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Front-face fluorescence spectroscopy as a tool to classify seven bovine muscles according to their chemical and rheological characteristics

A. Sahar [a], T. Boubellouta [a], J. Lepetit [b], É. Dufour [a,*]

[a] U.R. Typicité des Produits Alimentaires, ENITA Clermont Ferrand, BP 35, F-63370 Lempdes, France
[b] INRA-QuaPA, Site de Theix, 63122 Saint Genès Champanelle, France

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ABSTRACT

Potential of front-face fluorescence spectroscopy was evaluated to classify muscles according to their chemical and rheological characteristics. Seven bovine muscles (*Semitendinosus, Semimembranosus, Tensor fasciae latae, Rectus abdominis, Longissimus thoracis et lumborum, Triceps branchii* and *Infraspinatus*) were taken from 14 animals of the Charolais breed. Chemical characteristics and rheological properties of the meat were determined including dry matter, fat, collagen, protein, peak load, energy required to rupture and cooking loss. Emission spectra in the 305–400 nm, 340–540 nm and 410–700 nm ranges were recorded using front-face fluorescence spectroscopy by fixing the excitation wavelengths at 290, 322 and 382 nm, respectively. Analysis of variance (ANOVA) applied on chemical and rheological parameters showed that these muscles were significantly different ($P < 0.01$) from each other. Chemical and rheological data were divided into low, medium and high range groups for each variable. The results of PLSDA showed that 305–400 nm spectra were responsible for 67% (calibration), 53% (validation), 96% (calibration) and 55% (validation) of good classification for protein and cooking loss, respectively, while 340–540 nm spectra allowed 75% of good classification (validation samples) for fat content.

1. Introduction

Spectroscopic techniques have been used more and more in the agricultural and food industries recently. It has become increasingly clear that the application of spectroscopic methods to food analysis can alleviate problems in the processing and distribution of food and food products. Indeed, traditional analytical methods for food components are slow, relatively expensive, time-consuming, require highly skilled operators and are not easily adapted to on-line monitoring. Thus, a number of non-invasive and non-destructive instrumental techniques such as infrared and, more recently, fluorescence have been developed for the determination of product composition and texture. Fluorescence spectroscopy offers several inherent advantages for the characterization of molecular interactions and reactions (Dufour & Haertlé, 1990; Dufour, Genot, & Heartlé, 1994; Lakowicz, 1983; Longworth, 1971; Marangoni, 1992). First, it is 100–1000 times more sensitive than absorption spectroscopy. Second, fluorescent compounds are sensitive to their environment, enabling characterization of conformational changes. This technique is relatively low-cost and can be applied in both fundamental research and in the factory as on line sensors for monitoring food products (Karoui & Dufour, 2008).

A variety of biological tissues contain naturally occurring fluorophores, and emission from these compounds is called intrinsic fluorescence (or autofluorescence). It is known that myofibrils of muscles exhibit intrinsic fluorescence (Udenfriend, 1969). Meat consists of components which contain relatively strong fluorophores (tryptophan residues in proteins, vitamin A, riboflavin, NADH, pyridinoline in collagen, protoporphyrin IX, lipid oxidation products, etc.). As their excitation and emission wavelengths have been characterized (Egelandsdal, Dingstad, Togersen, Lundby, & Langsrud, 2005; Schneider et al., 2008; Skjervold et al., 2003; Veberg et al., 2006), it makes meat a good product for fluorescence studies (Egelandsdal, Wold, Sponnich, Neegard, & Hildrum, 2002; Swatland, 1987c, 1993, 1997; Swatland & Barbut, 1991; Wold, Lundby, & Egelandsdal, 1999). Fluorescence spectroscopy has been considered as a tool for quantification of the composites of meat (Wold et al., 1999), and to investigate the structural properties of meat and cheese (Dufour & Frencia, 2001; Dufour et al., 2000; Dufour, Devaux, Fortier, & Herbert, 2001; Egelandsdal, Kvaal, & Isaksson, 1996; Egelandsdal et al., 2002; Karoui & Dufour, 2006; Lopez & Dufour, 2001; Swatland, Madsen, & Nielsen, 1996). Experiments on meat products have shown the ability of this technique to determine collagen, elastin and adipose tissue (Egelandsdal et al., 2005; Swatland, 1987a, 1987b, 1987c). Another application

* Corresponding author. Address: Ministère de l'alimentation, de l'agriculture et de la pêche, DG Enseignement et Recherche, 1ter avenue de Lowendal, 75700 Paris 07 SP, France. Tel.: +33 1 49 55 85 53.
E-mail address: eric.dufour@agriculture.gouv.fr (É. Dufour).

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of fluorescence was the development of a suitable method to characterize meat tenderness (Dufour & Frencia, 2001). This ability of fluorescence spectroscopy to study the characteristic changes of structure/texture has also been reported to be of use to estimate properties such as lipid oxidation (Wold & Mielnik, 2000) and water-holding capacity (Brøndum et al., 2000).

However, the meat sector has no quality control methods for rapid online, as well as offline, analysis of meat products. Such a tool would allow enterprises to partition the meat market on the basis of meat tenderness, as Australian are starting to do with the MSA (Meat Standard Australia) system. The MSA system mainly relies on sensory measurements which are expensive, tedious and time consuming.

Despite the heterogeneity and anisotropy of meat, fluorescence spectroscopy has been shown to be a valuable method for the evaluation of meat quality (Dufour & Frencia, 2001; Skjervold et al., 2003). The aim of this study was to assess front-face fluorescence spectroscopy as a non-destructive and rapid method to classify meat samples according to their chemical and rheological characteristics. Multivariate analysis techniques such as partial-least square discriminant analysis (PLSDA) make it possible to model the relationship between spectra and the chemical and rheological characteristics of meat samples.

2. Materials and methods

2.1. Sample preparation

Fourteen carcasses (15 days of maturation) were taken from Charolais breed cattle. Seven muscles ($7 \times 14 = 98$ samples) were dissected from each carcass including *Semitendinosus* (ST), *Semimembranosus* (SM), *Tensor fasciae latae* (TF), *Rectus abdominis* (RA), *Longissimus thoracis et lumborum* (LT), *Triceps branchii* (TB) and *Infraspinatus* (IS). These muscles were trimmed of external fat, vacuum packed and stored at −20 °C.

2.2. Proximate analysis

Frozen samples of the muscles were thawed at −4 °C for 12 h and ground to determine the dry matter content, fat content, collagen and protein. Percentages of dry matter content (ISO 1442:1997 (F)) and fat content (Norme française 403:2001) were determined using an oven drying procedure (105 °C for 24 h) and an extraction procedure using petroleum ether in a Soxhlet apparatus, respectively. Collagen percentage was calculated by measuring the hydroxyproline contents and multiplying by a factor of 8 (ISO 3496:1994 (F)). Protein percentage was determined on 1 g of sample by the Kjeldahl method (ISO 937:1978 (F)). All observations were carried out in triplicate on different samples of each muscle.

2.3. Shear force

Vacuum packed samples (approximately $10 \times 10 \times 4$ cm) stored at −20 °C were thawed in a water bath at 10 °C for 1 h, just before the measurement. Pieces (3 cm thick in the direction perpendicular to the muscle fibres) were grilled simultaneously on both sides with an Infragrill E (Sofraca, France) until a core temperature of 55 + 5 °C was reached. Cooking loss was calculated by weighing the meat samples before and after heating.

Warner–Bratzler shear force was assessed on the grilled-meat samples ($1 \times 1 \times 3$ cm) perpendicular to the muscle fibres direction using a Warner–Bratzler shearing device, as defined by Honikel (1998). Peak load (N) and energy to rupture (J) of the muscle sample were determined. The measurement was repeated 10 times on different samples of the same muscle.

2.4. Fluorescence spectroscopy

Fluorescence spectra were recorded using a FluroMax-2 spectroflurimeter (Spex-Jobin Yvon, Longumeau, France) mounted with a front-surface sample holder and the incidence angle of the excitation radiation was set at 56° to ensure that reflected light, scattered radiation and depolarisation were minimized. For surface measurements, meat samples were mounted between two quartz slides. Emission spectra in the range 305–400 nm, 410–700 nm and 340–540 nm were recorded with excitation wavelengths of 290, 382 and 322 nm, respectively: these mainly addressed the fluorescence of tryptophan, pyridinoline/riboflavin and vitamin A molecules (Skjervold et al., 2003). But due to the numerous fluorophores present in meat, the recorded spectra were most probably not from a unique fluorophore. For each muscle sample, two spectra were recorded on different samples.

2.5. Pre-treatment of the spectra and statistical analysis

In order to reduce scattering effects, the fluorescence spectra were normalized by reducing the area under each spectrum to a value of 1 according to Bertrand and Scotter (1992). Thus, mainly the shifts of the maximum emission and the width changes of the spectra, retaining most of the structural information, were considered.

2.6. Analysis of variance (ANOVA)

Statistical analysis was carried out using STATISTICA software. Significance differences of chemical and rheological parameters among the seven bovine muscle samples were determined by analysis of variance (ANOVA) using the least square difference method (Fisher test). Differences were considered significant at the $P < 0.01$ level.

