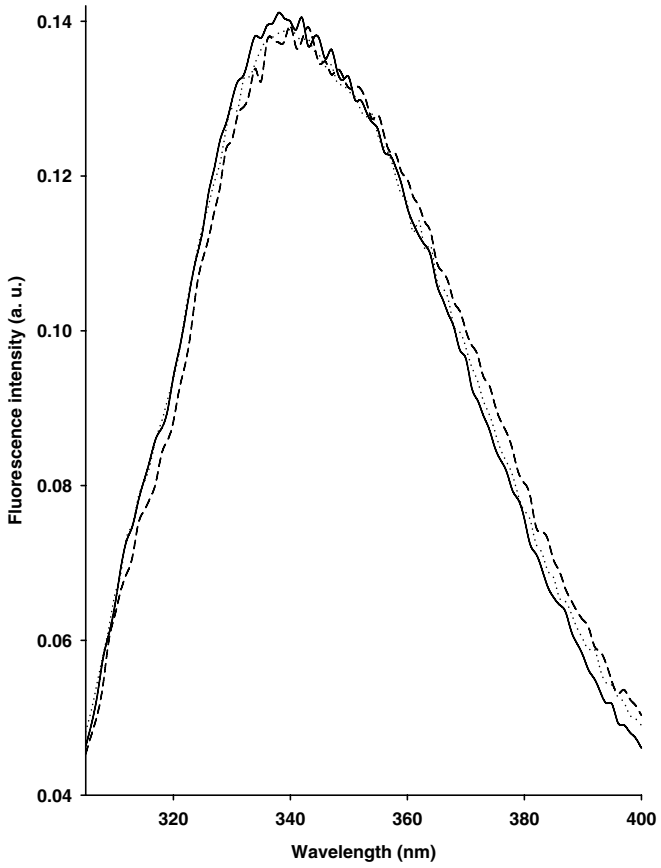

Fig. 1. Normalised tryptophan fluorescence spectra (excitation: 290 nm, emission: 305–400 nm) of cheeses produced during autumn period recorded at three different times throughout ripening: 2 (—), 30 (....) and 60 (- - -) days.

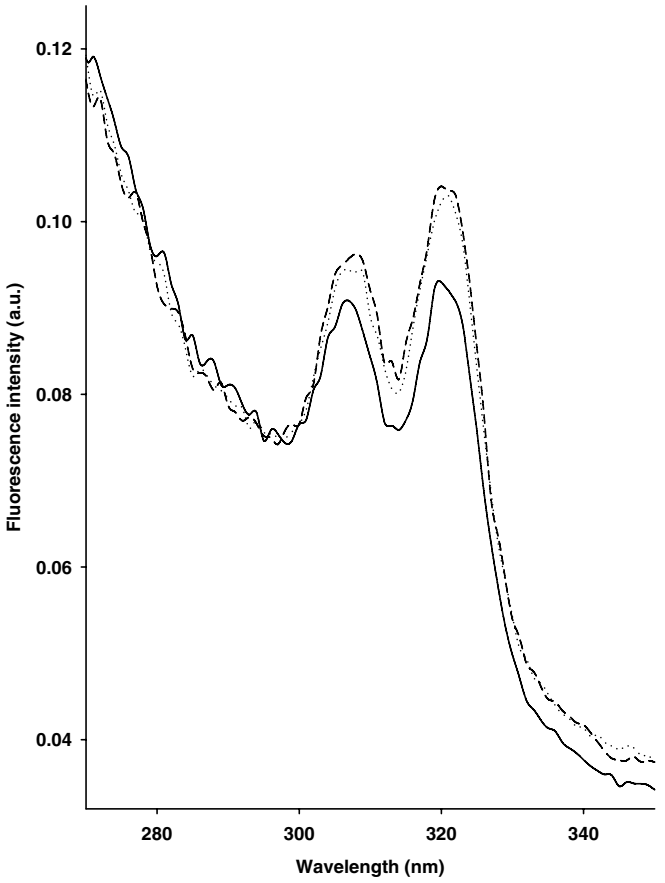

Fig. 2. Normalised vitamin A fluorescence spectra (emission: 410 nm, excitation: 250–350 nm) of cheeses produced during autumn period recorded at three different times throughout ripening: 2 (—), 30 (....) and 60 (- - -) days.

