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Front face fluorescence spectroscopy coupled with chemometric tools for monitoring the oxidation of semi-hard cheeses throughout ripening

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Abstract

The purpose of this study was to assess the potential of the 400–640 nm emission fluorescence spectra after excitation set at 380 nm to monitor the changes in spectra and oxidation of 24 semi-hard cheeses, produced during autumn ($n = 12$) and summer ($n = 12$) periods, throughout ripening. Emission fluorescence spectra were recorded at the surface and at the inner layers of cheeses at 2, 30 and 60 days of ripening. The factorial discriminant analysis (FDA) applied on the first 5 principal components (PCs) of the principal component analysis (PCA) carried out on the 400–640 nm emission spectral data set recorded at the surface layers of cheeses produced during autumn or summer period showed a good discrimination of cheeses. Less good classification was obtained from cheese samples cut at the inner layers. In a second step, the 400–640 nm emission fluorescence spectra recorded at the surface or the inner layers of cheeses produced during autumn and summer periods were gathered into one matrix and analysed by FDA. Considering fluorescence spectra recorded at the inner layers, correct classification was observed for 67.4% and 62.3% for the calibration and cross-validation samples, respectively. Better classification was obtained for cheeses cut at the surface layers since 93.7% and 90.3% of the calibration and cross-validation samples respectively, were correctly classified. The obtained results showed that the 400–640 nm emission fluorescence spectra were primarily affected by light and oxygen then by the physico-chemical changes that occurred throughout ripening.

Keywords: Semi-hard cheese; Ripening; Front-face fluorescence spectroscopy; Oxidation

1. Introduction

Cheeses are exposed to light from both natural and artificial sources throughout processing, packaging, and distribution as well as at the retail level. Understanding the structure of cheese and the degradation of lipids, proteins and vitamins provide useful information in determining what constitutes a quality product. During ripening, light induces degradation of these components inducing both formations of off-flavours and colour changes which may lead to loss in nutritional values and the formation of toxic products (e.g. cholesterol oxides). Indeed, it has been reported that exposure of milk to light, especially in the range of 415–455 nm, cause off-flavours in milk and degrades some of its riboflavin, vitamins C and A (Bosset, Sieber, & Gallman, 1994; Fanelli, Burlew, & Gabriel, 1985; Fellman, Dimick, & Hollender, 1991). These findings are of particular interest since dairy products are exposed to light with maximum radiant emission set at 450–500 nm, a wavelength range which induced lipid oxidation, the photo-degradation of riboflavin (Sattar, de Man, & Alexander, 1977) and vitamin A (Marsh, Kajda, & Ryley, 1994). This phenomenon induced the formation of off-flavour, colour changes and the decrease in nutritional value of dairy products as has been reported by Bosset et al. (1994).

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The role of vitamins as an important nutriment in the human diet is well known. Among them there is riboflavin (vitamin B_2) which is a water-soluble vitamin. The vitamin B_2 is very stable during thermal processing, storage and food preparation. But, both light and oxygen have been found to induce riboflavin degradation (Bosset et al., 1994; Mavattivi, Monetti, Vrhovšeket, Tonon, & Andrés-Lacueva, 2000; Sattar et al., 1977; Singleton, Aurand, & Lancaster, 1963; Skibsted, 2000). Thus, the analysis of riboflavin and flavin cofactors such as flavin adenine dinucleotide (FAD) and flavin mononucleotides (FMN) is of considerable importance to the food industry during manufacturing and storage.

At present, several procedures are available for determining the level of riboflavin in foodstuffs. Among them, there are high performance liquid chromatography (Esteve, Farré, Frígola, & Garcıa-Cantabella, 2001; Ollilainem, Mattila, & Varo, 1990; Reyes, Norris, Taylor, & Potts, 1988; Ribarova, Shishkov, Obretenova, & Metchkneva, 1987; Russell & Vanderslice, 1992; Van den Berg, Van Schaik, Finglas, & de Froidmont- Görtz, 1996; Vidal-Valverde & Reche, 1990) and capillary zone electrophoresis with laser induced detection (Cataldi, Nardiello, Carrara, Ciriello, & De Benedetto, 2003). These techniques suffer from a number of drawbacks since they are time consuming, expensive when looking at enzymatic and bio-chemical requirements and not easily adapted for online measurements. However, this is not the case for spectroscopic techniques when the capital cost is excluded. These optical techniques including the near infrared (NIR), mid infrared (MIR) and fluorescence spectroscopies can be used as low cost and rapid methods to provide results of several parameters within a few minutes. Consequently, a huge amount of money and time could be saved and a product with more consistent quality could be offered to the customers if these techniques are adopted in cheese industry.

In recent years, fluorescence spectroscopy has become popular for process monitoring in many industries, especially dairy products, due to the relative simplicity of the equipment required for scanning a spectrum. In addition, this technique provides information about fluorescent molecules and their environment in a wide variety of biological samples, and it is reported to be 100–1000 times more sensitive than other spectrophotometric techniques (Strasburg & Ludescher, 1995). In addition, several intrinsic fluorescent compounds (i.e., tryptophan, vitamin A, etc.) were found to be very sensitive to their environment at the molecular level (Dufour & Riaublanc, 1997). The main research and applications in the field of dairy products are usually carried out by using multivariate statistical analyses. In dairy products, fluorescence spectroscopy combined with chemometric tools has been applied in several studies to monitor structure changes in milk proteins and their physico-chemical environment during milk heating (Dufour & Riaublanc, 1997; Kulmyrzaev, Levieux, & Dufour, 2005), milk coagulation (Dufour, Lopez, Riaublanc, & Mouhous Riou, 1998; Herbert, 1999; Herbert, Riaublanc, Bouchet, Gallant, & Dufour, 1999), cheese ripening (Herbert et al., 2000; Mazerolles et al., 2001; Mazerolles, Devaux, Dufour, Qannari, & Courcoux, 2002). This technique has also been used to evaluate the quality of Danbo cheeses packaged in modified atmosphere and exposed to light for up to 84 days (Andersen, Vishart, & Holm, 2005). Finally, front face spectroscopy has been utilised to determine the geographic origin of milk (Karoui, Martin, & Dufour, 2005c), French Jura hard cheeses and Swiss Gruyère and l'Etivaz PDO cheeses (Dufour, Karoui, & Bosset, 2003; Karoui, Bosset, Mazerolles, Kulmyrzaev, & Dufour, 2005a) and of Emmental cheeses originating from different European countries, manufactured during winter and summer periods (Karoui et al., 2004a, 2004b, 2005a, 2005b, 2005c).