2.7. Discriminant analysis (PLSDA)

Partial-least square discriminant analysis (PLSDA) with validation were performed on normalized spectral data in MATLAB (The Mathworks Inc., Natic, MA, USA) using the 'Saisir' package available at the website: http://easy-chemometrics.fr. The aim of this technique is to predict the membership of an individual to a qualitative group defined as a preliminary (Vigneau, Qannari, Jaillais, Mazerolles, & Bertrand, 2006). For each chemical and rheological parameter, samples were classified into three groups, i.e., low, medium and high. The PLSDA assesses new synthetic variables called loading factors, which are linear combinations of the variables and allows a better separation of the centre of gravity of the considered groups. The method allows the individual samples to be reallocated within the various groups. Comparison of the predicted groups to the real group is an indicator of the quality of the discrimination and it is valued as the percentage of correct classifications. The spectral collection was divided into two groups: two-thirds of the investigated muscle samples were used for the calibration set and one-third for the validation set.

3. Results and discussion

3.1. Chemical and rheological parameters

The results of the chemical and rheologial analysis including proximate analysis (fat content, dry matter content, collagen and protein) and Warner–Bratzler shear force measurements (peak force, energy to rupture and cooking loss) of the seven beef muscles, i.e., *Semitendinosus* (ST), *Semimembranosus* (SM), *Tensor fasciae*

latae (TF), *Rectus abdominis* (RA), *Longissimus thoracis et lumborum* (LT), *Triceps branchii* (TB), *Infraspinatus* (IS), taken from 14 carcasses are reported in Table 1.

The results showed a wide range of variation for chemical and rheological parameters for a given muscle. For example, fat contents of IS muscles had a standard error of 2.45, while the standard errors for peak load varied from 12.11 to 17.68.

3.2. Proximate analysis

3.2.1. Dry matter content

Results from analysis of variance (ANOVA) using $P < 0.01$ showed that TF muscles contained the highest percentage of dry matter among the muscles, whereas the round muscles (ST and SM) contained the lowest dry matter percentages.

3.2.2. Fat content

As previously reported by Stetzer, Cadwallader, Singh, Mckeith, and Brewer (2008), fat contents were the highest in IS muscles (Table 1). The results were also in agreement with Brackebusch, McKeith, Carr, and McLaren (1991): among 16 muscles used for their studies, fat contents for five muscles (RA, IS, ST, SM and TB) were similar to our results. Among these five muscles they found that RA and IS contained the highest levels of fat while ST and SM contained the lowest. Similar results were found in the present work, but the fat percentages reported in their study were derived using a warm chloroform:methanol extraction. So their fat contents derived using this method would be expected to be higher than values obtained by ether extraction (Marchello, Dryden, & Ray, 1968).

Variation in the composition of beef muscles is well established. Von Seggern, Calkins, Johnsen, Brickler, and Gwartney (2005) showed that the fat content of meat increased as water content decreased. Similar results are found in the present studies, where muscles containing less fat (ST, SM) had higher dry matter contents, however, TF muscles containing the highest amounts of fat were lower in dry matter.

3.2.3. Collagen

Collagen contents were significantly higher in TF, TB and IS muscles than in SM and LT muscles and agreed with Torrescano, Sanchez-Escalante, Gimenez, Roncales, and Beltran (2003). These results are also in agreement with Von Seggern et al. (2005) who showed that chuck muscles (IS and TB) contained more collagen compared to round muscles (ST and SM). Stolowski et al. (2006) used seven major beef muscles from three Angus and Brahman breed crosses and showed that TB muscle contained significantly ($p < 0.05$) higher amounts of total collagen (mg/g) than SM and LT, as found in the present study.

3.2.4. Protein

As reported by Boles and Shand (2008) and Brackebusch et al. (1991), protein contents were significantly higher in ST and SM muscles than in IS muscles. Protein contents for the muscles, except TB, were inversely related to fat content.

3.3. Warner–Bratzler shear force measurement

Peak force values showed that ST muscles were significantly the toughest muscle followed by TB, while LT and IS muscles were the most tender among the seven muscles. In this study, IS muscles which contained the highest fat content (4.83%) had the lowest values for peak force (47.26 N). These findings are similar to Below, Brooks, McKenna, and Savell (2003). These authors also showed that muscles with high fat contents were usually tender. The results are in agreement with Torrescano et al. (2003), who found that TB and SM were the toughest muscle although LT was most tender among the 14 bovine muscles studied by them. Energy required to rupture the muscle appeared to be higher in ST, SM, TF RA and TB muscles than in LT and IS.

It also appeared that TF and IS muscles had the highest cooking losses, whereas RA muscles had lower cooking losses.

3.4. Fluorescence spectroscopy

3.4.1. Characteristics of spectra obtained from fluorescence spectroscopy

Results of fluorescence spectroscopy of three different muscles are shown in Fig. 1a–c. It can be seen that the spectra were different between muscles.

The fluorescence emission spectra recorded following excitation at 290 nm showed different shapes and intensities among the muscles with a maximum at about 330–333 nm, mainly originating from tryptophan residues in proteins (Dufour, Dalgalarrondo, & Adam, 1998; Dufour, Frencia, & Kane, 2003; Skjervold et al., 2003). This demonstrates that the tryptophan spectra of bovine muscles are fingerprints that allow their identification between different muscles. These results are in accord with Dufour and Frencia (2001). In their studies they used two muscles (LT and IS) and recorded the emission spectra by fixing the excitation wavelength at 290 nm. They demonstrated that it is possible to differentiate between these muscles from their emission spectra. They suggested that spectra were fingerprints of proteins present in the bovine muscles.

The origin of emission spectra scanned from 410 to 700 nm with an excitation wavelength of 382 nm, was more difficult to assign to a given fluorophore. These spectra showed a maximum intensity at about 465–473 nm, with a shoulder at about 480–483 nm. Another less intense peak was observed at about 635–645 nm with a shoulder at 597–600 nm. Previous reports by Egelandsdal et al. (2002) and Skjervold et al. (2003) have shown 380 nm to be the best excitation wavelength for quantification of connective tissue: in this

Table 1
Chemical and rheological parameters (Mean values and standard error) of seven beef muscles[a].

Beef muscles	*n*	Dry matter (%)	Fat content (%)	Collagen (%)	Protein (%)	Peak load (N)	Energy to rupture (J)	Cooking loss (%)
ST	24	$26.16^{b} + 1.09$	$1.6^{d} + 0.70$	$0.78^{ab} + 0.16$	$23.86^{a} + 1.59$	$81.20^{a} + 12.13$	$0.53^{a} + 0.09$	$33.18^{ab} + 3.41$
SM	22	$26.07^{b} + 0.78$	$1.14^{d} + 0.59$	$0.66^{b} + 0.14$	$23.88^{a} + 0.59$	$70.44^{bc} + 14.78$	$0.53^{a} + 0.11$	$33.61^{ab} + 3.41$
TF	24	$27.43^{a} + 1.78$	$3.71^{ab} + 2.02$	$0.87^{a} + 0.28$	$22.79^{bc} + 0.90$	$72.95^{abc} + 13.13$	$0.52^{a} + 0.08$	$34.50^{a} + 2.08$
RA	24	$26.83^{ab} + 1.89$	$3.40^{bc} + 1.92$	$0.76^{ab} + 0.20$	$23.23^{ab} + 0.87$	$64.75^{c} + 13.65$	$0.49^{a} + 0.12$	$26.58^{c} + 2.69$
LT	14	$27.12^{ab} + 1.50$	$2.34^{cd} + 1.23$	$0.64^{b} + 0.22$	$23.55^{ab} + 0.78$	$46.03^{d} + 12.11$	$0.35^{b} + 0.09$	$31.53^{b} + 1.45$
TB	26	$26.43^{ab} + 1.09$	$3.08^{bc} + 1.45$	$0.85^{a} + 0.24$	$22.30^{c} + 1.12$	$77.88^{ab} + 17.68$	$0.53^{a} + 0.12$	$32.75^{ab} + 2.32$
IS	18	$26.87^{ab} + 2.08$	$4.83^{a} + 2.45$	$0.87^{a} + 0.26$	$20.91^{d} + 0.95$	$47.26^{d} + 13.57$	$0.34^{b} + 0.09$	$34.43^{a} + 2.95$

[abcd]Means chemical and rheological parameters within a column with the same superscripts are not significantly different ($P < 0.01$).

[a] *Semitendinosus* (ST), *Semimembranosus* (SM), *Tensor fasciae latae* (TF), *Rectus abdominis* (RA), *Longissimus thoracis et lumborum* (LT), *Triceps branchii* (TB), and *Infraspinatus* (IS).