The excitation spectra of vitamin A located in the fat globules of cheeses showed two maxima located at 322 and 308 nm and a shoulder at 295 nm. It appeared that the shapes of vitamin A excitation spectra changed with the ripening time. Moreover, the ratio of fluorescence intensity $F.I._{322nm}/F.I._{295nm}$ increased throughout ripening, particularly between 2 and 30 days, from which the ratios changed slightly. Indeed, young cheeses (2 days old) had the lowest ratio, i.e., 1.26, while cheeses of 30 and 60 days old had the highest, i.e., 1.37. In addition, Fig. 2 showed that the maxima located at 308 and 322 nm shifted slightly towards higher wavelengths with cheese ripening. It has been reported that the fluorescent properties of the fluorophores are very sensitive to the changes of their environment and solvent viscosity (Dufour et al., 2000). The changes of $F.I._{322\,nm}/F.I._{295\,nm}$ ratios could be related to the change in the physical state of triglycerides in the fat globules. Indeed, Karoui et al. (2003) have observed a drastic decrease in the $F.I._{322\,nm}/F.I._{295\,nm}$ ratios of semi-hard and hard cheeses when the fluorescence spectra of these samples were recorded between 20 and 60 °C. Similar results were obtained during the heating of model systems (Dufour, Lopez, Riaublanc, & Mouhous Riou, 1998). From the obtained results, it was concluded that the viscosity of triglycerides changes with the ripening time and that crystallisation takes place throughout ripening. The present investigations were, also, in agreement with those of Dufour et al. (2000) who have reported by using front face fluorescence spectroscopy a decrease in the $F.I._{322\,nm}/F.I._{295\,nm}$ ratios of 16 semi-hard cheeses between 1 and 21 days of ripening. The same authors observed that the environment of methyl groups determined by mid infrared changed during the ripening of experimental semi-hard cheeses. They related this phenomenon to the change in the ratio of anti-symmetric stretching modes of methylene and methyl ($A\nu_{CH2}/A\nu_{CH3}$). Similar results were obtained from cheeses made with milk produced during summer period (data not shown).

Due to the slight difference between fluorescence spectra, particularly those of tryptophan, it was necessary to use chemometric tools to extract relevant information from the spectral data sets.

3.3. Cheese discrimination based on tryptophan fluorescence spectra throughout ripening

A number of 108 spectra were collected from the 12 investigated cheeses produced during autumn period

throughout ripening (2, 30 and 60 days ripening). A similar number of spectra were also obtained from cheeses produced during summer time. At this step, cheeses produced during autumn and summer seasons were analysed separately by using chemometric tools (PCA and FDA).

The map defined by the two first PCs of the PCA performed on cheeses produced during autumn period throughout ripening is shown in Fig. 3a. Although cheeses of 30 days old partly overlapped cheeses of 2 and 60 days old, a good discrimination between young cheeses (2 days old) which had mostly positive contribution according to the PC2 (accounting for 29.9% of the total variance) from those of 60 days old which exhibited mostly negative score values was observed. It was concluded that one or several phenomena take place during the ripening of cheeses at the molecular level.

Spectral patterns corresponding to the PCs 1 and 2 (Fig. 3b) provided information about the characteristic wavelengths which explained the discrimination between cheeses. The spectral pattern 1 indicates a broadening of cheeses located on the negative side of PC1. Since cheeses were discriminated according to the PC2, the study of the spectral pattern 2 is of relevant interest. The spectral pattern 2, indicated that the environment of tryptophan residues was relatively more hydrophilic for the old cheeses (60 days of ripening) than the young ones. This phenomenon could be ascribed to the partial proteolysis of caseins which results in an increase in the exposure of tryptophan residues to the solvent. Another explanation could arise from the changes in the protein-protein and protein-lipid interactions in the protein network during ripening (Herbert et al., 2000; Karoui & Dufour, 2003). Similar results were obtained from cheeses produced with milk produced during the summer period.