It is well known that riboflavin plays a key role in the photo-oxidation of dairy products. Thus, this vitamin could be considered as an indicator of early oxidation of dairy products as has been reported by Miquel Becker, Christensen, Frederiksen, and Haugaard (2003). However, few studies have been published concerning the determination of the effect of both light and modified atmosphere packaging on the degree of oxidation of riboflavin in dairy products during storage (Andersen et al., 2005; Christensen, Povlsen, & Sørensen, 2003; Miquel Becker et al., 2003; Mortensen, Sørensen, & Stapelfeldt, 2003b). In addition, all the above mentioned studies regarding the potential of front face fluorescence spectroscopy to monitor the oxidation of dairy products have been performed at the laboratory scale under extreme conditions (use of fluorescent lamp, presence or absence of O_2, CO_2, etc.) and at the retail stage. At our knowledge, no study has been performed throughout ripening of semi-hard cheese at the industrial plant. So, it would be interesting to validate the relevance of fluorescence spectra on representative cheese samples.

The objective of the current study was to evaluate the feasibility of using fluorescence spectroscopy as a nondestructive technique to monitor the oxidation at the molecular level of semi-hard cheeses, made with cow's milk and collected during both the grazing period (summer) and the stabling period (autumn), throughout ripening. In order to extract reliable information, the 400–640 nm emission fluorescence spectra (excitation = 380 nm) were recorded at the surface and the inner layers of cheeses at 2, 30 and 60 days ripening. Principal component analysis (PCA) and factorial discriminant analysis (FDA) were then applied to extract information contained in the fluorescence spectral data sets.

2. Materials and methods

2.1. Cheese samples

Twenty four different semi-hard (Raclette) cheeses manufactured during summer ($n = 12$) and autumn ($n = 12$) periods were produced from one manufacturer. The milk

samples were collected during both the grazing period (summer) and the stabling period (autumn). The Raclette cheeses (7 kg each) were manufactured using the same making procedure for the two grazing periods and ripened at the same 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 and 40 mm from the rind. The spectroscopic analyses were performed at 2, 30 and 60 days of ripening.

2.2. Fluorescence spectroscopy

Fluorescence spectra were recorded using a FluoroMax-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. The spectrofluorimeter was equipped with a thermostatically controlled cell and the temperature was controlled by a Haake temperature controller (Haake, Champlan, France). Slices of 2 cm length, 1 cm large and 0.2 cm thickness were prepared from the samples cut at the surface (20 mm from the rind) and at the centre (40 mm from the rind) layers. Spectra of cheese slices mounted between two quartz slides were recorded at 20 °C. The emission spectra (400–640 nm) were recorded with the excitation wavelength set at 380 nm. Considering these emission spectra recorded after excitation wavelength set at 380 nm was justified by the fact that they covered information related to the level of oxidation of some compounds in cheeses (i.e., riboflavin, lipid and other unknown fluorescent compounds) throughout ripening. 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 3 different slices.

2.3. Mathematical analysis of data

2.3.1. Principal component analysis

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). This mathematical treatment consists of dividing each row by the sum of the corresponding columns. In this case, mainly the shift of the peak maximum and the peak width changes in the spectra were considered following this normalisation. PCA was then applied to the normalised spectra to investigate differences between the cheese samples (Herbert et al., 2000; Jolliffe, 1986). The PCA transforms the original variables (wavelengths) into new axes, or principal components (PCs), which are orthogonal, so that the data set presented on these axes are uncorrelated with each others. 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 cheese samples (Herbert et al., 2000) reducing the dimension to two or three PCs, keeping most of the original information found in the data set.

2.3.2. Factorial discriminant analysis

FDA was performed on the first 5 PCs (which contain the whole information observed in the 400–640 emission spectral data set), resulting from the PCA applied to the emission fluorescence spectral data. The aim of this technique is to predict the membership of an individual sample to a qualitative group defined as a preliminary (Safar, Bertrand, Devaux, & Genot, 1994). A group was created for each ripening time of cheese, i.e., 2 days old, 30 days old and 60 days old. The method cannot be applied in a straightforward way to continuous spectra because of the high correlation 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 linear combinations of the selected PCs, and allows a better separation of the centres of gravity of the considered groups. The individual cheese samples can be reallocated within one of the three groups. For each cheese, the distance from the various centres 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: full cross-validation leaves out only one sample at a time; it is the original version of the method. As the number of observations was quite small (24 cheese samples), the FDA was validated by a segmented cross-validation. Segmented cross-validation leaves out a whole group of samples at a time (Karoui, Thomas, & Dufour, 2006). The PCA and FDA were performed using StatBoxPro (Grimmer Logiciels, Paris, France).

3. Results and discussion

3.1. Fluorescence spectra

It has been reported that fluorescence spectroscopy is a very sensitive technique able to measure trace substances in cheese samples containing one or more fluorescent chemical molecules (Karoui & Dufour, 2003). As most of the spectra represent very similar shapes and can therefore be very difficult to make visual discrimination, only one spectrum for samples recorded at 2, 30 and 60 days ripening is shown.

The 400–640 nm emission fluorescence spectra recorded after excitation at 380 nm at the surface (20 mm from the rind) of the investigated cheeses produced during autumn period showed some differences throughout ripening (Fig. 1). Similar results were obtained with cheeses made during summer time (data not shown). It appeared that young cheeses (2 days old) presented intense fluorescence at about 520 nm, and correspondingly low fluorescence in the violet region, while ripened cheeses (60 days old) had the lowest intensity at 520 nm and the highest one in the 405–480 nm spectral region. In addition, a slight shift to a lower wavelength (from 523 to 519 nm) was observed for cheeses of 30 and 60 days ripening, which might involve the formation of lumiflavin in these cheeses as has been reported by Wold, Jørgensen, and Lundby (2002).