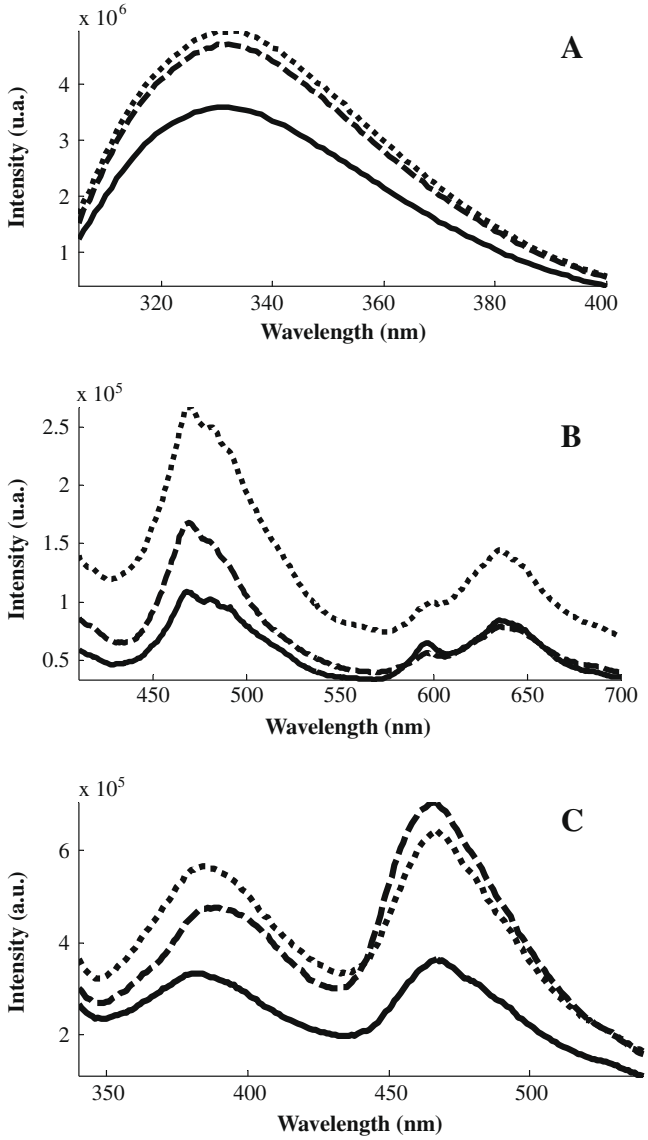

Fig. 1. Fluorescence spectra of three muscles of meat. (A) Emission spectra recorded between 305 and 400 nm with excitation wavelength set at 290 nm, (B) emission spectra recorded between 410 and 700 nm with excitation wavelength set at 382 nm, and (C) emission spectra recorded between 340 and 540 nm with excitation wavelength set at 322 nm. Tensor fasciae latae (···); Longissimus thoracis et lumborum (- - -); and Semimembranosus (—).

case, the maximum emission was observed at about 480 nm. Excitation at 380 nm is also used to investigate riboflavin in dairy products (Karoui & Dufour, 2006). It appears that cheeses present intense fluorescence at about 520 nm following excitation at 380 nm. Considering the spectra (Fig. 1b) in this study, the fluorescence of collagen was the main contributor, but riboflavin also contributed. The less intense peak observed at about 635–645 nm with a shoulder at 597–600 nm might be due to protoporphyrin IX, except that the usual wavelength used for excitation of this fluorophore is 420 nm (Schneider et al., 2008). But protoporphyrin IX may be slightly excited at 380 nm due to the large width of the fluorophore excitation spectrum.

Emission spectra from 340 to 540 nm with excitation measured at 322 nm showed two peaks: the weakest was located at 380–395 nm and the largest one at about 465–468 nm. Similar peaks assigned to vitamin A were found by Skjervold et al. (2003) in red meat and Veberg et al. (2006) in white meat. Dufour et al. (2000) found similar peaks for vitamin A fluorescence in cheese.

3.4.2. Classification of muscles according to their chemical and rheological characteristics

For discriminant analysis, the meat samples were divided into three groups (low, medium and high) according to the chemical and rheological data (Table 2). Indeed, the groups were not built according to the muscle type since the samples of a given muscle showed a wide range of variation for the considered chemical and rheological parameter (Table 1). Considering TF muscle, one sample contained 1.93% of fat, whereas others contained up to 5% of fat. So, regarding their fat content, the 24 TF data can be distributed into the three groups: low (8 samples), medium (10 samples) and high (6 samples). Similarly, the 18 IS data are divided into low (4 samples), medium (6 samples) and high (8 samples) groups according to their fat content.

PLSDA was applied on normalized fluorescence spectral data sets (calibration and validation data sets) recorded in the 305–400 nm, 340–540 nm and 410–700 nm ranges in order to assign a given meat sample to a chemical or rheological group. The spectral collections were divided into two groups: two-thirds of the investigated muscle samples were used for the calibration set and one-third for the validation set.

Calibration and validation results of discriminant analysis (PLSDA) carried out on 305–400 nm fluorescence spectra are reported in Table 3. The results showed that five factors of PLSDA gave 63% of good classification for dry matter content of muscles. In Table 1, it appears that TF samples had higher dry matter contents than LT and SM. The differences between these three muscles were also observed in the 305–400 nm fluorescence spectra (Fig. 1a): TF spectra showed higher fluorescence intensity than LT and SM. PLSDA with four factors showed 53% and 55% of good classification (validation set) for protein content and peak load, respectively. This is discussed by Damez and Clerjon (2008), who reviewed the use of fluorescence spectroscopy in meat and said that tryptophan is an important intrinsic fluorescent that can be used to assess the nature of the tryptophan microenvironment and that the information retained in tryptophan spectra can be correlated with meat tenderness. In case of cooking loss, 55% of good classification (validation set) can be achieved using 10 factors of PLSDA.

Table 2
Division of the considered samples into three groups[a] according to chemical and rheological data.

Physico-chemical parameters	Code	*n*	Range
Dry matter %	L	49	24.5–25.9
	M	74	26.0–27.9
	H	29	28.0–31.4
Fat content %	L	82	0.0–2.5
	M	48	2.6–4.9
	H	22	5.0–9.2
Collagen %	L	42	0.22–0.68
	M	85	0.69–0.99
	H	25	1.00–1.50
Protein %	L	23	18.7–21.5
	M	57	21.6–23.2
	H	72	23.3–25.0
Peak Load (N)	L	34	22–52
	M	64	53–75
	H	56	76–100
Energy to rupture (J)	L	37	0.20–0.39
	M	69	0.40–0.54
	H	46	0.55–0.77
Cooking loss %	L	36	22.8–30.4
	M	82	30.5–31.2
	H	34	31.3–34.9

[a] Low (L) medium (M), high (H), number of samples in a given group (*n*), lowest and highest values used to define a given group (range).

The results of discriminant analysis (PLSDA) applied on 410–700 nm fluorescence spectra are reported in Table 4. It can be seen that PLSDA with 10 factors showed 63%, 55% and 57% of good classification (validation set) for fat content, protein content and peak load, respectively.

The results of discriminant analysis (PLSDA) carried out on 340–540 nm fluorescence spectra showed that PLSDA using seven factors made it possible to obtain 75% of good classification (validation set) for muscle fat contents (Table 5). It proved that emission spectra recorded in the 340–540 nm range are good indicators of fat contents in bovine muscles. In addition, PLSDA using 10 factors demonstrated 59% and 53% of good classification (validation sets) for dry matter contents and energy to rupture, respectively.

Front-face fluorescence spectroscopy has the potential to dramatically reduce analytical time and cost of traditional measurements or new methods, such as MSA systems, used for the evaluation of meat quality. However, the robustness of the models for classification has to be improved for several variables. It will be improved by testing on a larger set of samples and by increasing the database. In addition, a larger database will allow us to expand the three groups (low, medium and high) for each type of muscle. These improvements should increase the robustness of the predictions.

Table 3
Results for calibration and validation sets of partial-least square discriminant analysis (PLSDA) carried out on 305–400 nm fluorescence spectra (rows: observed classifications; columns: predicted classifications).

Parameters	No. of PLSDA factors	Groups	Calibration				Validation			
			L	M	H	% of good classification	L	M	H	% of good classification
Dry matter content	5	L	27	3	3	82	9	5	2	56
		M	14	29	6	59	4	17	4	68
		H	4	3	12	63	1	3	6	60
Protein	4	L	12	2	1	80	5	2	1	63
		M	7	21	10	55	4	9	6	47
		H	3	10	35	73	2	9	13	54
Cooking loss	10	L	24	0	0	100	6	4	2	50
		M	2	52	1	95	3	18	6	67
		H	0	1	21	95	2	6	4	33
Peak load	4	L	13	6	2	62	6	5	0	55
		M	7	26	10	60	5	10	6	48
		H	7	7	23	62	2	5	12	63

Low (L), medium (M), high (H).

Table 4
Results for calibration and validation sets of partial-least square discriminant analysis (PLSDA) carried out on 410–700 nm fluorescence spectra (rows: observed classifications; columns: predicted classifications).

Parameters	No. of PLSDA factors	Groups	Calibration				Validation			
			L	M	H	% of good classification	L	M	H	% of good classification
Fat contents	10	L	39	7	8	72	21	4	3	75
		M	8	20	4	63	4	8	4	50
		H	4	2	9	60	3	1	3	43
Protein	10	L	8	6	1	53	5	2	1	63
		M	4	24	10	63	3	10	6	53
		H	5	12	31	65	4	7	13	54
Peak load	10	L	12	2	7	57	5	5	1	45
		M	4	27	12	63	3	12	6	57
		H	7	8	22	59	2	5	12	63

Low (L), medium (M), high (H).