In order to assess the ability of tryptophan fluorescence spectra to discriminate cheeses as a function of their ripen-

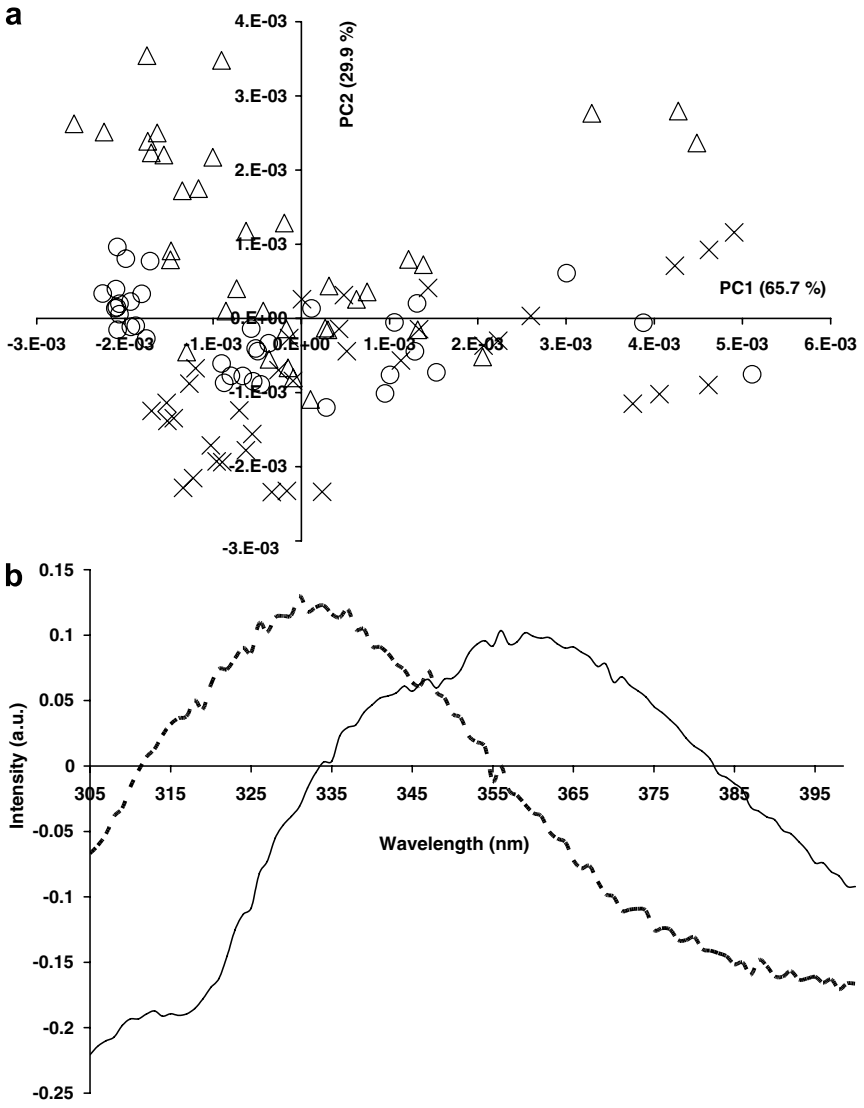

Fig. 3. (a) Principal component analysis (PCA) similarity map determined by principal components 1 (PC1) and 2 (PC2) for tryptophan fluorescence spectra of cheeses produced during autumn period after 2 (△), 30 (○) and 60 (×) days ripening. (b) Spectral patters for the principal components 1 (—) and 2 (- - -).

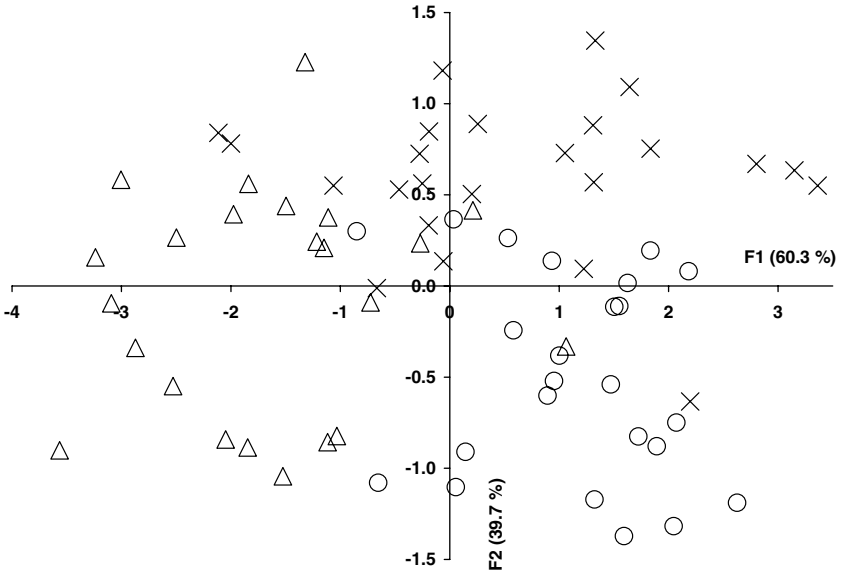

Fig. 4. Discriminant analysis similarity map determined by discriminant factors 1 (F1) and 2 (F2) of the factorial discriminant analysis (FDA) performed on tryptophan fluorescence spectra of cheeses produced during autumn period after 2 (△), 30 (○) and 60 (×) days ripening.

