Fluorescence Spectroscopy and Chemometrics in the Food Classification – a Review

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Abstract

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This review deals with the last few years' articles on various fluorescence techniques (conventional, excitation-emission matrix, and synchronous fluorescence spectroscopy) as a tool for the classification of food samples. Chemometric methods as principal component analysis, hierarchical cluster analysis, parallel factor analysis, and factorial discriminate analysis are briefly reminded. The respective publications are then listed according to the food samples: dairy products, eggs, meat, fish, edible oils, and others.

Keywords: chemometrics; fluorescence spectroscopy; food analysis

Fluorescence spectroscopy is a rapid, sensitive, and non-destructive analytical technique providing in a few seconds spectral signatures that can be used as fingerprints of the food products (dairy products, fishes, edible oils, wines, etc.). The application of fluorescence in food analysis has increased during the last decade, probably due to the propagated use of chemometrics. The study by Norgaard (1995) can serve as a general investigation of how to enhance the potential of fluorescence spectroscopy by chemometrics.

Since fluorescence spectra are typically composed of broad overlapping fluorescence bands containing chemical, physical, and structural information of all sample components, the analytical information contained in spectra is multivariate in nature and, therefore, non-selective. In addition, differences between the samples may cause very slight spectral differences that are difficult to distinguish. The analytical information contained in fluorescence spectra can be extracted by using various multivariate analysis techniques that relate several analytical variables to the properties of the analyte(s). The multivariate techniques most frequently used allow to group the samples with similar characteristics, to establish classification methods for unknown samples (qualitative analysis) or to perform methods determining some properties of unknown samples (quantitative analysis).

In the following sections, mathematical data pretreatments most frequently used and their specific purpose, reduction of variables with principal component analysis, multivariate classification techniques for qualitative analysis, and multivariate calibration methods for quantitative analysis are discussed. The applications are then listed according to the food samples: dairy products, eggs, fishes, edible oils, and miscellaneous.

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CHEMOMETRICS

Pretreatment of spectra

In conventional fluorescence spectroscopy, two basic types of spectra are usually measured. When a sample is excited at a fixed wavelength λex, an emission spectrum is produced by recording the emission intensity as a function of the emission wavelength λem. An excitation spectrum may be obtained when λex is scanned while the observation is conducted at a fixed λem. In food analysis, the emission spectra at a particular λex are typically studied. When a set of emission spectra at different λex is recorded, a three-dimensional landscape is obtained, the so-called fluorescence excitation-emission matrix (EEM). Recording EEMs (total excitation-emission matrix luminescence spectroscopy) enables to obtain more information about the fluorescent species present in the sample, because the bands arising in the wider axes are considered. The broad nature of conventional fluorescence spectrum and spectral overlap can be overcome and enhanced selectivity can be obtained using synchronous fluorescence scan (SFS). In SFS, the λex and λem are scanned simultaneously (synchronously), usually maintaining a constant wavelength interval, Δλ, between λex and λem. Besides the spectral overlap, the inner-filter effect, scattered light, and reflected light can also limit the applicability of conventional right-angle fluorescence spectroscopy (Figure 1) under certain conditions, e.g. high concentrations of the fluorescent species. To avoid these problems, the method of front-face fluorescence spectroscopy (Figure 1) can be used for bulk liquid samples and solid samples (Parker 1968; Hirschfeld 1978; Eisinger & Flores 1979; Blumberg *et al.* 1980; Hirsch & Nagel 1989; Genot *et al.* 1992a, b; Sóti *et al.* 1993; Herbert *et al.* 2000; Patra & Mishra 2002). The incidence angle of the excitation radiation is usually set at 56° (Table 1).

Often, several spectra of the same sample are recorded to verify reproducibility, and the average of those spectra is computed and used afterward. No special smoothing algorithms are needed when the multivariate data approach is used (Norgaard

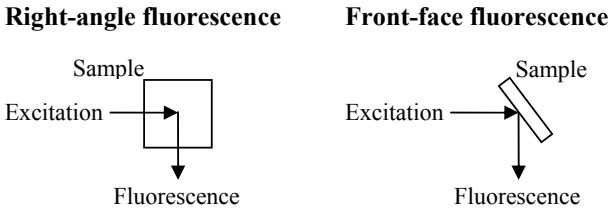

Figure 1. Right-angle and front-face fluorescence spectroscopy

Table 1. Fluorescence and multivariate analysis techniques in food classification

Food sample	Fluorescence technique	Multivariate analysis technique	References
Semi-hard cheese	front-face (56°)	PCA, FDA	Karoui & Dufour (2006)
	front-face (56°)	CCSWA	Karoui *et al.* (2006a)
Soft cheese	front-face (52°)	PCA, CCSWA	Karoui *et al.* (2007a)
	front-face (56°)	PCA, FDA, CCA	Karoui *et al.* (2003)
Eggs	front-face (22.5°)	PCA, FDA	Karoui *et al.* (2006d)
Fish	front-face (56°)	PCA, FDA	Karoui *et al.* (2006e)
Meat	front-face (180°), EEM	PCA, PLS, PARAFAC	Møller *et al.* (2003)
Olive oils	right-angle, EEM	PCA, PARAFAC	Guimet *et al.* (2004)
	right-angle, EEM	PCA, PARAFAC, LDA, PLSR	Guimet *et al.* (2005)
	right-angle, EEM	NMF, PARAFAC, LDA, PLSR	Guimet *et al.* (2006)
	right-angle, EEM, SFS	PCA, HCA	Poulli *et al.* (2005)
Vegetable oils	right-angle, EEM, SFS	kNN, LDA	Sikorska *et al.* (2005)
Honey	front-face (56°)	PCA, FDA	Karoui *et al.* (2007b)
Wines	front-face (56°)	PCA, FDA	Dufour *et al.* (2006)
Beers	right-angle, EEM	PCA, kNN, LDA	Sikorska *et al.* (2006)