From Fig. 1, three spectral regions of particular interest can be observed: the broad peak at about 520 nm is due to riboflavin as suggested by previous findings (Miquel Becker et al., 2003; Wold et al., 2002, 2005). Additionally, a significant difference in the fluorescence intensity at 522 nm between cheeses of 2 days old and those of 30 and 60 days ripening was observed ($P < 0.05$). The other region is in the range of 600–640 nm since two peaks located at 620 and 635 nm were found. These investigations were in agreement with those of Wold et al. (2005), who attributed these narrow peaks to porphyrin and chlorin compounds. The last region located between 405 and 480 nm showed some changes during the ripening time; up-to-date, 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 & Thayer, 1998). Finally, β-carotene absorbs in the region 400–500 nm. β-carotene can also undergo photodegradation (Hansen & Skibsted, 2000), which may influence the shape of riboflavin fluorescence spectra.

Regarding the 400–640 nm emission fluorescence spectra recorded at the inner layers of cheeses (40 mm from the rind), the shape of the spectra were similar to those observed at the surface layers (Fig. 2). In addition, similar results were obtained from cheeses produced during summer time (data not shown). This indicated that the fluorophores found at the surface layers were also at the inner layers of cheeses. However, differences between the spectra

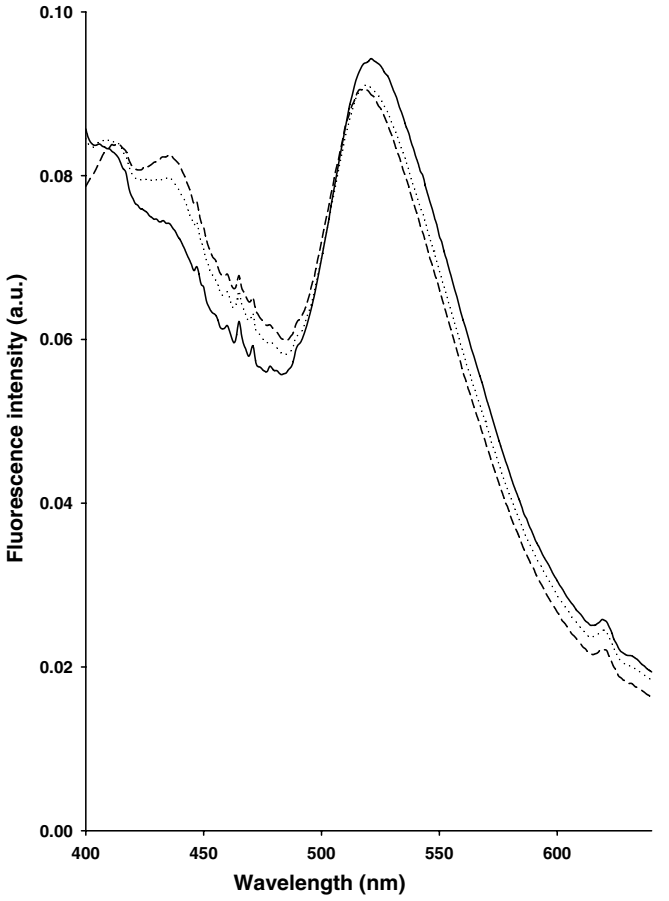

Fig. 1. Normalised fluorescence emission spectra recorded following excitation at 380 nm on the surface layers (20 mm from the rind) of cheeses produced during autumn period after 2 (—), 30 (···), and 60 (---) days ripening.

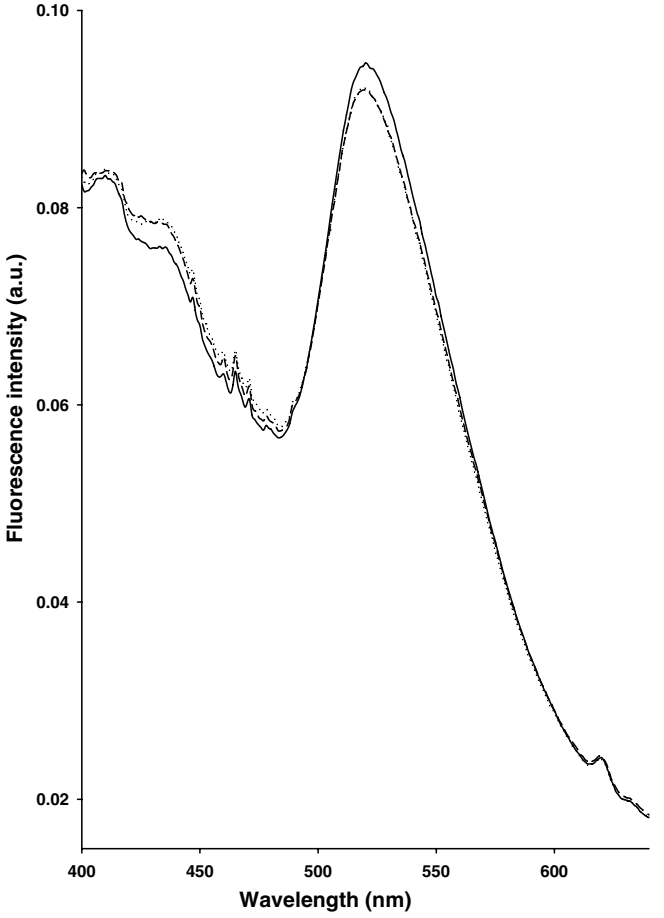

Fig. 2. Normalised fluorescence emission spectra recorded following excitation at 380 nm on the inner layers (40 mm from the rind) of cheeses produced during autumn period after 2 (—), 30 (···), and 60 (---) days ripening.

(Fig. 2) were quite slighter comparing to those observed at the surface layers (Fig. 1) of the investigated cheeses. Additionally, less fluorescence intensity was observed for cheeses recorded at the surface layers than those at the centre layers, especially for those of 30 and 60 days old. This indicate that the quantity of riboflavin at the inner layers of cheeses were higher than those at the surface, in agreement with the findings of Marsh et al. (1994) who reported that loss in riboflavin level was higher in the surface layers than that at the inner layers for cheeses exposed to sun light. This difference in the amount of riboflavin between the surface and inner layers could be explained by the fact that in dairy plants, cheeses were exposed to light with maximum radiant emission at 450–500 nm, a wavelength range most detrimental to riboflavin photodegradation (Sattar et al., 1977).