ing time, FDA was applied to the first 5 PCs of the PCA performed on the tryptophan fluorescence spectra of cheeses produced during the autumn period. Before applying the FDA, 3 groups were defined: cheeses of 2 days old, cheeses of 30 days old and cheeses of 60 days old. The calibration map defined by the discriminant factors 1 and 2 took into account 100% of the total variance with discriminant factor 1 accounting for 60.3% of the total variance (Fig. 4).

Considering discriminant factor 1, young cheeses (2 days old) were observed on the left, while the other cheeses were observed mostly on the right. The discriminant factor 2 discriminated cheeses of 30 days old which had mostly negative score values from ripened cheeses (60 days old) which had positive scores. Similar results were obtained for cheeses produced during summer time.

Correct classification was observed for 80.6% and 63.9% for the calibration (data not shown) and validation samples (Table 2), respectively. Similar results were obtained for cheeses produced during the summer period since the percentage of samples correctly classified was 69.4% and 66.7% for the calibration (data not shown) and validation (Table 2) data set, respectively. The best classification was obtained for young cheeses since 75% and 100% correct classifications were obtained for cheeses produced during autumn and summer periods, respectively. Cheeses of 30 and 60 days of ripening were much more misclassified.

Finally, the first 5 PCs of the PCA performed on tryptophan fluorescence spectra scanned on cheeses produced during autumn and summer periods were pooled into one matrix, and this new table was analysed by FDA. This concatenation approach enables us to test the ability of tryptophan fluorescence spectra to discriminate cheeses throughout ripening independently of their production seasons, which correspond to the reality of cheeses found in the market. The map defined by the first two discriminant factors showed similar results to those observed with tryptophan fluorescence spectra recorded on cheeses produced during autumn or summer period. The percentage of samples correctly classified by the FDA was 66% and 63.9% for the calibration (data not shown) and validation (Table 2) spectra, respectively. No group of cheese was completely correctly classified. The relatively low level of correct classification limits the use of this intrinsic probe as a valuable tool to evaluate the age of cheese throughout ripening independently of their production season. However, tryptophan fluorescence spectra had demonstrated its ability to give valuable information about the molecular environment of cheeses throughout ripening.

3.4. Cheese discrimination based on vitamin A fluorescence spectra throughout ripening

PCA was applied to the vitamin A fluorescence spectra recorded on cheeses produced during the summer period throughout ripening. The similarity map defined by the PC1 and PC2 accounted for 97.9% of the total variance with PC2 accounting for 1.3% and allowed a good discrimination of cheeses according to their ripening stage (Fig. 5). Indeed, according to the PC2, positive values were observed for young cheeses (2 days of ripening), whereas negative scores were observed for ripened cheeses (60 days old). In addition the three groups of cheeses were quite well separated. Similar results were obtained for cheeses produced during autumn period.

The difference observed in the vitamin A fluorescence spectra were related to the changes of lipid structures and protein-lipid interactions, but the interpretation at the molecular level was more difficult. Indeed, less information is known on the relation between the shape of the vitamin

Table 2
Classification table for cheeses produced during autumn and summer periods based on tryptophan and vitamin A fluorescence validation data sets