1995). Obviously raw spectra are used as acquired in right-angle geometry without any pretreatment. Front-face fluorescence spectra are normalised by reducing the area under each spectrum to the value of 1 in order to reduce scattering effects and to delete the differences between the spectra due to different amounts of sample (Bertrand & Scotter 1992). This mathematical procedure 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 are considered. First or second derivative algorithm is sometimes tested to enhance differences between spectra and to resolve the overlapping bands (Sikorska *et al*. 2006).

Reduction of variables by principal component analysis

There is a need for variable-reduction methods because of the vast amount of spectral information provided by fluorescence spectrophotometers, the substantial number of samples required to construct classification and calibration models, and the high number of correlated variables in the spectral data. The best known and most widely used variable-reduction method is the principal component analysis (PCA). PCA is a mathematical procedure which decomposes the data matrix with n rows (samples) and p columns (variables, e.g. wavelengths) into the product of a scores matrix, with n rows (samples) and $d < p$ columns (principal components, PCs), and a loadings matrix, with $d < p$ rows (principal components, PCs) and p columns (variables). The scores are the positions of the samples in the space of the principal components and the loadings are the contributions of the original variables to the PCs. All PCs are mutually orthogonal, and each successive PC contains less of the total variability of the initial data set. Usually only a limited number $d < p$ of PCs are retained as the variability in the others is due to noise. This reduces the dimensionality of the data considerably, enabling effective visualisation, classification, and regression of multivariate data (Geladi 2003).

Multivariate classification for qualitative analysis

In qualitative analysis, the sample properties that have to be related to spectral variations have discrete values that represent the product identity or the product quality, e.g. "original" or "blend", "fresh" or "non-fresh". To solve the selectivity and interference problems of fluorescence spectra, multivariate classification methods are used for grouping the samples with similar discrete values (characteristics).

Multivariate classification methods, also known as pattern-recognition methods, are subdivided in "supervised" and "non-supervised" learning algorithms, depending on whether or not the class to which the samples belong is known (Geladi 2003).

"Non-supervised" methods

"Non-supervised" methods, also known as exploratory methods, do not require any *a priori* knowledge about the group structure in the data, but instead produce the grouping, i.e. clustering, them selves. This type of analysis is often very useful at an early stage of the investigation to explore subpopulations in a data set, e.g. different freshness of a product. Cluster analysis can be performed with simple visual techniques, such as hierarchical cluster analysis (HCA) or PCA. HCA involves the assessment of similarity between the samples based on their measured properties (variables). The samples are grouped in clusters in terms of their nearness in the multidimensional space. The results are presented in the form of dendograms to facilitate the visualisation of the sample relationships (Poulli *et al.* 2005). PCA is usually the first step in fluorescence data exploration (Table 1). The main goal of PCA is to find relationships between different parameters (samples and variables) and/or the detection of possible clusters within the samples and/or variables. PCA performed on fluorescence spectra makes it possible to draw the similarity maps of the samples and to get the spectral patterns. The spectral patterns corresponding to the principal components provide information about the characteristic peaks which are the most discriminating for the samples observed on the maps. While the similarity maps allow the comparison of the spectra in such a way that two neighbouring points represent two similar spectra, the spectral patterns exhibit the fluorescence bands that explain the similarities observed on the maps.

In most of the fluorescence studies, bilinear model as two-way PCA has been used, i.e., PCA is

applied separately to each excitation or emission wavelength, where it gives one score matrix and one leading matrix for each excitation or emission wavelength. A trilinear model PARAFAC (parallel factor analysis) ideally decomposes the fluorescence landscapes (excitation emission matrices) presented in a three-way array into trilinear components according to the number of fluorophores present in the samples (Bro 1997). PARAFAC gives one score matrix and two leading matrixes. The retrieved scores and loadings can then be directly related to the relative concentrations (scores) and the fluorescence characteristics of the present fluorophores. Thus, the excitation and emission loadings can be used in interpretation and identification of the fluorescence phenomena found. It means that, where PCA gives the abstract latent variables, PARAFAC produces the true understanding phenomena (Munck *et al.* 1998).

In some cases, non-negative matrix factorisation (NMF) may be more suitable than PCA. With NMF, only positive solutions (values) can be obtained and thus this method provides a more realistic approximation to the original data than PCA that allows positive and negative values (Guimet *et al*. 2006).

The complexity of the food products can lead to collecting several (chemical, microbiological, etc.) data tables on the same samples. For exploring all these data sets, it is common to perform a multidimensional analysis (PCA, PARAFAC) on each data table and thereafter sum up the conclusions thus obtained. Other authors propose to run a multidimensional analysis on a data table obtained by merging all of them in one matrix. These two approaches obviously can not allow studying the relationships among the collected data tables.