Even without any chemical reference analyses, it is possible to suggest probable reactions leading to the changes in fluorescence spectra throughout ripening. Indeed, it is well known that cheeses are sensitive to exposure to light (Deger & Ashoor, 1987); these authors reported a reduction of riboflavin content in cheddar cheeses after 12 days of storage time. This reduction was found to be in the range of 24–44% depending on the light intensity. Light induced oxidation is located in the product surface and future research should investigate the kinetics of migration of free radicals and oxidation products into the inner layers of cheeses. It is well known that when riboflavin was exposed to light, it is photochemically degraded into different forms of lumichrome and lumiflavin (Skibsted, 2000). Light with high quantum energy, e.g., lower wavelength light in the visible/UV-spectrum, has the potential for the most sever effects (Becbölet, 1990). Other fluorescent compounds could also contribute to the shapes of the 400–640 nm emission spectra scanned on cheeses during ripening. Indeed, it is well known that vitamin A has excitation maximum around 325 nm and emission maximum between 470 and 510 nm (Duggan, Bowman, Brodie, & Udenfriend, 1957; Wolfbeis, 1985). Riboflavin absorbs light between 400 and 510 nm and thus could absorb some of the light emitted by vitamin A. The same phenomenon could be observed for β-carotene which absorb in the 400–500 nm. This fluorescence transfer between a donor and acceptor could influence the shape of emission fluorescence spectra. Lipid oxidation of cheeses during ripening could also contribute to the change observed on the spectra after excitation set at 380 nm. Indeed, it has been reported that oxidised whole milk powder had an excitation maximum at 365 nm and an emission maximum of between 435 and 440 nm (Liang, 1999).

3.2. Multivariate statistical analysis of the investigated cheeses

As most of the spectra represent very similar shapes and can therefore visually hardly be distinguished, univariate analysis of fluorescence spectra, i.e., the intensity of fluorescence at one given excitation/emission wavelength pair is not appropriate. Multidimensional statistical analysis such as PCA and FDA make it possible to extract information from spectral data bases.

3.2.1. Emission fluorescence spectra recorded at the surface and inner layers of cheeses throughout ripening

PCA was performed on the spectra recorded at the surface layers of the 12 cheeses produced during summer period at 2, 30 and 60 days ripening. A total of 108 spectra (12 cheeses × 3 ripening times × 3 repetitions) were analysed. The scores scatter plot of PC1 versus PC2 of emission fluorescence spectra represented 96.3% of the total variance with a predominance of PC1 accounting for 70.6% of the total variance (Fig. 3A). Examining the two-dimensional scores plot in the space defined by the PC1 and the PC2, a good discrimination of cheese samples was observed throughout ripening. Indeed, considering the PC2 accounting for 25.7% of the total variance, cheese samples of 2 days old had positive score values, while those of 60 days old had negative score values.

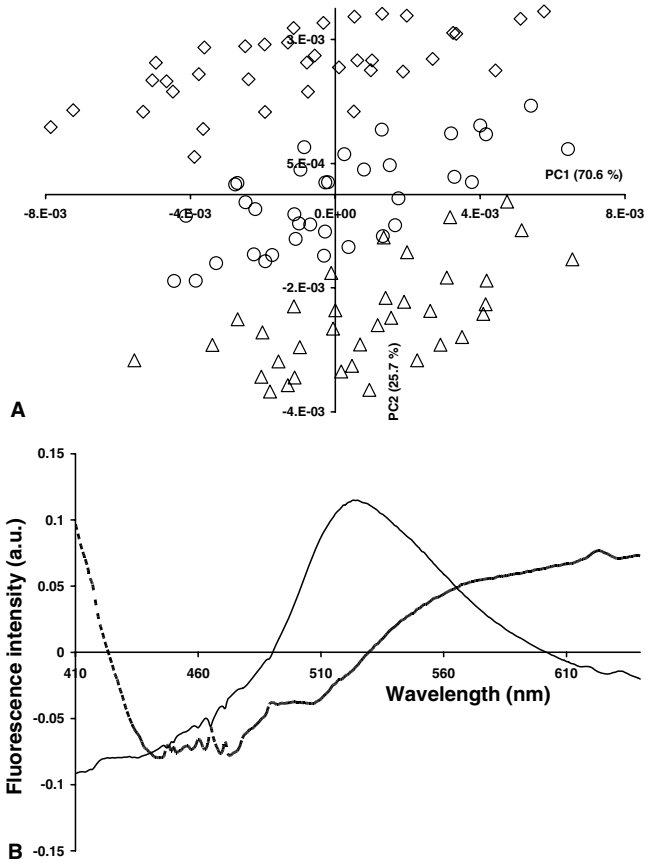

Fig. 3. Principal component analysis similarity map (score plot) determined by principal component 1 (PC1) and principal component 2 (PC2) (A) and spectral pattern (loading) corresponding to PC1 (—) and PC2 (···) (B), for emission fluorescence spectra recorded after excitation set at 380 nm at the surface layers (20 mm from the rind) of semi-hard cheeses produced during summer period after 2 (◇), 30 (○) and 60 (△) days ripening.

Spectral patterns (loadings) 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, & Riau-blanc, 1999). The spectral pattern 1 associated with the PC1 presented a positive peak around 523 nm (Fig. 3B). This peak corresponds to the riboflavin compound. Since cheese samples were essentially discriminated according to the PC2 throughout ripening, the study of the spectral pattern 2 is of particular interest. This spectral pattern exhibited a broad negative peak between 443–472 nm. These compounds were ascribed to the photodegradation of riboflavin to the lumichrome and lumiflavin products, which have been reported to exhibit emission maxima in the ranges 444–479 nm and 516–522 nm (Wold et al., 2005). The broad peak observed at 443–472 nm could also be due to the formation of fluorescent oxidation products which are due to the reaction between amino groups and aldehydes formed during the oxidation mechanism as has been stated by Wold et al. (2002). These products have been reported to present maximum emission spectra in the range of 400–470 nm after excitation range of 340–370 nm (Kikugawa & Beppu, 1987). The spectral pattern 2 exhibited also a narrow positive peak at 623 nm corresponding to porphyrin and chlorin. Similar results were found with cheeses produced during autumn period (data not shown).