Table 5
Results for calibration and validation sets of partial-least square discriminant analysis (PLSDA) carried out on 340–540 nm fluorescence spectra (rows: observed classifications; columns: predicted classifications).

Parameters	No. of PLSDA factors	Groups	Calibration				Validation			
			L	M	H	% of good classification	L	M	H	% of good classification
Fat contents	7	L	40	8	6	74	23	3	2	82
		M	6	19	7	59	5	9	2	56
		H	0	4	11	73	0	1	6	86
Dry matter	10	L	23	4	6	70	10	5	1	63
		M	8	37	4	76	8	12	5	48
		H	0	3	16	84	0	2	8	80
Energy to rupture	10	L	16	4	4	67	9	3	1	69
		M	5	32	9	70	6	9	8	39
		H	5	3	23	74	1	5	9	60

Low (L), medium (M), high (H).

4. Conclusion

Fluorescence spectroscopy seems to be a valuable method for discrimination among different meat samples according to their quality. In the present study, the results of partial-least square discriminant analysis (PLSDA) applied on 305–400 nm fluorescence spectra showed 67% and 96% (53% and 55% for validation sets) of good classification for protein content and dry matter content, respectively. Good results were also observed using 340–540 nm fluorescence spectra.

The recent development of very small spectrofluorimeters, as well as of fibre optic probes and LED emitting in the UV range, is leading to the development of integrated apparatus (Karoui & Dufour, 2008). The most attractive advantages of these laptop spectrofluorimeters are that no preliminary sample preparation is needed prior to measurement and results are obtained rapidly (a couple of seconds) compared with conventional techniques. Thus, front-face fluorescence in conjunction with chemometric tools has great potential to become a useful quality control method in rapid online, as well as offline, analysis of meat products.

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POTENTIEL DE LA SPECTROSCOPIE DE FLUORESCENCE POUR DISCRIMINER DIFFERENTS MUSCLES ET POUR PREDIRE CERTAINS PARAMETRES PHYSICO-CHIMIE DE LA VIANDE

SAHAR A[1]., BOUBELLOUTA T[1]., LEPETIT J[2]., DUFOUR É[1].

[1]UR Typicité des Produits Alimentaires, ENITA Clermont, Clermont Université, Site de Marmilhat, BP 35 – 63370 LEMPDES
[2]INRA-QuaPA, site de Theix, 63122 Saint Genès Champanelle

Introduction

La spectroscopie de fluorescence donne des informations sur la présence de fluorophores et sur leur environnement dans les échantillons. Le spectre de fluorescence d'un produit est caractéristique et spécifique de ce produit et constitue une empreinte. Comme cela est pratiqué depuis longtemps dans le domaine de la spectroscopie infrarouge, il est envisageable d'appliquer des méthodes d'analyse statistique multidimensionnelle aux spectres de fluorescence pour extraire l'information pertinente et développer des méthodes de prédiction de la qualité des produits. Le principal objectif de cette étude est d'évaluer le potentiel de la spectroscopie de fluorescence pour identifier différents muscles et pour prédire certains de leurs paramètres physico-chimiques.

Matériel et méthodes

Echantillonnage : Soixante six échantillons provenant de 14 carcasses (15 jours de maturation) de génisses de race 'charolaise' sur lesquelles ont été prélevés trois muscles - 'bavette de flanchet' ('B', n=24), 'rond de gîte' ('R', n=24), et 'paleron' ('P', n=18), ont été utilisés pour cette étude.
Physico-chimie : Les matières sèches des échantillons ont été déterminées par la méthode de référence (ISO 1442), les matières grasses en utilisant la méthode de Soxhlet (norme française V 04-403), les protéines au moyen de la méthode de Kjeldhal (ISO 937) et le collagène en mesurant l'hyroxyproline (ISO 3496). Les mesures ont été répétées trois fois sur des éprouvettes différentes pour chaque échantillon de muscle. Les mesures de texture ont été réalisées par la méthode de cisaillement selon la procédure décrite par Honikel [1]. Les paramètres suivants ont été déterminés, perte de poids (%), force maximale (N), déplacement à la force maximale (mm), énergie à la rupture (J) et contrainte maximale de cisaillement (N/cm^2). Les mesures ont été répétées 10 fois sur des éprouvettes différentes pour chaque échantillon de muscle.
Spectroscopie de fluorescence : Les spectres de fluorescence ont été enregistrés au moyen d'un spectrofluorimètre FluroMax-2 (Spex-Jobin Yves, Longjumeau, France). Les spectres d'émission de résidus tryptophane des protéines (305-400 nm), de la vitamine A (340-540 nm) et de la riboflavine (410-700 nm) ont été enregistrés après excitation à des longueurs d'onde fixées à 290, 322 et 382 nm, respectivement. Les mesures ont été répétées deux fois sur des éprouvettes différentes pour chaque échantillon de muscle.
Traitement statistique: Les jeux de données ont été évaluées par des méthodes chimiométriques telles la PLSDA – une méthode discriminante, et la PLS avec cross validation - une méthode prédictive. Le logiciel MatLab (The Mathworks Inc., Natic, MA, USA) a été utilise et les routines mises en oeuvre sont accessibles à l'adresse suivante : http://easy-chemometrics.fr.

Résultats et discussion

Les résultats (moyenne pour chaque muscle) des analyses physico-chimique sont présentés tableau 1. Le paleron (P) est caractérisé par la teneur en matières grasses (4,83%) la plus élevée, la quantité de protéines (20,91%) la plus faible, une force maximale égale à 47,26N, une énergie à la rupture de 0,34J et une valeur de cisaillement de $39,53N/cm^2$. Au contraire, le rond de gîte muscle (R) présente la plus faible valeur en matières grasses (1,6%) et les valeurs les plus élevées pour les protéines (23,86%), la force maximale (81,2N), l'énergie à la rupture (0,53J) et le cisaillement (69,06 N/cm^2). Les données physico-chimiques diffèrent d'un muscle à l'autre permettant d'envisager une discrimination.

Muscle	n	Matières grasses %	Matières sèche %	Collagène %	Protéines %	Perte de poids (%)	Force maximale (N)	Déplacement à force maximale (mm)	Energie à la rupture (J)	Cisaillement (N/cm^2)
R	24	1,60	26,16	0,78	23,86	33,18	81,20	19,07	0,53	69,06
B	24	3,40	26,83	0,76	23,23	26,58	64,75	18,05	0,49	54,75
P	18	4,83	26,87	0,87	20,91	34,43	47,26	18,92	0,34	39,53

Tableau 1 : Résultats des analyses physico-chimiques réalisées sur les trois muscles : R= rond de gîte, B= bavette de flanchet, P= paleron (n=nombres d'échantillons).

Les spectres d'émission de résidus tryptophane des protéines montrent des maxima à environ 330-332 nm (Figure 1). Par ailleurs les spectres de la riboflavine sont caractérisés par un maximum à 468-470 nm, ainsi que par un autre pic de moindre intensité à environ 645 nm qui présente un épaulement à environ 598 nm (Figure 1). Les allures de ce deuxième pic et de l'épaulement varient d'un échantillon à l'autre. Enfin, les spectres de la vitamine A montrent deux pics localisés à 467-469 nm et à 380-395 nm (Figure 1).

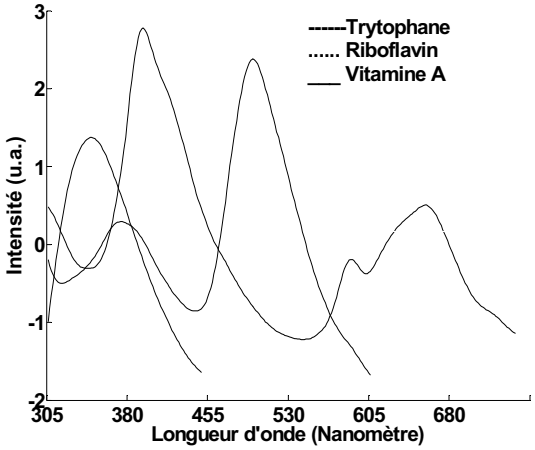

Figure 1 : Spectres d'émission de fluorescence de la viande : (- - -) tryptophane, (…….) riboflavine, et (_____) vitamine A.

Les résultats des analyses discriminantes (PLSDA) réalisées sur les données montrent des pourcentages de bonne classification de respectivement 100%, 97%, 91% et 95% pour les spectres du tryptophane, les spectres de la riboflavine, les spectres de la vitamine A et les paramètres physico-chimiques.

Les résultats de prédiction des paramètres physico-chimiques au moyen de la régression PLS avec cross validation appliquées sur les données de calibration des trois muscles R+B+P montrent une relativement bonne prédiction du collagène (R^2C=0,91) à partir des spectres des tryptophanes, une prédiction relativement correcte de l'énergie à la rupture (R^2C=0,55) et de la matière sèche (R^2C=0,51) à partir, respectivement, des spectres de la riboflavine et de la vitamine A. Par contre les R^2V pour ces paramètres présentent des valeurs très faibles (respectivement R^2V=0,32, 0,15 et 0,26 pour les trois paramètres) ; indiquant la faible robustesse des modèles de prédiction (Tableau 2).