The relations between the information collected by the various methods have been described by two methods, common components and specific weights analysis (CCSWA) and canonical correlation analysis (CCA). The goal of CCA is to find the maximal correlation between the chosen linear combination of the first set of variables and the chosen linear combination of the second set of variables. Maximally correlated pairs of variables may then be identified with linear combinations and are called the canonical variables. CCSWA consists of determining the common space of representation for all the data sets. Each table has a specific weight associated with each dimension for this common space. This information is not given by CCA (Mazerolles *et al*. 2002).

"Supervised" methods

In the "supervised" methods (also known as discriminant analysis), each spectrum is formerly assigned to a definite class, so a qualitative data is added to the quantitative spectral data. For this purpose, comprehensive libraries of spectra that represent the natural variation of each product have to be constructed in a calibration process. Most of the classification methods can operate either in the wavelength space or in a dimension-reduced factor space. Principal component or partial least squares (PLS) analyses are often applied to spectral datasets prior to discriminant analysis as tools for dataset size and co-linearity reduction. Spectral data are then treated to highlight the relation between these data and the class considered. Various methods can be used, like linear discriminant analysis (LDA) (Sikorska *et al*. 2005), factorial discriminate analysis (FDA) (Karoui & Dufour 2003), or K-nearest neighbours (kNN) (Sikorska *et al*. 2005). In any case, the purpose is to form weighted linear combinations of the data to minimise within class variance and to maximise between class variance. The distance between classes characterises the partition obtained. The validity of the method can be verified by comparison of the distances. The distances between classes means have to be clearly superior to the distances within classes. Another way to validate the discrimination is to test it. If the samples studied are numerous enough, they can be separated into two sets: a training set to elaborate the method (calibration), and a test set to validate it. The classification rules are later used for allocating new or unknown samples to the most probable subclass.

In fluorescence spectrometry, factorial discriminate analysis (FDA) is usually the second step in mathematical analysis of the data (Table 1). 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. Thus, in the first step, step-wise discriminant analysis is performed to select the most relevant PCs for the discrimination of variables following the qualitative classes initially defined. From the selected variables, 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 classes considered. The individuals can be reallocated within various classes. For each individual, the distance from the various centres of gravity of a class is calculated. The individual is assigned to the class where the distance between the centres of gravity is the shortest. The comparison of the assigned class and the real class is an indicator of the quality of discrimination. Similarity maps and patterns can be drawn, in analogy to those for PCA.

Multivariate calibration for quantitative analysis

The multivariate regression methods most frequently used in quantitative fluorescence analysis are partial least-squares regression (PLSR) and principal component regression (PCR). Both can be used in specific spectral regions or the whole spectrum, and they allow more information to be included in the calibration model. PCR uses the principal components provided by PCA to perform regression on the sample property to be predicted. PLSR finds the directions of greatest variability by comparing both spectral and target property information with the new axes, called PLSR components or PLSR factors. Thus, the main difference between the two methods is that the first principal component or factor in PCR represents the widest variations in the spectrum, whereas in PLSR it represents the most relevant variations showing the best correlation with the target property values. In both cases, the optimum number of factors used to build the calibration model depends on the sample properties and the analytical target (Geladi 2003).

APPLICATIONS

Dairy products

Aromatic amino acids, nucleic acids, tryptophan residues of proteins, riboflavin, and vitamin A are the best known fluorescent molecules in dairy products.

Fluorescence spectrum recorded on a cheese sample following excitation at 290 nm and 380 nm and emission between 400–600 nm included information on several fluorophores and may be considered as a characteristic fingerprint which allows the sample to be identified (Karoui & Dufour 2003). Tryptophan residues in proteins are excited at 290 nm with maximum emission at about 345 nm. The emission of tryptophan is highly sensitive to its local environment and is thus often used as a reference group for protein structure changes, binding of ligands, and protein-protein associations. The emission fluorescence region (305–450 nm) allows the study of the fluorescent Maillard-reaction products (maximum emission at 440 nm) (Karoui *et al*. 2007a). Riboflavin has a strong and broad fluorescence emission peak in the region of 525–531 nm (excitation wavelength 380 nm). In ultraviolet light, riboflavin is degraded into two fluorescent products: lumichrome and lumiflavin with emission maxima between 444–479 nm and 516–522 nm, respectively. The reduction of the fluorescence at about 525 nm might reflect the photo-degradation of riboflavin (Andersen *et al*. 2006; Karoui *et al*. 2007b). The excitation spectra of vitamin A recorded between 250 and 350 nm with the emission wavelength set at 410 nm provide information on the development of protein fat globule interactions during milk coagulation. The shape of the vitamin A excitation spectrum was correlated with the physical state of the triglycerides in the fat globule (Karoui *et al*. 2007a).

Since the cheeses differ in their manufacturing processes, geographic locations, animal feeds and breeds, as well as the conditions of milk production, it is apparent that their structures at the molecular level and, as a consequence, the environments of the intrinsic cheese fluorophores are different.

During ripening, the main components of cheese are subject to physical and chemical changes, which determine the fluorescence properties of the final product. Young cheese (2 days old) had the highest fluorescence intensity around 520 nm, whereas 60-day old cheese had the highest intensity in the 420–480 nm spectral region and the lowest one at 520 nm (Karoui *et al*. 2007b). As a result of lumiflavin formation, a blue shift around 520 nm is observed for cheeses of 30 and 60 days ripening (Wold *et al*. 2002). Lipid oxidation of cheese throughout ripening can also contribute to the change observed in the spectra after excitation set at 380 nm. The shift of the tryptophan maximum emission to larger wavelength range of the ripened cheese (60 days ripening) can be explained by the exposing of more tryptophan residues to the aqueous phase. The excitation spectra of vitamin A showed the greatest difference during cheese ripening. This high sensitivity was attributed to the change in the environment, solvent viscosity,

and the physical state of triglycerides in the fat globules (Karoui *et al.* 2006a).