In a second step, the ability of the investigated emission fluorescence spectra to differentiate cheeses throughout ripening was investigated by applying FDA on the first 5 PCs of the PCA performed on the 400–640 nm emission fluorescence spectra recorded at the surface layers. Three groups (2, 30 and 60 days old) were created for the investigated cheese samples before applying the FDA. The map of the cross-validation spectra defined by the discriminant factors 1 and 2 of the FDA is shown in Fig. 4. This figure shows a good discrimination of cheeses according to their ripening time. Indeed, considering discriminant factor 1 accounting for 99.2% of the total variance, positive score values were observed for young cheeses (2 days old), while ripened cheeses (60 days old) were observed on the left. Cheeses of 30 days ripening had co-ordinates close to the origin and were well separated from the other cheeses.

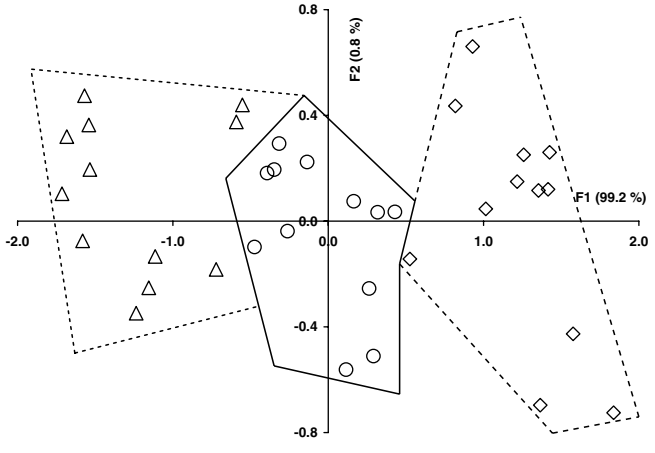

Fig. 4. Discriminant analysis similarity map of the cross-validation data sets determined by discriminant factors 1 (F1) and 2 (F2) of the factorial discriminant analysis (FDA) performed on emission fluorescence spectra recorded after excitation set at 380 nm at the surface layers (20 mm from the rind) of semi-hard cheeses produced during summer period after 2 (◇), 30 (○) and 60 (△) days ripening.

Correct classification was observed for 100% and 91.7% of the calibration and the cross-validation spectra, respectively (Table 1). Regarding the cross-validation data set, 100% correct classification was observed for spectra recorded on cheese samples of 30 days old. Considering the spectra scanned on cheese samples of 2 days old, only one spectrum was misclassified. Two spectra recorded on cheeses of 60 days old group were classified as belonging to 30 days old group. Similar results were obtained with cheeses produced during summer period since 96% and 91.7% of calibration and cross-validation spectra were correctly classified (data not shown). It was concluded that the 400–640 nm emission fluorescence spectra recorded at the surface layers following excitation set at 380 nm can be considered as a promising tool for monitoring the oxidation of cheeses throughout ripening.

In a second step, PCA was applied to the 108 spectra recorded at the inner layers (40 mm from the rind) of cheeses produced during autumn period throughout ripening. The score plots of the first two PCs show only a slight

Table 1
Classification table for semi-hard cheese samples produced during autumn period at the surface (20 mm from the rind) and inner (40 mm from the rind) layers throughout ripening based on riboflavin fluorescence calibration and cross-validation data sets collected

		Predicted[a]	Ripening time (days)		
	Real[b]	2	30	60	% correct classification
Surface layers		Calibration fluorescence spectra			
	2	24	–	–	100
	30	–	24	–	100
	60	–	–	24	100
	Total	–	–	–	100
		Cross-validation fluorescence spectra			
	2	11	1	–	91.7
	30	–	12	–	100
	60	–	2	10	83.3
	Total	–	–	–	91.7
Inner layers		Calibration fluorescence spectra			
	2	22	2	–	91.7
	30	3	10	11	41.7
	60	1	6	17	70.8
	Total	–	–	–	68.1
		Cross-validation fluorescence spectra			
	2	3	9	–	25
	30	–	1	11	8.3
	60	–	1	11	91.7
	Total	–	–	–	41.7

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

discrimination between cheese samples of 2 days old and those of 30 and 60 days old (data not shown).

FDA was then applied to the first 5 PCs of the PCA performed on the 400–640 nm emission fluorescence spectra. Correct classification was observed for 68.1% and 41.7% for the calibration and cross-validation spectra, respectively (Table 1). Considering cross-validation data set, only spectra recorded on cheeses of 60 days old were quite satisfactory classified, since 91.7% of them were correctly classified. However, the 400–640 nm emission fluorescence spectra failed to discriminate cheeses of 2 and 30 days old. Considering cheeses of 30 days old, 11 of 12 spectra were classified as belonging to 60 days old group. For spectra recorded on young cheeses (2 days old), only 25% of them were correctly classified. From the results obtained, it was shown that misclassification occurred, on the one hand, between cheeses of 2 days old and 30 days old, and on the other hand, between cheeses of 30 days old and 60 days old. No misclassification was observed between young cheeses (2 days old) and ripened cheeses (60 days old). Throughout ripening, it seemed that riboflavin component was to be primarily affected by oxygen and light as reported by Marsh et al. (1994). The physico-chemical modification that takes place during ripening could have an effect but seemed to be smaller than those of light and oxygen. The poor results obtained from spectra scanned at the inner layers of the investigated cheeses showed that the 400–640 nm emission spectra could not be used as a powerful tool for monitoring the oxidation of the investigated cheeses throughout ripening, which is not the case for those recorded at the surface layers.

It is well known that riboflavin is generally bound to proteins. Riboflavin binding protein – a monomeric, two-domain protein – has been originally purified from hens egg white (Wasylewski, 2000). In plasma, it has been shown that riboflavin is bound to proteins, predominantly albumin, but also to immunoglobulins. Like others 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 group for the oxidation in dairy products, as well as an indicator group for protein conformation and interaction changes in cheese matrix during ripening since it interacts with proteins. This was not clearly found in this research for cheeses cut at the inner layers and further research will be needed during the ripening of semi-hard cheeses.