Muscle	Tryptophane et collagène						Riboflavine et énergie à la rupture					Vitamine A et matière sèche				
	n	F	R^2C	RMSEC	R^2V	RMSEV	F	R^2C	RMSEC	R^2V	RMSEV	F	R^2C	RMSEC	R^2V	RMSEV
R+B+P	66	6	0,91	0,06	0,32	0,20	5	0,55	0,09	0,15	0,13	7	0,51	1,20	0,26	1,47
R	24	6	1	0,01	0,64	0,14	7	0,93	0,02	0,57	0,07	7	0,96	0,21	0,94	0,72
B	24	4	0,97	0,04	0,57	0,09	5	0,85	0,04	0,39	0,07	6	0,88	0,75	0,79	1,23
P	18	3	0,99	0,04	0,71	0,44	2	0,86	0,04	0,57	0,17	6	0,97	0,28	0,82	4,28

Tableau 2 : Régression PLS réalisée sur : (R+B+P) trois muscles ensemble et un seul muscle, R 'rond de gîte' B 'bavette de flanchet' et P 'paleron'. n= nombres des échantillons, F= facteur de PLS, R^2C= Coefficient de détermination de calibration, RMSEC=Root mean square error of calibration, R^2V= Coefficient de détermination de validation, RMSEV=Root mean square error of validation

Les régressions PLS ont également été réalisées sur des jeux de données de calibration et de validation ne renfermant que les données d'un seul muscle (R, B ou P). De meilleures prédictions ont été trouvées pour les taux de collagène de chacun des muscles (R, R^2C=1 & R^2V=0,64, B, R^2C=0,97 & R^2V=0,57, P, R^2C=0,99 & R^2V=0,71) à partir des spectres des tryptophanes. Enfin, les spectres de la riboflavine et de la vitamine A permettent de relativement bien prédire respectivement l'énergie à la rupture (R, R^2C=0,93 & R^2V=0,57, B, R^2C=0,85 & R^2V=0,39, P, R^2C=0,86 & R^2V=0,57) et la matière sèche (R, R^2C=0,96 & R^2V=0,94, B, R^2C=0,88 & R^2V=0,79, P, R^2C=0,97 & R^2V=0,82).

Conclusion

La spectroscopie de fluorescence frontale couplée aux méthodes chimiométriques présente un potentiel important dans le développement de méthodes rapides et non destructives pour l'identification et la caractérisation des muscles. Cette méthode apparaît comme un bon outil pour l'identification des différents muscles ; en particulier, les spectres des tryptophanes conduisant à 100% de bonne classification.

Par ailleurs, la PLS appliquée sur les données spectrales de calibration des trois muscles R+B+P donne en général de bons R^2C, alors que les données de validation conduisent à des R^2V généralement faibles ; suggérant des modèles peu robustes. A contrario, la PLS appliquée sur les données spectrales de calibration et de validation d'un seul muscle (R ou B ou P) donne en général de bons R^2C et de bons R^2V ; démontrant la robustesse des modèles de prédiction. Les différences structurales caractérisant les 3 muscles étudiés ne permettent pas de développer un modèle général. Par contre la constitution de banques de données spectrales par muscle est une piste que nous approfondissons en vue de développer des applications dans le domaine de la mesure rapide et non destructive de la qualité de la viande.

Références bibliographiques

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Synchronous Front-Face Fluorescence Spectroscopy Coupled with Parallel Factors (PARAFAC) Analysis to Study the Effects of Cooking Time on Meat

AMNA SAHAR, TAHAR BOUBELLOUTA, STÉPHANE PORTANGUEN, ALAIN KONDJOYAN, AND ÉRIC DUFOUR

ABSTRACT: In this study, the potential of synchronous front-face fluorescence coupled with chemometrics has been investigated for the analysis of cooked meat. Bovine meat samples (thin slices of 5 cm diameter) taken from *Longissimus dorsi* muscle were cooked at 237 °C for 0, 1, 2, 5, 7, and 10 min under control conditions. Synchronous front-face fluorescence spectra were collected on meat samples in the excitation wavelength range of 250 to 550 nm using offsets (Δλ) of 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, and 160 nm between excitation and emission wavelengths. The synchronous fluorescence landscape containing 360 spectra was analyzed using PARAFAC. The best PARAFAC model presented 2 components since core consistency values for the first 2 components were 100% and the explained variance was 67.98%. The loading profiles of 1st and 2nd components had an optimal Δλ of 70 and 40 nm, respectively, allowing to determine the excitation (exc.) and emission (em.) maxima wavelengths of 1st (fluorescence band at about exc.: 340 to 400/em.: 410 to 470 nm, and peak at exc.: 468/em.: 538 nm) and 2nd (exc.: 294 nm/em.: 334 nm) components. As the loading profile of the 1st component of PARAFAC was assigned to Maillard-reaction products formed during cooking, the profile of the 2nd component corresponded with the fluorescence characteristics of tryptophan residues in proteins. Loadings and scores of the PARAFAC model developed from the synchronous fluorescence spectra enabled to get information regarding the changes occurring in meat fluorophores during cooking of meat at 237 °C from 0 to 10 min.

Keywords: cooking time, meat, PARAFAC, synchronous fluorescence spectroscopy

Introduction

Meat is a complex mixture of chemical constituents and rich in proteins, making it susceptible to denaturation of proteins and formation of Maillard-reaction compounds during thermal treatments. Cooking of meat products may also generate low levels of mutagenic/carcinogenic heterocyclic amines because of some naturally present compounds like creatine, free amino acids, and monosaccharides, affecting the nutritional quality of meat.

There are growing needs to control the quality of food products in minimum possible time, which led to replace the time consuming and expensive conventional methods with some reliable, rapid, and less expensive techniques. In this regard, fluorescence spectroscopy seems to be a good candidate due to its high sensitivity to physicochemical changes occurring in food (Rizkallah and others 2008). When meat is subjected to thermal treatment, many changes occur in meat like denaturation of protein (tryptophan residues in proteins), degradation of some fluorophores (tryptophan residues in proteins, vitamins), and development of some new fluorophores (Maillard-reaction products, heterocyclic amines), which consequently changes the fluorescence signals of meat. Front-face fluorescence spectroscopy has already been used to evaluate the progress of Maillard reaction in milk (Birlouez-Aragon and others 2001; Kulmyrzaev and Dufour 2002), seeds (Yaacoub and others 2009), and infant formulas (Birlouez-Aragon and others 2005).

Synchronous fluorescence spectroscopy (SFS) has been proved to be a very useful method for the analysis of complex food matrices, for authenticity and detailed chemical characterization (Christensen and others 2006). In SFS the excitation wavelength, $-\lambda$exc, and the emission wavelength, $-\lambda$em, are scanned synchronously with a constant wavelength interval ($\Delta\lambda = \lambda em - \lambda exc$), which makes it possible to narrow the spectral bands and get information on several fluorophores in a given spectrum.

Such kind of 3-dimensional (3D) data set comprises of excitation and emission matrix (samples × offsets [Δλ] ×excitation wavelengths) can be analysed using a decomposition model like parallel factors analysis or PARAFAC, which decompose the data set into scores and loadings, enabling a more thorough interpretation of data.

Recently, 3-way fluorescence spectroscopy combined with PARAFAC modelling was successfully applied for quantitative and qualitative analysis of complex mixtures and food products (Nikolajsen and others 2003; Rodriguez-Cuesta and others 2003; Trevisan and Poppi 2003; Andersen and others 2005; Ni and others 2006; Diez and others 2007). Ni and others (2008b) used synchronous fluorescence and UV–VIS spectroscopy to study the interactions between the tetracycline antibiotic, aluminium ions, and DNA with

MS 20090489 Submitted 5/31/2009, Accepted 8/14/2009. *Authors Sahar, Boubellouta, and Dufour are with U.R. "Typicité des Produits Alimentaires," ENITA Clermont, Clermont Univ., BP 35, F-63370 Lempdes, France. Authors Portanguen and Kondjoyan are with UR QuaPA, INRA Theix, F-63122 Saint Genès Champanelle, France. Direct inquiries to author Dufour (E-mail: eric.dufour@agriculture.gouv.fr).*

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the aid of the Methylene Blue dye probe. These researchers had used PARAFAC for the decomposition of the 3-way data set. In other research, Ni and others (2008a) studied the interactions of Isoprocarb and sodium 2-isopropylphenate with bovine serum albumin. In their study, 3D plots of synchronous spectra were used, and the multivariate statistical technique, PARAFAC, was applied to decompose this 3-way data array.

Such multi-way models have been applied to auto-fluorescence landscapes of food systems as well, like sugar (Bro 1999), meat (Moller and others 2003), fish oil (Pedersen and others 2002), milk (Boubellouta and Dufour 2008), yogurt (Christensen and others 2005), cheese (Christensen and others 2003), wines (Airado-Rodriguez and others 2009), and edible oils (Guimet and others 2004; Sikorska and others 2004).

The aim of this study was to show the potential of synchronous fluorescence spectroscopy coupled with PARAFAC to detect the main fluorescent compounds present in meat and to determine the effects of cooking kinetics on these compounds.