Due to the complexity of cheese ripening, several data tables can be recorded on the same sample. All the data sets are then analysed by chemometric methods like PCA, FDA or CCSWA.

The emission fluorescence spectra (400–640 nm) following excitation at 380 nm recorded at the surface layers of semi-hard cheese, produced during summer and autumn periods, can be used as a useful probe for monitoring the oxidation of cheese throughout ripening (2, 30, and 60 days). PCA and FDA were applied to extract the information contained in the fluorescence spectra. Correct classification of 93.7% and 90.3% of the calibration and cross-validation data sets, respectively, was observed for the cheese samples cut at the surface layers and produced during summer and winter periods. In the inner layers of cheese, only 67.4% and 62.5% correct classification was observed for the calibration and cross-validation spectra, respectively (Karoui *et al.* 2007b).

Recently, spectroscopic techniques coupled with CCSWA were used as an accurate tool to monitor the molecular changes that occur in cheese throughout ripening (Mazerolles *et al.* 2002, 2006; Kulmyrzaev *et al.* 2005). The CCSWA showed its ability to describe the overall information collected from fluorescence and physico-chemical data tables and to extract relevant information at the molecular level throughout ripening of semi-hard cheese. Tryptophan, riboflavin, and vitamin A fluorescence spectra were scanned on 12 cheeses on 2, 30, and 60 days of ripening. From the CCSWA performed on four data sets, it appeared that the relationships between all these tables led to the formation of two common components which allowed a global characterisation of the various brand cheese products throughout ripening (Karoui *et al.* 2006a).

Fluorescence spectroscopy can provide useful fingerprints, allowing the identification of cheese according to their manufacturing processes and sampling zones. The discrimination between the sampling zones and manufacturing processes were better with the vitamin A than with the tryptophan or riboflavin fluorescence spectra. Tryptophan, riboflavin, and vitamin A fluorescence spectra were recorded in two sampling zones (external and central) of 15 retail soft cheeses, for which the manufacturing processes were different. The 15 cheeses were discriminated using PCA or CCSWA. Using the PCA performed separately on each of the probe data sets, the best result was obtained from the vitamin A fluorescence spectra. CCSWA was then applied to the tryptophan, riboflavin, and vitamin A fluorescence spectra. The results showed that the CCSWA methodology allowed the use of all the spectroscopic information given by the three intrinsic probes in a very efficient way. The spectral patterns allowed information on the protein structure, protein-protein and protein-fat globule interactions, and the degree of riboflavin degradation to be derived at the molecular level (Karoui *et al.* 2007a).

The quality parameters of cheese such as rheological properties may be derived from the fluorescence data (Kulmyrzaev *et al.* 2005). Changes at the molecular and macroscopic levels of ripened soft-cheese samples, for which the manufacturing process was varied, were studied from the surface to the centre of the cheese using dynamic low amplitude strain rheology and front-face fluorescence spectroscopy. Protein tryptophan emission spectra and vitamin A excitation spectra were recorded in samples cut from the surface to the centre. The values of storage modulus (G'), loss modulus (G''), tan (δ), and strain were determined for all the samples. For each cheese, the data sets containing fluorescence spectra and rheology values were analysed by PCA, FDA, and CCA. The discriminant ability of the data was investigated by applying FDA to the first 10 PCs of the PCA performed on the vitamin A and tryptophan fluorescence spectra. From the tryptophan fluorescence data sets, 94% and 87.7% correct classifications were observed for the calibration and validation groups, respectively. A better classification (100% and 96% for the calibration and the validation groups) was obtained from the vitamin A spectra. Correlations between the rheological data (G') and the fluorescence spectra (tryptophan, vitamin A) were considered to improve the understanding of the relationship between the shape of fluorescence spectra and rheology characteristics of cheese. CCA was performed on tryptophan spectra/rheology data and on vitamin A spectra/rheology data. It appeared that tryptophan fluorescence spectra and vitamin A spectra were highly correlated with the rheological properties. These correlations indicated that the phenomena observed at the molecular (fluorescence) and the macroscopic (rheology) levels are related to the texture of cheese since fluorescence spectroscopy and dynamic testing

rheology allowed the discrimination of the cheese samples as a function of their location in the cheese (Karoui & Dufour 2003).

Using partial least square, tryptophan fluorescence spectra recorded at 20°C on 2-day-old cheese predicted storage modulus (G'), loss modulus (G''), strain, tan (δ), and complex viscosity (η*) measured at 80°C on 60-day-old cheeses with correlation coefficients of 0.98, 0.97, 0.98, 0.98, and 0.97, respectively. Riboflavin fluorescence spectra gave slightly lower correlation coefficients of 0.88, 0.88, 0.92, 0.87, and 0.88, respectively (Karoui & Dufour 2006).

Near/mid-infrared and fluorescence spectroscopy combined with chemometric methods has a potential for determining the geographic origin of different cheeses. CCSWA was performed on the physico-chemical, infrared, and tryptophan fluorescence data sets of Emmental cheese of different European geographic origins produced during winter. The results showed that spectroscopic techniques may provide useful fingerprints and allow the identification of Emmental cheese according to the geographic origin and the production conditions. In addition, tryptophan fluorescence spectra allowed a good discrimination of Emmental cheeses made from raw milk or from thermised milk (Karoui *et al.* 2005a).