3.2.2. Global analysis of the emission fluorescence spectral data sets recorded on cheeses during autumn and summer periods

Although many factors influence the final quality of semi-hard cheeses i.e., milk origin, milk treatment, season, type and amount of starter added, manufacture conditions, ripening time and temperature, the potential of the 400–640 nm emission fluorescence spectra to discriminate cheese samples produced during autumn and summer periods at the surface layers throughout ripening was assessed. The 216 spectra recorded on the 24 cheeses produced during summer and autumn periods at the surface layers were therefore pooled into one matrix and PCA was applied to this new table. The map defined by the two first PCs showed a good discrimination between young cheeses (2 days old) and those of 30 and 60 days old (data not shown).

In a second step, FDA was applied to the first 5 PCs of the PCA. Three groups were created independently of their manufacturing periods as explained herein above (2, 30 and 60 days ripening). The cross-validation map defined by discriminant factors 1 and 2 represented 100% of the total variance with discriminant factor 1 accounting for 98.5% of the total variance (Fig. 5). Considering discriminant factor 1, cheeses of 2 days old were observed on the far right, whereas those of 60 days old were located on the far left. Again, cheeses of 30 days old exhibited co-ordinates close to the origin. Similar results were observed for the calibration data sets (data not shown).

Correct classification was observed for 93.7% and 90.3% of the calibration and cross-validation spectra, respectively. The obtained results showed that the 400–640 nm emission fluorescence spectra can be used as an indicator for monitoring the oxidation that occurred at the surface layers of the investigated cheeses throughout ripening. Table 2 gives the classification of the calibration and cross-validation spectra for the three groups. For the cross-validation data sets, this table illustrates 95.8% correct classification for spectra recorded on cheeses of 2 and 30 days old. Considering cheeses of 60 days old, five spectra were classified as belonging to 30 days old group. Again, it is noteworthy that no misclassification was occurred between spectra recorded on young (2 days old) and matured (60 days) cheeses in the calibration and cross-validation data sets.

The same approach was applied to the inner layers of the 24 cheeses. The FDA applied to the first 5 PCs did not allow a good discrimination of cheeses throughout ripening.

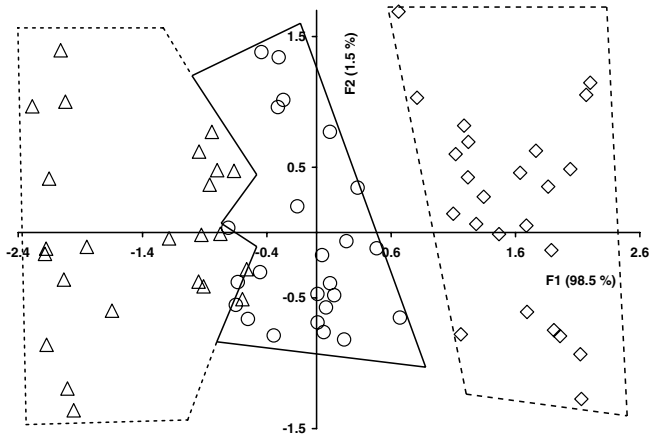

Fig. 5. Discriminant analysis similarity map of the cross-validation data sets determined by discriminant factors 1 (F1) and 2 (F2) of the factorial discriminant analysis (FDA) performed on emission fluorescence spectra recorded after excitation at 380 nm at the surface layers (20 mm from the rind) of semi-hard cheeses produced during summer and autumn periods after 2 (◇), 30 (○) and 60 (△) days ripening.

Table 2
Classification table for semi-hard cheese samples produced during autumn and summer periods at the surface (20 mm from the rind) and inner (40 mm from the rind) layers throughout ripening based on riboflavin fluorescence calibration and cross-validation data sets

		Predicted[a]	Ripening time (days)		
	Real[b]	2	30	60	% correct classification
Surface layers		Calibration fluorescence spectra			
	2	44	4	–	91.7
	30	–	48	–	100
	60	–	5	43	89.6
	Total	–	–	–	93.7
		Cross-validation fluorescence spectra			
	2	23	1	–	95.8
	30	1	23	–	95.8
	60	–	5	19	79.2
	Total	–	–	–	90.3
Inner layers		Calibration fluorescence spectra			
	2	38	7	3	79.2
	30	6	28	14	58.3
	60	1	16	31	64.6
	Total	–	–	–	67.4
		Cross-validation fluorescence spectra			
	2	19	2	3	79.2
	30	8	10	6	41.7
	60	2	6	16	66.7
	Total	–	–	–	62.5

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

Indeed, only a slight discrimination of young cheeses (2 days old) from those of 30 and 60 days old was observed. Cheeses of 30 and 60 days ripening were confused on the map. This was confirmed on the percentage of spectra correctly classified. Indeed, only 67.4% and 62.5% correct classification was observed for the calibration and cross-validation spectra, respectively. Misclassification was observed between spectra recorded on cheeses of 2, 30 and 60 days old. The obtained results show again that the 400–640 nm emission fluorescence spectra scanned after excitation wavelength set at 380 nm cannot be considered as a valuable tool for monitoring the oxidation process at the inner layers of the investigated cheeses throughout ripening.

4. Conclusion

This study demonstrated that the 400–640 nm emission fluorescence spectra recorded at the surface layers of semi-hard cheeses (Raclette) following excitation set at 380 nm, produced during summer and autumn periods, could be used as a useful probe for monitoring the oxidation of cheeses throughout ripening. The oxidation process seemed to be primarily affected by both oxygen and light independently of the production season of cheeses. Indeed, correct classification of 93.7% and 90.3% of the calibration and cross-validation data sets, respectively was observed for cheese samples cut at the surface layers and produced during summer and winter periods. However, the 400–640 nm emission spectra failed for monitoring oxidation process in cheeses samples cut at the inner layers throughout ripening. The validity of the obtained results still remains to be tested on a larger set of samples covering a more variety of cheeses.