Materials and Methods

Cooking of meat samples

Longissimus thoracis muscle was taken from the carcass of an 18-mo-old heifer immediately after slaughter. Meat samples (thin slices of 5 cm diameter) were cooked between 218 and 245 °C (average = 237 °C) for 1, 2, 5, 7, and 10 min under control conditions. Jets of superheated steam mixed with air were applied on very thin slices (1 mm to 2 mm) of meat. Samples (5 cm diameter) were prepared in quadruplicate for each time of the cooking kinetic. Kondjoyan and Portanguen (2008) gave a detailed description of the experimental apparatus used for cooking, with functional analysis. The samples were vacuum packed and stored at 4 °C for further analysis.

Synchronous fluorescence spectra

Synchronous fluorescence spectra were collected in the 250 to 550 nm excitation wavelength range using offsets of 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, and 160 nm between excitation and emission monochromators. Fluorescence spectra were recorded using a FluoroMax-2 spectrofluorimeter (Spex-Jobin Yvon, Longjumeau, France) mounted with a front-surface sample-holder and the incidence angle of the excitation radiation was set at 56° to ensure that reflected light, scattered radiation, and depolarization phenomena were minimized. The spectra were collected at room temperature. The experiment was repeated for 4 times for each cooking time, so a total of 360 synchronous fluorescence spectra (6 different times of cooking × 4 repetitions × 15 offsets) were recorded.

Chemometrics

Principal component analysis (PCA). PCA was applied to the synchronous spectra to investigate differences in the spectra. This statistical multivariate treatment makes it possible to draw similarity maps of the samples showing differences/similarities between the spectra, and to get spectral patterns (that can be interpreted like spectra) showing the most discriminant wavelengths (Dufour and Riaublanc 1997b; Herbert and others 1999; Dufour and others 2000).

Parallel factor analysis (PARAFAC). PARAFAC is a chemometric decomposition method, and is a generalization of principal component analysis (PCA) to higher-order arrays (Burdick 1995).

The PARAFAC Toolbox (Bro 1997) used in this study is available online at the following address: http://www.models.kvl.dk. PARAFAC decomposes N-order array ($N \geq 3$) into a sum of the outer products of N loading components (Rutledge and Bouveresse 2007). The spectral data were arranged in a 3-way array with samples on the 1st mode, $\Delta\lambda$ on the 2nd mode and excitation wavelength on the 3rd mode. The number of PARAFAC components necessary to reconstruct the data is an important parameter (Rutledge and Bouveresse 2007). Several methods can be used to determine the appropriate number of PARAFAC components (Louwerse and others 1999; Smilde and others 2004). In this study a diagnostic known as core consistency diagnostic or CORCONDIA has been used (Bro and Kiers 2003). The core consistency diagnostic is often used as a measure of percentage of agreement of the PARAFAC models with ideal trilinearity and guides the choice of appropriate number of PARAFAC components to be considered (Bro 1997). When the core consistency drops from a high value (above about 60%) to a low value (below about 50%), it indicates that an appropriate number of components has been attained (Moberg and others 2001). In addition, the nonnegativity has been applied in 3 modes to build the components of the models. Imposing nonnegativity constraint on decomposition model parameters of fluorescence 3-way spectral data is common practice since both the spectral intensities and fluorophore concentrations are known to be positive (Bro and others 2002). Chemometric analyses were performed using MATLAB (The Mathworks Inc., Natick, Mass., U.S.A.).

Results and Discussion

Synchronous spectra recorded on meat samples

The 3D landscapes were constructed in such a way that x-axis represents the synchronous excitation wavelength (nm), y-axis shows the wavelength interval ($\Delta\lambda$), while z-axis are plotted by linking points of equal fluorescence intensity. The 3D plot obtained from raw meat sample (Figure 1A) showed a prominent peak at about 295 nm for $\Delta\lambda = 40$ nm, originating from the fluorescence of protein tryptophan residues (Boubellouta and Dufour 2008). However a modification of intense peaks can be observed in 3D landscape of meat sample, which were cooked at 237 °C for 10 min (Figure 1B). Here the peak at about 290 nm, which was prominent in the raw meat sample became less intense, while a new intense peak emerged at about 470 nm and presented a maximum of fluorescence intensity for $\Delta\lambda = 70$ nm. This peak appeared gradually when the meat samples were cooked from 1 to 10 min.

Synchronous spectra for $\Delta\lambda = 60$ nm recorded for the meat samples cooked at 237 °C for 0, 2, and 7 min showed differences in the shapes of the spectra, that is, when the intensity of the band centered at about 290 nm decreased, the intensity of the band in the 350 to 550 nm region increased (Figure 1C). The disappearing band in the 290 nm region corresponded to the degradation/modification of tryptophan residues with the increasing cooking time (Gatellier and others 2008). At the opposite, the increase with cooking time of the band centered in the 350 to 550 nm region was associated with the formation of Maillard compounds (Estévez and others 2008; Yaacoub and others 2009).

The synchronous fluorescence spectra recorded at $\Delta\lambda = 60$ nm for the meat samples cooked for 2 min showed an intense peak at about 295 nm (maximum emission at 355 nm), which is not prominent in other spectra recorded for $\Delta\lambda = 120$ and 160 nm (Figure 2A). Another pattern of synchronous fluorescence spectra considering the previous 3 $\Delta\lambda$ could be seen for the meat samples cooked for 5 min (Figure 2B), that is, the intensities of bands in the spectra increase and decrease as a function of $\Delta\lambda$. In addition of the intense band observed at about 470 nm, another band appeared at

350 to 380 nm, as well as some less intense peaks can be seen in the region starting from 380 to 500 nm.

SFS makes it possible to narrow the spectral bands compared to excitation or emission spectra, and to have information on the fluorescent properties of several intrinsic fluorophores on a given spectrum.

Principal component analysis (PCA) of meat samples

PCAs were applied on 2 data sets ($\Delta\lambda = 40$ nm and $\Delta\lambda = 70$ nm) of synchronous spectra recorded on meat samples cooked from 0 to 10 min to investigate the potential of synchronous spectra to discriminate these samples as a function of cooking time and to retrieve additional information from different offset values. The PCA similarities maps defined by the principal components 1 and 2 for synchronous spectra at $\Delta\lambda = 40$ nm (Figure 3A) and $\Delta\lambda = 70$ nm (Figure 3B) showed that in both cases, the first 2 principal components (PCs) accounted for more than 96.7% of the total variance with a predominance of component 1 (92.4% for $\Delta\lambda = 40$ nm and 87.1% for $\Delta\lambda = 70$ nm). A discrimination of meat samples cooked for 0, 1, and 2 min from the other samples cooked for 5, 7, and 10 min was observed according to PC 1 for the data set with offset $\Delta\lambda = 40$ nm. Here samples cooked for less than 2 min were observed on the left side of PC1 and the samples cooked for more than 5 min were present on the right side. Considering spectral pattern 1, the most discriminant wavelength was observed at 294 nm and can be assigned to the fluorescence of protein tryptophan residues (Figure 3C). The intensity of the band at 294 nm was the highest for raw meat and decreased with the increase of the cooking time suggesting a drastic decrease of tryptophan content for 10 min cooking. A positive peak was also observed at 460 nm that might correspond to Maillard-reaction compounds. For the data set with $\Delta\lambda = 70$ nm, a slightly different trend appeared since the meat samples cooked for 2 min exhibited coordinates close to the origin according to PC 1 (Figure 3B). The spectral pattern associated with PC1 showed a negative band at 294 nm (Figure 3D) and, as