Tryptophan fluorescence data and infrared spectral data are fingerprints that allow an accurate identification of Emmental cheese according to their manufacturing periods (summer and winter). Cheeses produced during winter and summer were pooled into one matrix and PCA was applied separately to three infrared spectral regions and to the tryptophan fluorescence spectra. The two-dimensional score plots for the tryptophan fluorescence spectra showed a good discrimination between the cheese manufactured in winter or in summer. Similar results were observed with three mid-infrared spectral regions. In order to assess the potential of infrared and fluorescence spectroscopy to determine the geographic origin of cheese independently of the seasonal period of manufacture, FDA was performed on the first twenty PCs of the PCA performed on the four data tables corresponding to three infrared spectral regions and tryptophan fluorescence spectra. Before applying FDA, five groups were created for the cheese (Austria, Finland, Switzerland, France and Germany), independently of the manufacturing period. When FDA was applied to either the infrared or fluorescence spectral data, the geographical classification was not satisfactory. Therefore, the first twenty PCs of the PCA extracted from each data set (infrared and tryptophan fluorescence spectra) were pooled into a single matrix and analysed by FDA. Correct classification of 89% and 76.7% of the calibration and cross-validation data sets, respectively, was observed (Karoui *et al.* 2004).

The results of FDA performed on the mid-infrared spectra, fluorescence emission spectra, following excitation at 250 and 290 nm, and fluorescence excitation spectra following emission at 410 nm showed a good discrimination of the cheeses from three different regions in Jura (France). The discrimination between cheeses manufactured at different altitudes in Switzerland was better with the fluorescence spectra than with the mid-infrared spectra (Karoui *et al.* 2005b).

Fluorescence spectroscopy in combination with chemometrics is a fast method for monitoring the oxidative stability and quality of yogurt (Miquel Becker *et al.* 2003). PARAFAC analysis of the fluorescence landscapes with excitation wavelengths from 270 nm to 550 nm and emission wavelengths in the range of 310–590 nm exhibited three fluorophores (tryptophan, riboflavin, and lumichrom) present in yogurt, all strongly related to the storage conditions. Regression models based on PARAFAC scores, PLS, and multiway PLS between fluorescence landscopes and the riboflavin content were compared and yielded only minor differences with respect to the prediction error (Christensen *et al.* 2005).

Eggs

The fluorescence spectroscopy is a promising approach to determining the egg freshness. The emission spectra of different eggs showed two maxima located at 635 and 672 nm after excitation at 405, 510, 540 and 557 nm, related to the pigments of porphyrin nature and porphyrin derivatives of florin and oxoflorin. The intensity at 672 nm of a fresh egg is stronger than that of an old one (Karoui *et al.* 2006b).

The fluorescence of thick and thin egg albumens was evaluated as a possible rapid method for the monitoring of egg freshness. The fluorescence emission spectra of tryptophan residues of proteins and fluorescent Maillard reaction products were recorded directly on thick and thin albumen samples within 2–29 days of storage. PCA performed on

tryptophan spectra showed mainly discrimination between 1, 2, 3, and 4 weeks of storage. The ability of tryptophan fluorescence spectra to differentiate between thick or thin albumen samples according to their storage time was investigated by applying FDA to the first 5 PCs of the PCA performed on tryptophan fluorescence spectra. Before applying FDA, four groups were created for the investigated eggs (stored for 1, 2, 3, and 4 weeks). Correct classification amounting to 62.8% and 54.3% was observed for the calibration and the validation of thick albumen samples, respectively. The percentage of thin albumen samples correctly classified by the FDA was 67.2% and 69.1% for the calibration and validation spectra, respectively. Considering fluorescent Maillard reaction products, the percentage of samples correctly classified into four groups by the FDA was 97.4% and 91.4% for the calibration and validation thick albumen samples, respectively. It was concluded that fluorescent Maillard reaction products could be considered as fingerprints that may allow the discrimination between fresh and aged eggs (Karoui *et al*. 2006c).

Recently, Karoui *et al*. (2006d) confirmed the high potential of vitamin A fluorescence spectra as a useful tool for monitoring egg freshness. Tryptophan and vitamin A fluorescence spectra were investigated in search for potential markers of egg yolk freshness during storage. Comparison of the results obtained from the FDA indicated that better calibration as well as validation results were obtained from the vitamin A excitation fluorescence spectra than from the tryptophan emission fluorescence spectra. Using the vitamin A fluorescence spectra, correct classification was observed for 94.9% and 91.4% for the calibration and the validation sets, respectively. The first five PCs of the PCA extracted from each data set (tryptophan and vitamin A fluorescence spectra) were pooled into a single-matrix and analysed by FDA. Correct classification was obtained for 97.5% of the calibration and 96.3% of the validation spectra.

Meat

Autofluorescence for the analysis of meat was first proposed in 1986 as a method for quality control of meat and fish products based on their intrinsic fluorescence characteristics (Jensen *et al*. 1986). The method was based on excitation at 340 nm and the fact that bone, cartilage, connective tissues, and meat possess different fluorescent properties. Only weak fluorescence signals are obtained with pure meat at this excitation wavelength, whereas the undesired substances (fat, bone, cartilage, and connective tissue) all give a considerable fluorescence emission signal. The emission spectra of these compounds are different in shape but they all have a peak with a maximum at 390 nm and a shoulder peak with emission at 455 nm (bone, cartilage, and connective tissue) or 475 nm (fat), which can probably be assigned to different types of collagen and NADH.