Front-face fluorescence spectroscopy has the potential to dramatically reduce analytical time and cost of traditional measurement used for the evaluation of light induced oxidation. As the 400–640 nm emission fluorescence spectra are located in the visible range, its measurement may be a valuable and cheap method based on marketed laptop spectrofluorimeter running in the visible range for monitoring the oxidation process of cheeses at the surface layers throughout ripening.

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References

Andersen, C. M., Vishart, M., & Holm, V. K. (2005). Application of fluorescence spectroscopy in the evaluation of light-induced oxidation in cheese. *Journal of Agricultural and Food Chemistry, 53*, 9985–9992.

Becbölet, M. (1990). Light effects on food. *Journal of Food Protection, 53*, 430–440.

Bertrand, D., & Scotter, C. N. G. (1992). Application of multivariate analyses to NIR spectra of gelatinized starch. *Applied Spectroscopy, 46*, 1420–1425.

Bosset, J. O., Sieber, R., & Gallman, P. U. (1994). Influence of light transmittance of packaging materials on the shelf life of milk and milk products – a review. In M. Mathlouthi (Ed.), *Food packaging and preservation* (pp. 222–268). Glasgow: Blackie Academic and Professional.

Cataldi, T. R. I., Nardiello, D., Carrara, V., Ciriello, R., & De Benedetto, G. E. (2003). Assessment of riboflavin and flavin content in common food samples by capillary electrophoresis with laser-induced fluorescent detection. *Food Chemistry, 82*, 309–314.

Christensen, J., Povlsen, V. T., & Sørensen, J. (2003). Application of fluorescence spectroscopy and chemometrics in the evaluation of processed cheese during storage. *Journal of Dairy Science, 86*, 1101–1107.

Deger, D., & Ashoor, S. H. (1987). Light-induced changes in taste, appearance, odor, and riboflavin content of cheese. *Journal of Dairy Science, 70*, 1371–1376.

Dufour, E., Karoui, R., & Bosset, J. O. (2003). Utilisation de la fluorescence frontale intrinsèque de fromages de type L'Etivaz AOC et Gruyère AOC pour reconnaître leur origine géographique. *Travaux de Chimie Alimentaire et d'Hygiène, 94*, 379–393.

Dufour, E., Lopez, C., Riaublanc, A., & Mouhous Riou, N. (1998). La spectroscopie de fluorescence frontale: une approche non invasive de la structure et des interactions entre les constituants des aliments. *Agoral, 10*, 209–215.

Dufour, E., Subirade, M., Loupil, F., & Riaublanc, A. (1999). Whey proteins modify the phase transition of milk fat-globule phospholipids. *Le Lait, 79*, 217–228.

Dufour, E., & Riaublanc, A. (1997). Potentiality of spectroscopic methods for the characterisation of dairy products. I. Front-face fluorescence study of raw, heated and homogenised milks. *Le Lait, 77*, 657–670.

Duggan, D. E., Bowman, R. L., Brodie, B. B., & Udenfriend, S. (1957). A spectrophotometric study of compounds of biological interest. *Archives of Biochemistry and Biophysics, 68*, 1–14.

Esteve, M.-J., Farré, R., Frígola, A., & García-Cantabella, J.-M. (2001). Simultaneous determination of thiamin and riboflavin in mushrooms by liquid chromatography. *Journal of Agricultural and Food Chemistry, 49*, 1450–1454.

Fanelli, A. J., Burlew, J. V., & Gabriel, M. K. (1985). Protection of milk packaged in high density polyethylene against photodegradation by fluorescent light. *Journal of Food Protection, 48*, 112–117.

Fellman, R. L., Dimick, P. S., & Hollender, R. (1991). Photo-oxidative stability of vitamin A fortified 2% low fat milk and skim milk. *Journal of Food Protection, 52*, 113–116.

Fox, J. B., & Thayer, D. W. (1998). Radical oxidation of riboflavin. *International Journal of Vitamin Research, 68*, 174–180.

Hansen, E., & Skibsted, L. H. (2000). Light-induced oxidative changes in a model dairy spread. Wavelength dependence of quantum yields. *Journal of Agricultural and Food Chemistry, 48*, 3090–3094.

Herbert, S. (1999). Caractérisation de la structure moléculaire et microscopique de fromages à pâte molle. Analyse multivariée des données structurales en relation avec la texture, thesis, Ecole Doctorale Chimie Biologie de l'Université de Nantes, France.

Herbert, S., Mouhous Riou, N., Devaux, M. F., Riaublanc, A., Bouchet, B., Gallant, J. D., et al. (2000). Monitoring the identity and the structure of soft cheeses by fluorescence spectroscopy. *Le Lait, 80*, 621–634.

Herbert, S., Riaublanc, A., Bouchet, B., Gallant, D. J., & Dufour, E. (1999). Fluorescence spectroscopy investigations of acid- and rennet-induced milk coagulation of milk. *Journal of Dairy Science, 82*, 2056–2062.

Jolliffe, I. T. (1986). *Principal component analysis*. New York: Springer.

Karoui, R., Bosset, J. O., Mazerolles, G., Kulmyrzaev, A., & Dufour, E. (2005a). Monitoring the geographic origin of both experimental french jura hard cheeses and Swiss Gruyère and L'etivaz PDO cheeses using mid-infrared and fluorescence spectroscopies. *International Dairy Journal, 15*, 275–286.

Karoui, R., & Dufour, E. (2003). Dynamic testing rheology and fluorescence spectroscopy investigations of surface to centre differences in ripened soft cheeses. *International Dairy Journal, 13*, 973–985.

Karoui, R., Dufour, E., Pillonel, L., Picque, D., Cattenoz, T., & Bosset, J. O. (2004a). Determining the geographic origin of Emmental cheeses produced during winter and summer using a technique based on the concatenation of MIR and fluorescence spectroscopic data. *European Food Research and Technology, 219*, 184–189.

Karoui, R., Dufour, E., Pillonel, L., Picque, D., Cattenoz, T., & Bosset, J. O. (2004b). Fluorescence and infrared spectroscopies: a tool for the determination of the geographic origin of Emmental cheeses manufactured during summer. *Le Lait, 84*, 359–374.