Predicted[a] Real[b]	2 days	30 days	60 days	% Correct classification
Autumn period				
Tryptophan fluorescence spectra				
2 days	**9**	3-	3	75
30 days	–	**6**	6	50
60 days	1	3	**8**	66.7
Total	–	–	–	**63.9**
Vitamin A fluorescence spectra				
2 days	**10**	2	–	83.3
30 days	2	**8**	2	66.7
60 days	2	8	**2**	33.3
Total	–	–	–	**61.1**
Concatenation: Tryptophan and vitamin A fluorescence spectra				
2 days	**11**	1	–	91.7
30 days	2	**9**	1	75
60 days	–	1	**11**	91.7
Total	–	–	–	**86.1**
Summer period				
Tryptophan fluorescence spectra				
2 days	**12**	–	–	100
30 days	2	**7**	3	58.3
60 days	3	4	**5**	41.7
Total	–	–	–	**66.7**
Vitamin A fluorescence spectra				
2 days	**10**	2	–	83.3
30 days	1	**10**	1	83.3
60 days	–	4	**8**	66.7
Total	–	–	–	**77.8**
Concatenation: Tryptophan and vitamin A fluorescence spectra				
2 days	**12-**	–	–	100
30 days	–	**9**	3	75
60 days	1	4	**7**	58.3
Total	–	–	–	**77.8**
Autumn + summer periods				
Concatenation: tryptophan				
2 days	**19**	2	3	79.2
30 days	3	**13**	8	54.2
60 days	4	6	**14**	58.3
Total	–	–	–	**63.9**
Concatenation: Vitamin A				
2 days	**20**	4	–	83.3
30 days	6	**14**	4	58.3
60 days	2	10	**12**	50
Total	–	–	–	**63.9**
Concatenation: Tryptophan and Vitamin A				
2 days	**24**	–	–	100
30 days	1	**17**	6	70.8
60 days	1	6	**17**	70.8
Total	–	–	–	**80.6**

[a] The number of cheeses predicted from the model.
[b] The number of real cheeses.

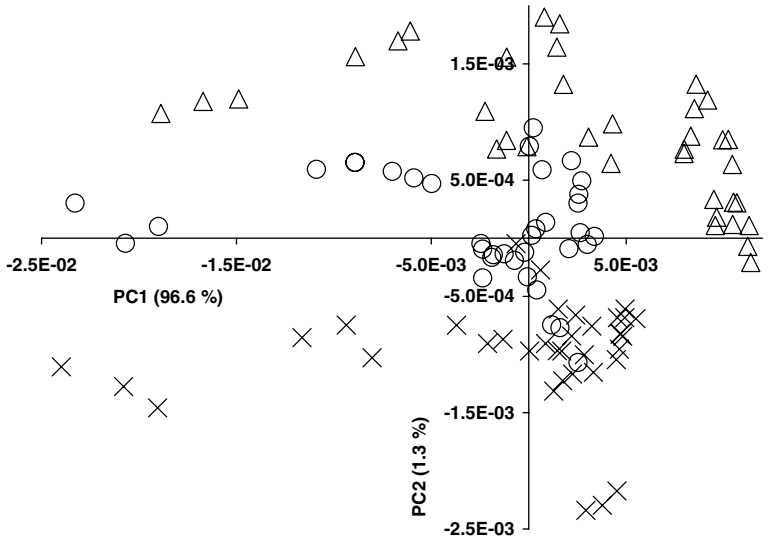

Fig. 5. Principal component analysis (PCA) similarity map determined by principal components 1 (PC1) and 2 (PC2) for vitamin A fluorescence spectra of cheeses produced during summer period after 2 (△), 30 (○) and 60 (×) days ripening.

A spectra and the organisation of the lipids than between the shape of the tryptophan spectra. Regarding the spectral pattern 1, an opposition between a negative peak located at 326 nm and a positive one at 299 nm was observed (data not shown). A similar spectral pattern was obtained during the ripening of experimental semi-hard cheeses (Dufour et al., 2000). It was concluded that the fluorescence properties of vitamin A in triglycerides depends on the changes of the state viscosity of triglycerides.

In a second step, the discriminant ability of the vitamin A was assessed by applying the FDA on the first 5 PCs of the PCA carried out on the vitamin A fluorescence spectra. The approach described in the above section was also used in this case, e.g., three groups were created. The map defined by the first two discriminant factors accounted for 100% of the total variance with discriminant factor 1 accounting for 87.5% of the total variance (Fig. 6). Considering the discriminant factor 1, young cheeses (2 days old) were observed on the far right, whereas ripened cheeses (60 days old) were observed on the far left. Cheeses of 30 days old present co-ordinate close to the origin and were well discriminated from the other cheese samples. Similar results were obtained for cheeses produced during the autumn period.