The studies on meat autofluorescence were primarily focused on the measurements of collagen in connective and adipose tissues, but protein fluorescence and suggestions for some fluorescent oxidation compounds were also reported (Swatland 1987, 1994, 2000, 2001). Most of these studies were carried out with a univariate data analytical approach; univariate regression models were calculated between the desired quality parameters and single wavelengths or extracted fluorescence peak features.

Egelandsdal *et al.* (2002) later applied the bilinear methods PCA and PLS in the evaluation of autofluorescence emission spectra of meat obtained from selected excitation wavelengths in the UV region between 300–400 nm. Fluorescence emission spectra assigned to fluorescent oxidation products were found to correlate with lipid oxidation and rancidity of meat. Moreover, tryptophan fluorescence (excitation 290 nm) was correlated with the texture of meat emulsions and sausages and meat tenderness. Also autofluorescence images reflecting the collagen fluorescence were used for quantification of the intramuscular fat content and connective tissue in beef as well as for mapping the lipid oxidation in chicken meat. All the described multivariate fluorescence studies of meat used bilinear models to evaluate single-emission spectra.

The only multiway study of autofluorescence of meat reported so far was on dry-cured Parma ham which was monitored throughout processing and aging (Møller *et al*. 2003). A PARAFAC decomposition of the recorded fluorescence landscapes revealed the presence of five fluorophores, of which tryptophan was assigned to be the dominating one. The remaining four components were more difficult to assign, one was suggested to arise from salting, and the others were related to the oxidation products.

To understand better the formation of the fluorescent compounds in meat undergoing lipid

oxidation, some different model systems were studied by fluorescence spectroscopy (Veberg *et al*. 2006). In each system, the formation of fluorescence resulting from reactions between protein and carbonyl compounds at 4°C in biological systems was investigated. Aldehydes were added to minced turkey, pork, and cod meat. When excited at 382 nm, different combinations of aldehydes resulted in broad and slightly differently shaped fluorescence spectra and the overall intensity increased logarithmically for 14 days. The fluorescence intensity increased in accordance with the degree of aldehyde saturation, and aldehydes with double bond in 2,4 position gave generally a higher intensity than the aldehyde with double bond in 2,6 position. Diferences in the spectral shape originating from different aldehydes suggest that fluorescence may be able to detect and quantify oxidation related to different groups of aldehydes. For turkey, the intensities were in the following order: 2,4-heptadienal > 2,4-nonadienal > MDA > 2-hexenal > 2,6-nonadienal. Pork revealed almost the same order except for 2,4-nonadienal and MDA, which switched places. The intensity order for cod was the same as for turkey.

The fluorescence responses of wholesome and unwholesome chicken carcasses were characterised and further evaluated for the detection and classification of wholesome and unwholesome chicken carcasses (Moon *et al*. 2006). Fluorescence characteristics from epidermal layers in the breast areas from chicken carcasses were dynamic from nature. Emission peaks and ridges (maxima) were observed at 386, 444, 472, 512, and 554 nm and valleys (minima) were observed at 410, 460, 484, and 538 nm. One of major factors affecting the line shapes of the fluorescence responses from chicken carcass skin layers was absorption by hemoglobin. With the use of the normalised ratio spectra (NRS) approach, oxyhemoglobin was shown to be a major constituent in chicken carcasses affecting the fluorescence emission line shapes. With the use of simple fluorescence band ratios as a multivariate model, wholesome and unwholesome chicken carcasses were correctly classified with 97.1% and 94.8% accuracies, respectively.

Fish

During freezing, storage and thawing, fish muscle may undergo protein denaturation and lipid oxidation. Fluorescence spectrometry was used to measure lipid oxidation products during frozen fish storage (Aubourg & Medina 1999; Duflos *et al*. 2002).

Important fluorophores of fish muscle include aromatic amino acids and nucleic acids (excitation 250 nm, emission 280–480 nm), tryptophan residues (excitation 290 nm, emission 305–400 nm) of proteins, and NADH (excitation 336 nm, emission 360–600 nm). The aromatic amino acids and nucleic acids fluorescence emission spectra recorded on cod, mackerel, salmon, and whiting fillets on 1, 5, 8, and 13 days of storage, as well as its tryptophan and NADH fluorescence spectra, are fingerprints that allow the evaluation of fish freshness (Dufour *et al*. 2003).

NADH fluorescence spectra may be considered as a promising probe for the reliable differentiation between frozen-thawed and fresh fish. The NADH emission spectra (excitation 340 nm) of fresh fish showed a maximum at 455 nm and a shoulder at 403 nm, while frozen-thawed fish was characterised by a maximum at 379 nm and a shoulder at 455 nm. It appeared that the shape of NADH emission spectra correlated with the treatment applied to the fish. Considering tryptophan fluorescence spectra, the normalised emission spectra of fresh fish samples exhibited a maximum at about 326 nm while frozen-thawed samples had a maximum at 330 nm. As most of the spectra represent very similar shapes (especially for tryptophan fluorescence spectra), PCA and FDA were used to extract information from spectral data bases. In the first step, PCA was applied separately to the tryptophan or NADH fluorescence spectra. From the NADH spectra, PCA results showed a good discrimination between fresh and frozen-thawed fish samples. In the second step, FDA was applied to the first five PCs of the PCA performed on the two data sets. A better classification was obtained from NADH fluorescence spectra since 100% correct classifications were obtained for the calibration and validation spectra (Karoui *et al*. 2006e).