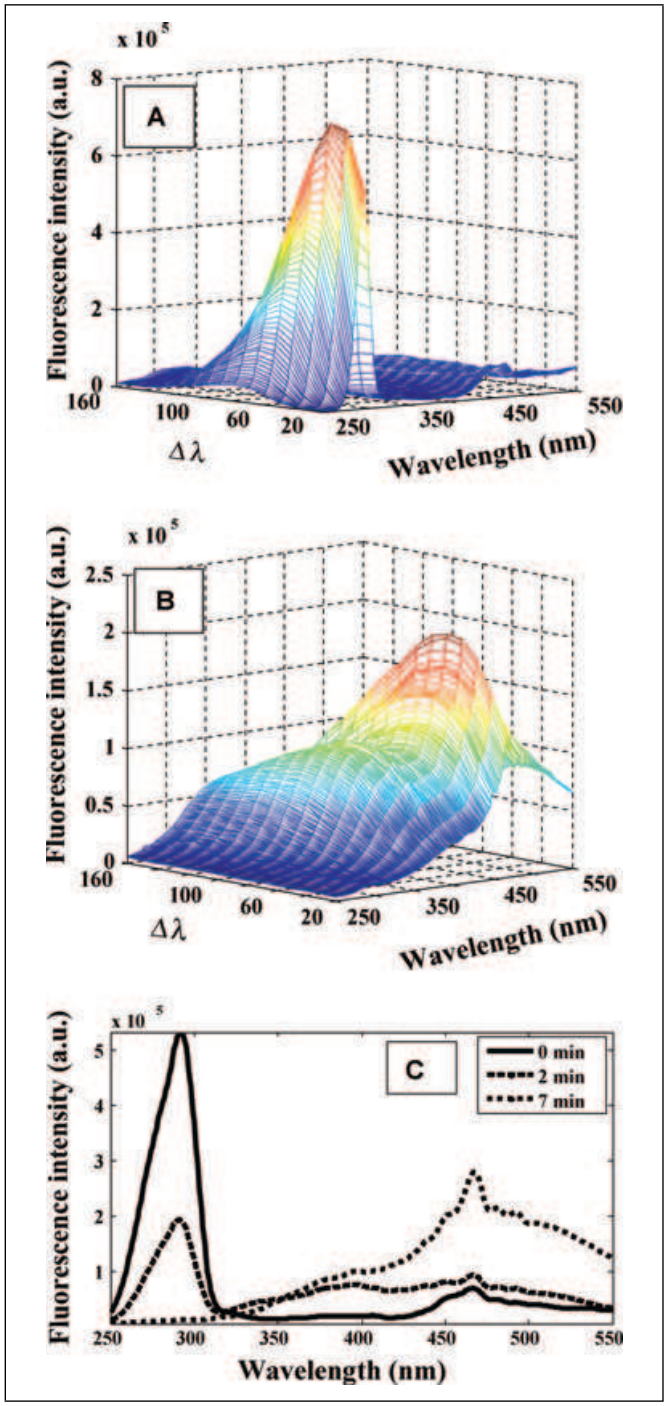

Figure 1—Synchronous fluorescent landscape of meat: (A) 3D for uncooked meat samples, (B) 3D for meat samples cooked for 10 min, and (C) 2D for meat samples cooked for 0, 2, and 7 min ($\Delta\lambda = 60$ nm).

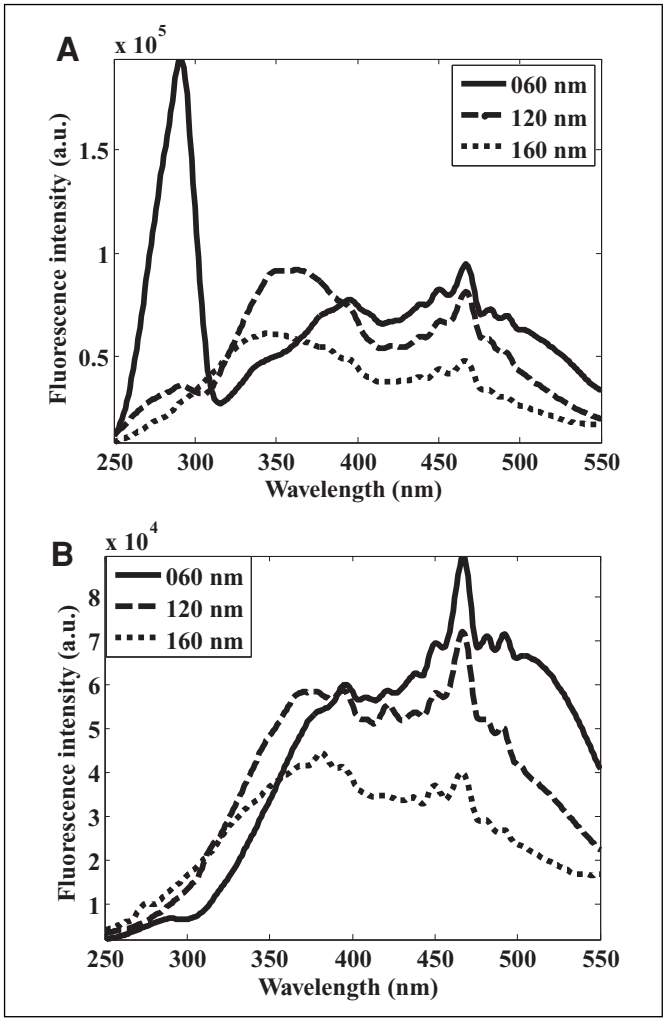

Figure 2—Synchronous fluorescence spectra of meat recorded for $\Delta\lambda = 60$, 120, and 160 nm for the meat samples cooked at 237 °C for (A) 2 and (B) 5 min.

described previously, can be assigned to the fluorescence of protein tryptophan residues. A positive peak was also observed at 460 nm that should correspond to Maillard-reaction products. Regarding PC 2, the spectral pattern exhibited 2 positive peaks at 280 and 365 nm, and a negative one at 300 nm. The opposition between the positive peak at 280 nm and the negative one at 300 nm may be attributed to the change in the environment of protein tryptophans in relation with protein denaturation. It has been shown that the emission of tryptophan is shifted towards higher wavelengths when proteins are denatured (Dufour and Riaublanc 1997a). Considering the band at 365 nm, it may be assigned to the fluorescence of Maillard-reaction compound that accumulated between 0 and 2 min, and that were transformed in other compounds fluorescing at higher wavelengths (460 nm according to spectral pattern associated with PC1) for longer cooking time.

These results confirmed that the synchronous spectra retained information related to the molecular structure of meat allowing discriminating samples as a function of cooking time. More interestingly, the differences observed on the 2 similarity maps (Figure 3A and 4B) indicated that synchronous spectra recorded at 2 different offsets contained complementary molecular information.

Calculation of the parallel factor (PARAFAC) analysis models on meat samples

The objective of PARAFAC is to resolve the synchronous fluorescence signal into the contribution of each of the fluorescent compounds present in the spectral data set of meat, that is, to estimate the profiles of fluorophores directly from the synchronous fluorescence landscapes measurements.

To investigate how many components are needed, different models were fitted using number of components varying from 1 to 5. The obtained results indicated that 2 components were suitable, since core consistency equal to 100% and explained variance amounting to 67.89% were observed. Adding the 3rd component, the core consistency decreased to 14% (87.02% of explained variance) (Table 1), suggesting that the model with 3 components was unstable and over fitted.

Considering the loading profiles of PARAFAC (Figure 4C), the 1st component with maximum excitation at about 468 nm and an optimal $\Delta\lambda$ of 70 nm (Figure 4B) corresponded to the fluorescence spectra of components that were formed during cooking of meat. This fluorophore was not described well in literature and might be referred to Maillard-reaction products. Ait Ameur (2006) found a similar peak in cookies (λexc = 470 nm and λem = 532 to 536 nm) and named it 530NF (530 nm neo-formed fluorescence). The researcher correlated it with hydroxymethylfurfural (HMF) content of the cookies. While HMF is nonfluorescent, this correlation would be indirect. An other less intense fluorescence band at an excitation range of 340 to 400 nm (λem 410 to 470 nm) could be observed in this loading profile, which might correspond to neo-formed compounds resulting from lipid oxidation (Estévez and others 2008). Similar fluorescent band had also been observed by Yaacoub and others (2009) in roasted nuts and they attributed this fluorescence zone to neo-formed compounds that were formed from proteins and lipidic secondary aldehydes by cooking.

The loading profile (Figure 4C) of the 2nd component showed maximum excitation at about 294 nm, which corresponded quite