Karoui, R., Dufour, E., Pillonel, L., Schaller, E., Picque, D., Cattenoz, T., et al. (2005b). The potential of combined infrared and fluorescence spectroscopies as a method of determination of the geographic origin of Emmental cheeses. *International Dairy Journal, 15*, 287–298.

Karoui, R., Martin, B., & Dufour, E. (2005c). Potentiality of front-face fluorescence spectroscopy to determine the geographic origin of milks from Haute-Loire department (France). *Le Lait, 85*, 223–236.

Karoui, R., Thomas, E., & Dufour, E. (2006). Utilization of rapid technique based on front-face fluorescence spectroscopy for differentiating between fresh and frozen-thawed fish fillets. *International Food Research, 39*, 349–355.

Kikugawa, K., & Beppu, M. (1987). Involvement of lipid oxidation products in the formation of fluorescent and cross-linked proteins. *Chemistry and Physics of Lipids, 44*, 277–296.

Kulmyrzaev, A., Levieux, D., & Dufour, E. (2005). Front-face fluorescence spectroscopy allows the characterization of mild heat treatment applied to milk. Relations with the denaturation of milk proteins. *Journal of Agricultural and Food Chemistry, 53*, 502–507.

Liang, J. H. (1999). Use of intrinsic fluorescence in the measurement of oxidative deterioration in milk powders. *Food Science and Agriculture Chemistry, 1*, 250–256.

Marsh, R., Kajda, P., & Ryley, J. (1994). The effect of light on the vitamin B_2 and the vitamin A content of cheese. *Nahrung, 38*, 527–532.

Mavattivi, F., Monetti, A., Vrhovšeket, U., Tonon, D., & Andrés-Lacueva, C. (2000). High-performance liquid chromatographic determination of the riboflavin concentration in white wines for predicting their resistance to light. *Journal of Chromatography A, 888*, 121–127.

Mazerolles, G., Devaux, M. F., Duboz, G., Duployer, M. H., Riou, N., & Dufour, E. (2001). Mouhous infrared and fluorescence spectroscopy for monitoring protein structure and interaction changes during cheese ripening. *Le Lait, 81*, 509–527.

Mazerolles, G., Devaux, M. F., Dufour, E., Qannari, E. M., & Courcoux, Ph. (2002). Chemometric methods for the coupling of spectroscopic techniques and for the extraction of the relevant information contained in the spectral data tables. *Chemometrics and Intelligent Laboratory Systems, 63*, 57–68.

Miquel Becker, E., Christensen, J., Frederiksen, C. S., & Haugaard, V. K. (2003). Front-face fluorescence spectroscopy and chemometrics in analysis of yogurt: rapid analysis of riboflavin. *Journal of Dairy Science, 86*, 2508–2515.

Mortensen, G., Sørensen, J., & Stapelfeldt, H. (2003b). Effect of modified atmosphere packaging and storage conditions on photooxidation of sliced Havarti cheese. *European Food Research and Technology, 216*, 57–62.

Ollilainem, V., Mattila, P., & Varo, P. (1990). The HPLC determination of total riboflavin in foods. *Journal of Micronutritional Analysis, 8*, 199–207.

Reyes, E. S. P., Norris, K. M., Taylor, C., & Potts, D. (1988). Comparison of paired ion liquid chromatographic method with AOAC fluorimetric and microbiological methods for riboflavin determination in selected foods. *Journal of the Association of Official Analytical Chemists, 71*, 16–19.

Ribarova, F., Shishkov, S., Obretenova, N., & Metchkneva, L. (1987). Comparative determination of riboflavin in milk HPLC and lumiflavin methods. *Die Nahrung, 31*, 77–79.

Russell, L. F., & Vanderslice, J. T. (1992). Non-degradative extraction and simultaneous quantitation of riboflavin, flavin mononucleotide, and flavin adenine dinucleotide in foods by HPLC. *Food Chemistry, 43*, 151–162.

Safar, M., Bertrand, P. R., Devaux, M. F., & Genot, C. (1994). Characterization of edible oils, butters, and margarines by Fourier transform infrared spectroscopy with attenuated total reflectance. *Journal of the Association of Official Analytical Chemists, 71*, 371–377.

Sattar, A., de Man, J. M., & Alexander, J. C. (1977). Light induced degradation of vitamins. I. Kinetic studies on riboflavin decomposition in solution. *Canadian Institute of Food Science and Technology Journal, 10*, 61–64.

Singleton, J. A., Aurand, W., & Lancaster, F. W. (1963). Sunlight flavor in milk. I. A study of components involved in the flavor development. *Journal of Dairy Science, 46*, 1051–1611.

Skibsted, L. H. (2000). Light induced changes in dairy products. *BIDFD, 346*, 4–9.

Strasburg, G. M., & Ludescher, R. D. (1995). Theory and application of fluorescence spectroscopy in food research. *Trends in the Food Science and Technology, 6*, 69–75.

Van den Berg, H., Van Schaik, F., Finglas, P. M., & de Froidmont- Görtz, I. (1996). Third EU MAT intercomparison on methods for the determination of vitamins B-1, B-2 and B-6 in food. *Food Chemistry, 57*, 101–108.

Vidal-Valverde, C., & Reche, A. (1990). Reliable system for the analysis of riboflavin in foods by high performance liquid chromatography and UV detection. *Journal of Liquid Chromatography, 13*, 2089–2101.

Wasylewski, M. (2000). Binding study of riboflavin-binding protein with riboflavin and its analogues by differential scanning calorimetry. *Journal of Protein Chemistry, 19*, 523–528.

Wold, J. P., Jørgensen, K., & Lundby, F. (2002). Non-destructive measurement of light-induced oxidation in dairy products by fluorescence spectroscopy and imaging. *Journal of Dairy Science, 85*, 1693–1704.

Wold, J. P., Veberg, A., Nilsen, A., Iani, V., Juzenas, P., & Moan, J. (2005). The role of naturally occurring chlorophyll and porphyrins in

light-induced oxidation of dairy products. A study based on fluorescence spectroscopy and sensory analysis. *International Dairy Journal, 15*, 343–353.

Wolfbeis, O. S. (1985). Fluorescence of organic natural products. In S. G. Schulman (Ed.), *Molecular luminescence spectroscopy* (pp. 167–370). New York: Wiley.