Edible oils

The fluorescence emission spectra of olive oils are related to its composition and stability. Virgin olive oils are quite stable against oxidation because of the antioxidant activity of phenolic compounds and vitamin E. In addition, chlorophylls protect oils in the darkness. Therefore, the fluorescent

spectra of virgin olive oils at the excitation wavelength of 365 nm shown a peak around 681 nm, due to chlorophylls, and three other peaks (two of low intensity at 445 nm and 475 nm, and one more intense at 525 nm), which can be attributed to vitamin E. Refining processes decrease the vitamin E and chlorophylls contents, so refined oils (pure, olive-pomace oil) are more liable to undergo oxidation processes. These changes are reflected in their emission fluorescence spectra, in which these oxidation products give a wide peak between 400 nm and 500 nm (excitation at 365 nm). The chlorophylls peak is much more intense than the peaks of the rest of the species in oils (Kyriakidis & Skarkalis 2000).

Both PCA and PARAFAC applied to EEMs of the two main groups of olive oils (virgin and pure) show clear differences between these types of oils. Chlorophylls had a strong influence on the models because of their high fluorescence intensity. Differentiation between the two types of oils is better when the chlorophylls fluorescence region is not included in the models. In this case, oxidation products are the species that contribute most to the separation between the two groups (Guimet *et al*. 2004).

Extra virgin olive oil is the highest-quality and the most expensive type of olive oil. It is sometimes adulterated with olive-pomace oil (OPO). Guimet *et al*. (2005) developed a fast screening method based on EEMs for detecting adulteration with OPO at 5% level in extra virgin olive oil. Adulteration causes mainly an increase of fluorescence at emissions below 500 nm. The authors applied unfold PCA and PARAFAC for exploratory analysis and quantified OPO adulteration around 5% level using the PLSR method, obtaining a prediction error of 1.2%.

The capabilities of NMF used together with LDA for discriminating between the different types of oils were also studied. The discrimination results obtained with the NMF-LDA were compared to those obtained using two other methods (PARAFAC combined with LDA and discriminant multiway PLSR). NMF-LDA and discriminant multiway PLSR yielded a better discrimination between commercial olive oils (virgin, pure, and olive-pomace) than PARAFAC-LDA. However, discriminant multiway PLSR was the best method for discriminating between extra virgin olive oils and those adulterated with olive-pomace oils. The main advantage of NMF with respect to discriminant multiway PLSR is that the basic functions are more interpretable than the PLS loadings, because they are positive and correspond to parts of the spectra that can be more easily related to the fluorescent compounds of the oils (Guimet *et al*. 2006).

Virgin olive oils are classified and priced according to acidity. Virgin olive oil that has acidity lower than 3.3 degrees (% (w/w) free fatty acid content calculated as oleic acid) is suitable for consumption without any treatment. Virgin olive oils of higher acidities (lampante olive oils), are refined in order to become edible. The comparison of SFS spectra with total luminescence spectra (EEM) collected from both edible and lampante olive oils showed a reduced spectral complexity and an approximately 7-fold amplification of fluorescence bands in SFS spectra. The wavelength interval $\Delta\lambda$ = 80 nm shows the best differentiation between olive oils. For this interval value, both edible and lampante virgin olive oil spectra show strong intensity bands at 660 nm and 605 nm. However, edible virgin olive oil shows one low intensity band at 370 nm while lampante virgin olive oil shows medium intensity bands at 385 and 450 nm. In order to compare the sets of SFS spectra, PCA and HCA were performed. Maximum differentiation between oils was found in the spectral range of 429–545 nm originating from oleic acid. Both HCA and PCA provide very good discrimination between the two virgin olive oil classes, however, PCA allows for 100% correct classification while HCA for 97.3% (Poulli *et al.* 2005).

Few papers were published in recent years on the use of fluorescence in vegetable oils. Sayago *et al*. (2004) applied fluorescence spectroscopy for detecting hazelnut oil adulteration in virgin olive oils.

Total luminescence and SFS techniques were tested to characterise and differentiate edible oils, including soybean, sunflower, rapeseed, peanut, olive, grape seed, linseed, and corn oils. Total luminescence spectra of all oils studied as solutions in n-hexane exhibit an intense peak (λex/λem, 290/320 nm) attributed to tocopherols. Some of the oils exhibit a second long-wavelength peak (λex/λem, 405/670 nm), belonging to pigments of the chlorophyll group. Additional bands were present arising from unidentified compounds. Similarly, bands attributed to tocopherols, chlorophylls, and unidentified fluorescent components were detected in the SFS spectra. The spectral profiles of SFS spectra of different oils vary significantly between different oils samples. In order to compare the

sets of SFS spectra of different oils, kNN method and LDA were performed. The kNN method was applied using the entire spectra as input, while the linear discrimination method used six excitation and emission wavelength pairs as input. Both methods provided a very good discrimination between the oil classes with a low classification error (Sikorska *et al*. 2005).

Fluorescence spectrometry and PLSR can be used as a rapid technique for evaluating the quality of heat-treated extra virgin olive (Cheikhousman *et al*. 2004) and rapeseed oils (Mas *et al*. 2004).