Correct classification was obtained for 97.2% and 77.8% for the calibration (data not shown) and validation (Table 2) samples, respectively. Less good classification was obtained for cheeses produced during the autumn period

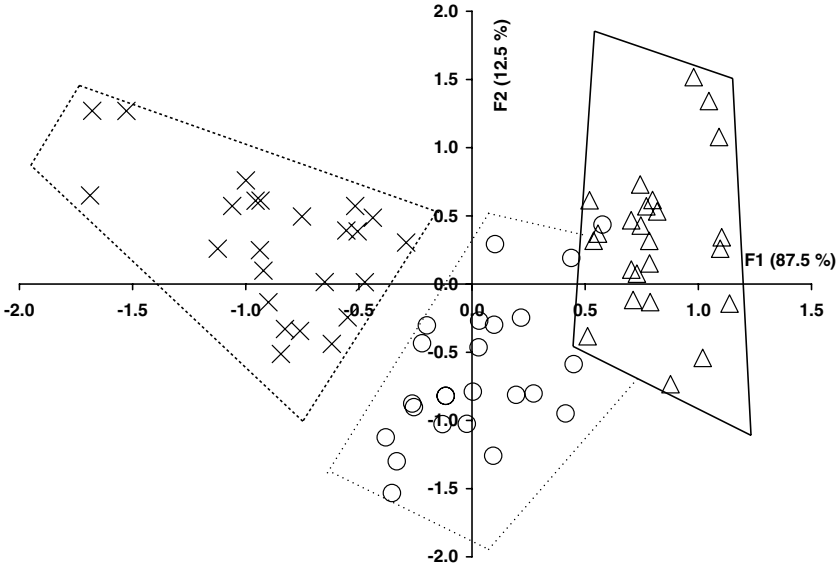

Fig. 6. Discriminant analysis similarity map determined by discriminant factors 1 (F1) and 2 (F2) of the factorial discriminant analysis (FDA) performed on vitamin A fluorescence spectra of cheeses produced during summer period after 2 (△), 30 (○) and 60 (×) days ripening.

since only 76.1% and 61.1% of the calibration (data not shown) and validation (Table 2) samples were correctly classified, respectively. However, the percentage of samples correctly classified was better with vitamin A than with tryptophan fluorescence spectra, particularly for cheeses produced during the summer period. The present findings were in agreement with those of Herbert et al. (2000) who reported that vitamin A fluorescence spectra was a useful probe for discriminating soft cheeses as a function of their ripening time.

Finally, the first 5 PCs of the PCA performed on vitamin A fluorescence spectra scanned on cheeses produced during autumn and summer periods were gathered into one matrix and analysed by FDA. The map defined by the two first discriminant factors allowed a good discrimination of cheeses as a function of their ripening time (data not shown). Indeed, the percentage of samples correctly classified using vitamin A spectra was 83.3% and 63.9% of the calibration (data not shown) and the validation (Table 2) data set, respectively.

3.5. Joint analysis of the tryptophan and vitamin A fluorescence spectra of cheeses throughout ripening

It is well known that tryptophan and vitamin A fluorescence spectra give specific information on the tertiary structure of proteins and the physical state of the triglycerides (Herbert et al., 2000; Karoui & Dufour, 2003). Cheese can be considered as a complex system which presents a set of different properties (Mazerolles, Hanafi, Dufour, Bertrand, & Qannari, 2006) and contains many fluorescent molecules. The data sets obtained for the investigated probes during this study contain information on the cheeses which can be complementary. Consequently, for exploring all these information, it is common to perform a multidimensional analysis on a data table obtained by merging all of them in one matrix. This combined analysis can be performed by using the technique of concatenation. Thus, the first 5 PCs of the PCA applied to each of the two data sets (tryptophan and vitamin A) were gathered into one matrix (108 spectra and 10 variables) and this table was analysed by FDA.

Considering cheeses produced during the autumn period, the map defined by the two first discriminant factors is shown in Fig. 7. The investigated cheeses were well discriminated as a function of their ripening time. Indeed, considering discriminant factor 1 which accounted for 60.5% of the total variance, young cheeses (2 days old) were observed on the right, whereas cheeses of 30 and 60 days ripening were observed on the left. The discriminant factor 2 separated cheeses of 30 days old which exhibited negative score values from ripened cheeses (60 days old). Similar results were obtained from cheeses produced during summer period (Fig. 8). From the obtained results, it appeared that such coupling is appropriate for the discrimination of cheeses according to their ripening stage.