Miscellaneous

Fluorescence spectroscopy in combination with PCA and PLSR provides information on oxidative changes in peanuts, pork scratchings, oatmeal, and muesli. The differences between fresh and oxidised samples are most evident with pork scratchings and oatmeal. For pork scratchings, an increase in fluorescence at λex/λem around 370/450 nm could be due to lipid oxidation, and a decrease at λex/λem around 470/530 nm could be related to riboflavin. The increase at excitation 430 nm (emission 470–510 nm) could be due to increased oxidation, as this region has been shown to be related to fluorescent protein-bound lipid peroxidation products. PLSR models were constructed between fluorescence data and the contents of free radicals and volatiles (acetaldehyde, pentanal, or hexanal). For pork scratchings, the fluorescence technique was able to monitor the progressing level of oxidation with good correlations to both free radicals and hexanal. With the other three products, the results were generally inferior except for good correlations between fluorescence spectra and free radicals in peanuts (Jensen *et al*. 2004).

The presence of fluorophores (aromatic amino acids, vitamins, cofactors and phenolic compounds) in honey makes fluorescence spectroscopy a valuable technique to determine the botanical origin of honey (Ruoff *et al*. 2005). Honey fluorescence spectra from seven floral origins, namely acacia, alpine rose, chestnut, rape, honeydew, alpine polyfloral and lowland polyfloral were recorded. The emission spectra (excitation 250 nm; emission 280–480 nm) allowed the study of the fluorescence of aromatic amino acids, furosine, 5-hydroxymethylfurfural (HMF) and phenolic compounds. It was concluded that emission spectra (280–480 nm) are fingerprints allowing good identification of the botanical origin of honey. The emission spectra (emission 305–500 nm) of tryptophan residues in protein after excitation at 290 nm may be considered as a characteristic fingerprint. Emission spectra (emission 300–600nm) recorded after excitation at 373 nm showed a maximum located around 445 nm which is related to furosine and HMF. The FDA applied separately to the first 10 PCs of the PCA performed on each data table can lead to some discrimination between the honey types investigated. However, it obviously cannot allow studying all the information contained on these tables. Thus, the first ten PCs of the PCA extracted from each data set were gathered together into one matrix and analysed by FDA. Correct classification of 100% and 90% was observed for the calibration and the validation samples, respectively (Karoui *et al*. 2007c).

Phenolic acids, stilbenes, anthocyanins, flavanols, and tannins are the best known fluorescent molecules in wines. The nature and amounts of these molecules differ from one grape variety to another. Wine processing and ageing also have effects on the phenolic compounds. In addition, wines contain many other compounds (e.g., proteins) that may fluoresce. The fluorescence spectra may provide useful fingerprints and mainly allow the identification of wines according to their variety and typicality. The ability of the phenolic acids emission (275–450 nm) and excitation (250–350 nm) spectra to differentiate between wines produced in France and Germany was investigated by applying FDA to the principal components of the PCA performed on the excitation fluorescence data or emission fluorescence data. Two groups were created for the investigated wines, i.e., Gamay wines and Domfelder wines. Correct classification amounting to 93.2% and 100% was observed for the emission fluorescence data set and the excitation fluorescence data set, respectively. The ability of emission and excitation spectra to differentiate between typical and non-typical Beaujolais wines was investigated by applying FDA to the principal components of the PCA performed on the excitation fluorescence data or emission fluorescence data. Two groups were created for the investigated wines, i.e., typical wines and outsider wines. Correct classification amounting to 95% and 87% was observed for the emission fluorescence data set and the excitation fluorescence data set, respectively (Dufour *et al*. 2006).

Fluorescence spectroscopy allows monitoring the changes in the chemical composition of beers during storage. A pronounced decrease of fluorescence features ascribed to riboflavin was observed in samples exposed to light as compared to those kept in the dark. PCA of SFS spectra ($\Delta\lambda$ = 10 nm or $\Delta\lambda$ = 60 nm) revealed clear clustering of samples according to the storage conditions. Two statistical methods were employed: the kNN method, which uses entire spectra, and the LDA method, for which only six selected excitation/emission wavelength pairs were extracted from the spectra. Successful classification of differently stored samples was accomplished using both the kNN and LDA (Sikorska *et al*. 2006).

The application of fluorescence spectroscopy to the food classification is still relatively recent. In those reports which do exist, however, the potential of the technique is clear, especially when it is combined with powerful multivariate analysis tools. In support to the theoretical advantages of working with EEMs or SFS as opposed to conventional spectra, some selectivity improvements of EEMs or SFS do seem to exist in certain instances. The principal advantages of fluorescence spectroscopy, pointed out by almost all authors, are its rapidity and sensitivity (100–1000 times more sensitive than other spectrophotometric techniques). In addition, fluorescent compounds are extremely sensitive to their environments at the molecular level. Thus, fluorescence spectroscopy can be used as an accurate tool to monitor the molecular changes which occur during food handling, processing, or storing.

List of abbreviations

EEM – excitation-emission matrix
SFS – synchronous fluorescence scan
PCA – principal component analysis
PCs – principal components
HCA – hierarchical cluster analysis
PARAFAC – parallel factor analysis
NMF – negative matrix factorisation
CCSWA – common components and specific weights analysis
CCA – canonical correlation analysis
PLS – partial least squares
LDA – linear discriminant analysis
FDA – factor discriminate analysis
kNN – k-nearest neighbours
PLSR – partial least squares regression
PCR – principial component regression
NRS – normalised ratio spectra
OPO – olive – pomace oil
HMF – 5-hydroxymethylfurfural

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