Regarding cheeses produced during the autumn period, the percentage of samples correctly classified into three groups by the FDA was 95.8% and 86.1% for the calibration (data not shown) and validation (Table 2) samples, respectively. Quite similar results was obtained from the cheeses produced during summer period since the percentage of samples correctly classified was 98.6% and 77.8% for the calibration (data not shown) and validation (Table 2) samples, respectively. From the Table 2, it was shown that cheeses of 2 days old were well discriminated from old cheeses (60 days old), while some misclassification still occurred between cheeses of 30 and 60 days ripening.

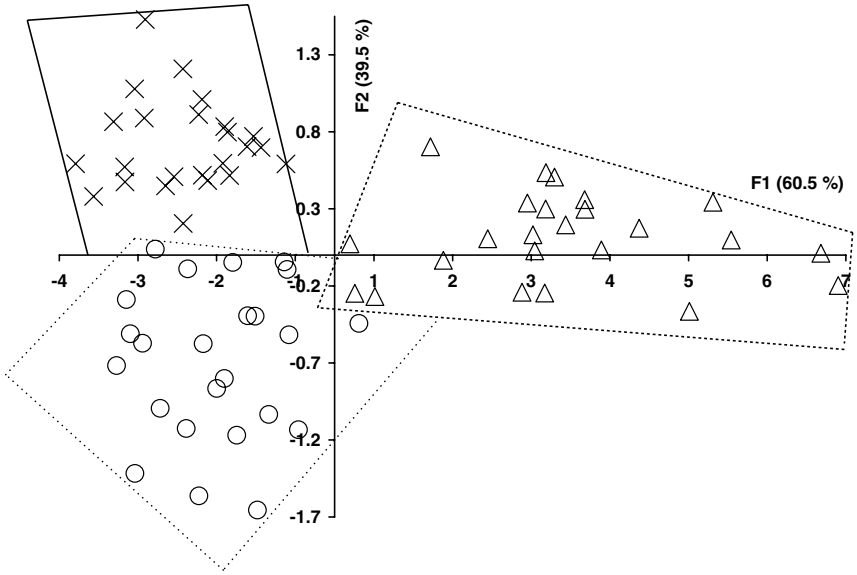

Fig. 7. Discriminant analysis similarity map determined by discriminant factors 1 (F1) and 2 (F2). Factorial discriminant analysis (FDA) was performed on the 10 concatenated PCs corresponding to the PCA performed on the tryptophan and vitamin A fluorescence spectra of cheeses produced during autumn period after 2 (△), 30 (○) and 60 (×) days ripening.

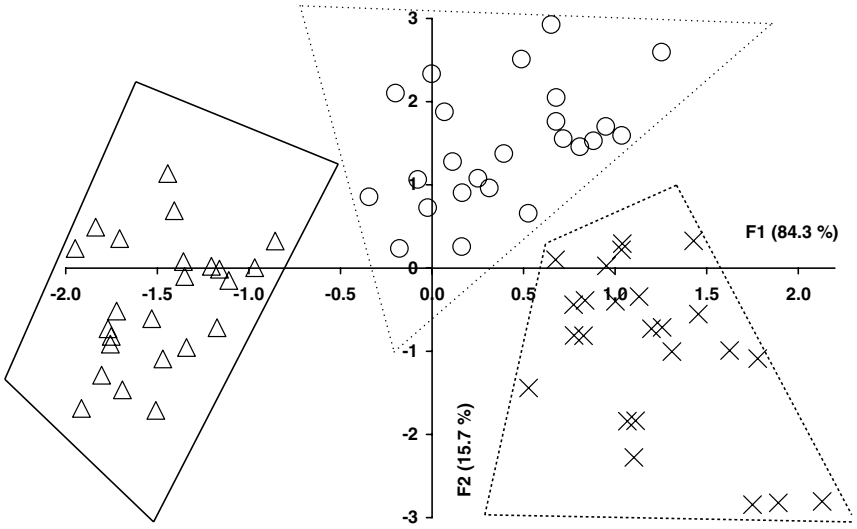

Fig. 8. Discriminant analysis similarity map determined by discriminant factors 1 (F1) and 2 (F2). Factorial discriminant analysis (FDA) was performed on the 10 concatenated PCs corresponding to the PCA performed on the tryptophan and vitamin A fluorescence spectra of cheeses produced during summer period after 2 (△), 30 (○) and 60 (×) days ripening.

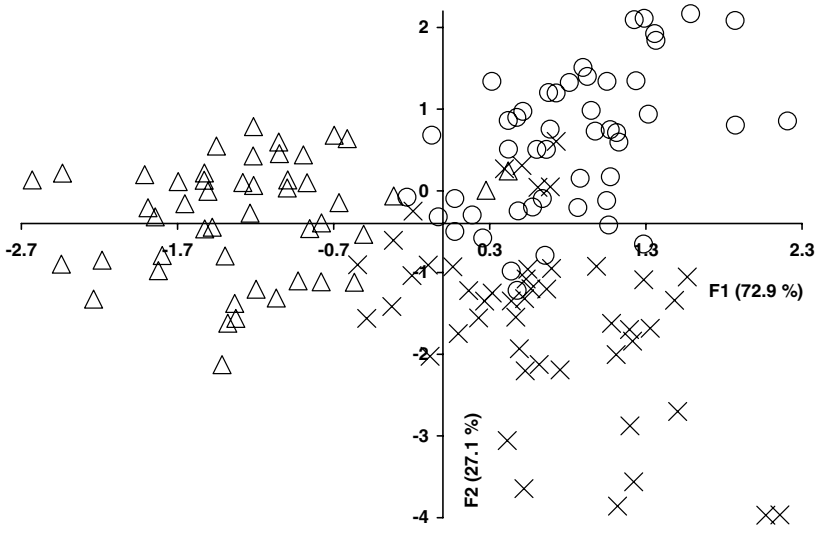

Fig. 9. Discriminant analysis similarity map determined by discriminant factors 1 (F1) and 2 (F2). Factorial discriminant analysis (FDA) was performed on the 10 concatenated PCs corresponding to the PCA performed on the tryptophan and vitamin A fluorescence spectra of cheeses produced during autumn and summer periods after 2 (△), 30 (○) and 60 (×) days ripening.

In order to assess the potential of tryptophan and vitamin A fluorescence spectra to discriminate cheeses according to their ripening time independently of their production periods, the first five PCs of the PCA performed on the tryptophan (108 samples and 5 variables) and vitamin A (108 samples and 5 variables) recorded on cheeses produced during the autumn period (matrix of 108 individuals and 10 variables) and those scanned on cheeses produced during the summer period (matrix of 108 individuals and 10 variables) were polled into one matrix, and this new table (matrix of 216 individuals and 10 variables) were analysed by FDA. The map defined by the discriminant factors 1 and 2 is shown in Fig. 9. A good discrimination of cheeses independently of their production times was observed. Indeed, considering discriminant factor 1, young cheeses presented negative score values, whereas the other cheeses had mostly positive scores. The discriminant factor 2 differentiated between cheeses of 60 days ripening which exhibited mostly negative scores from those of 30 days ripening (presented mostly positive values).

Correct classification was observed for 87.5% and 80.6% for the calibration (data not shown) and validation (Table 2) samples, respectively. Table 2 showed 100% correct classification for young cheeses. Considering cheeses of 30 days old, 7 cheeses were misclassified: 1 cheese sample with 2 days old group and 6 others with 60 days ripening group.

Six ripened cheeses (60 days old) were classified as belonging to 30 days group and 1 other was assigned to young cheese group (2 days old).

4. Conclusion

Although the concatenation statistical technique used in the present study did not produce 100% correct classification for all groups, the results obtained are promising considering the significant effect of the season on the characteristics of cheeses. Using the two data sets (tryptophan and vitamin A spectra) together allowed us to use the spectroscopic information collected for the two production periods (summer and autumn) in an efficient way. Although many factors influence the final quality of semi-hard cheeses (milk origin, milk treatment, season, type and amount of starter added, manufacturing conditions, ripening time and temperature), the investigated study confirmed the potential of this joint analysis (concatenation) of the spectral data sets obtained by using tryptophan and vitamin A fluorescence spectra, in order to determine the ripening stage of cheeses. Thus, it can be legitimately hoped that the models can be used to characterise Raclette cheeses of unknown age. In addition, other varieties of cheese can be promptly submitted to the protocol described here.

The simplicity of the front face fluorescence spectroscopy offers rich opportunities for efficient characterisation of cheeses at a very low cost. In addition, this technique has the potential to dramatically reduce analytical time and cost compared to the enzymatic and bio-chemical measurements.

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