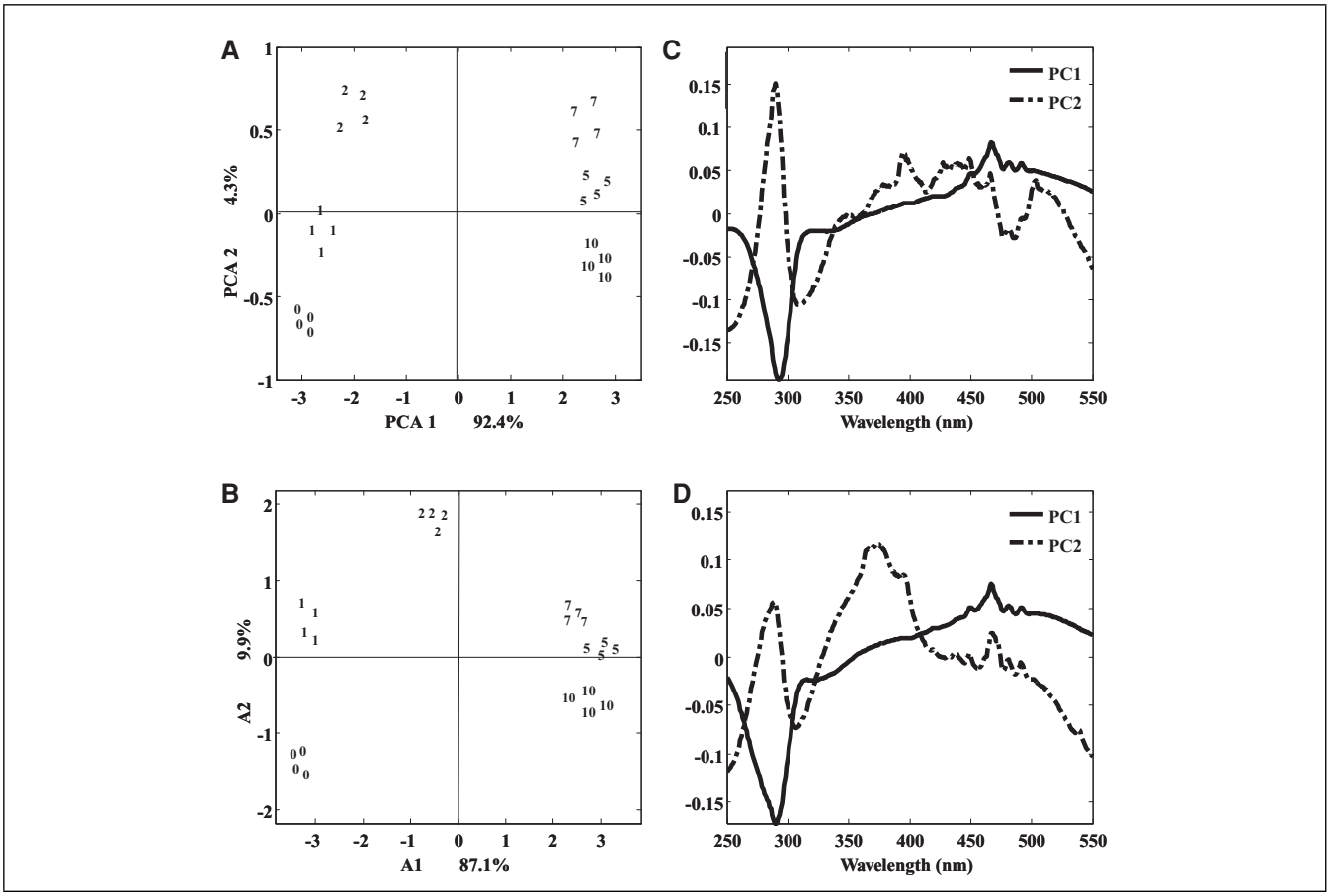

Figure 3—PCA similarity maps and their loadings defined by PC1 and PC2 for the synchronous spectra of meat samples cooked from 0 to 10 min recorded with $\Delta\lambda$ = 40 nm (A and C), and $\Delta\lambda$ = 70 nm (B and D), respectively.

well with the characteristics of tryptophan fluorescence spectra (Boubellouta and Dufour 2008), which is present in meat proteins. The optimal $\Delta\lambda$ of 40 nm (Figure 4B, Table 2) agreed with the emission maxima of tryptophan residues located at 334 nm.

Table 1—Core consistency values and the explained variance resulting from PARAFAC models derived from synchronous spectra recorded on cooked meat samples.

	Number of components of PARAFAC models				
	1	2	3	4	5
Core consistency (%)	100	100	14	0	0
Explained variance (%)	44.89	67.98	87.02	92.61	95.96

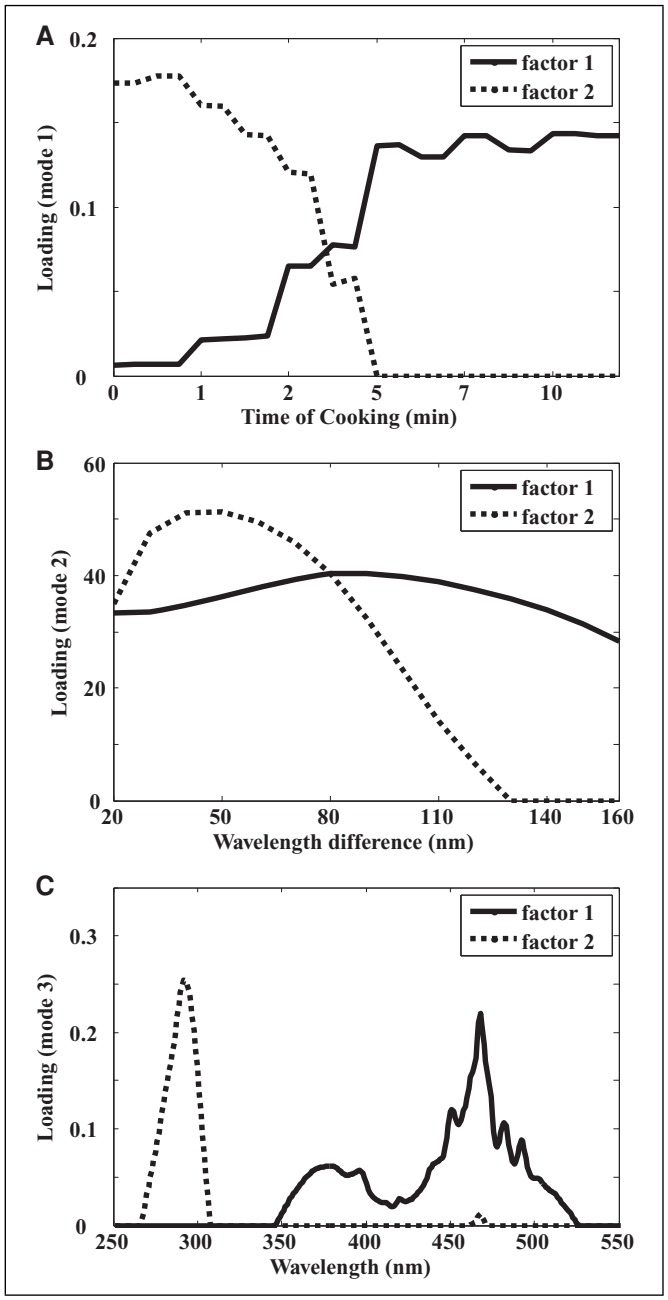

Figure 4—Two-component nonnegativity constraint model derived from the synchronous fluorescence spectra of meat cooked from 0 to 10 min. (A) Concentration mode, (B) $\Delta\lambda$ profile, and (C) loading profiles.

Table 2—Emission and excitation maxima of the 2 components of the PARAFAC model derived from synchronous spectra recorded on cooked meat samples.

	Component number of PARAFAC model	
	1	2
λ_{max} excitation (nm)	468	294
λ_{max} emission (nm)	538	334

The loading in sample mode (Figure 4A) represents the concentration mode for each fluorophore. The loading values are arranged in such a way that the changes of the properties of fluorophores can easily be caught throughout the cooking kinetic of meat samples (Boubellouta and Dufour 2008). Considering the 1st component of PARAFAC model, which corresponded to the compounds formed during the cooking kinetic, the profile of the estimated concentrations showed a significant increase with increasing time of cooking. Regarding the loading in sample mode of component 2 corresponding to tryptophan, a large decrease was observed when meat samples were submitted to cooking for 1 and 2 min while the samples that were cooked for 5, 7, and 10 min showed negligible or zero tryptophan. Tryptophan is very sensitive to oxidation at high temperature, which causes the loss of almost all tryptophan fluorescence in the meat sample when it was cooked for more than 5 min. This might happen due to pyrolisis of tryptophan, which may lead to the formation of heterocyclic amines (Bordas and others 2004).

Conclusions

This exploratory study demonstrates the potential of synchronous front-face fluorescence and chemometrics for the analysis of meat cooking kinetics. It has been shown that recorded synchronous fluorescence spectra allow to reveal information at molecular level about meat cooked for different times. PARAFAC analysis provided the mathematical decomposition of data sets. From the results of PARAFAC analysis, 2 components were found and it was shown that the 1st one can be attributed to Maillard-reaction compounds and the 2nd to tryptophan residues in proteins. In addition, this analytical method provides a simultaneous determination of the fluorescence level (that is, loading in sample concentration) of these 2 compounds. This investigation underlines the potential of synchronous fluorescence spectroscopy in combination with chemometrics as a nondestructive innovative method that can be applied to meat products for monitoring quality during cooking kinetics. The next step will be to evaluate the potential of synchronous fluorescence spectra coupled with regression methods to predict the amount of some neo-formed compounds formed during cooking.

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Potential of synchronous fluorescence spectroscopy coupled with chemometrics to determine the heterocyclic aromatic amines in grilled meat

A. SAHAR[a], S. PORTANGUEN[b], A. KONDJOYAN[b], É. DUFOUR[a,*]

[a]U.R. "Typicité des Produits Alimentaires", ENITA Clermont, Clermont Université, BP 35, F-63370 Lempdes, France

[b]UR QuaPA, INRA Theix, F-63122 Saint Genès Champanelle, France

* Present address of the corresponding author: Pr DUFOUR É, Ministère de l'alimentation, de l'agriculture et de la pêche, DG Enseignement et Recherche, 1ter avenue de Lowendal, 75700 Paris 07 SP, France

E-mail address: eric.dufour@agriculture.gouv.fr;

Tel.: +33.1.49.55.85.53

Abstract

In this study, the potential of synchronous front-face fluorescence spectroscopy (SFS) coupled with chemometric techniques was investigated for the determination of heterocyclic aromatic amines (HAA) in cooked meat samples. Bovine meat samples (1-2 mm thick, 5 cm diameter) from *Longissimus thoracis* muscle were cooked at an average temperature of 237 ^{o}C for 5, 7 and 10 minutes. Four HAA (4.8 DiMeIQx, MeIQx, IQx and PhIP) were determined on the cooked meat samples using classical LC-APCI-MS/MS method. In parallel, SFS spectra were recorded using a spectrofluorimeter on the same cooked meat samples in an excitation wavelength range of 250-550 nm using offsets of 20, 30, 40, 50, …….,160 nm between excitation and emission monochromators. The three dimensional synchronous fluorescence data set was analysed using PARAFAC (parallel factor) analysis and N-PLS (n-way partial least square) regression method. PARAFAC analysis allowed to capture the fluorescence changes occurring in meat during cooking: the best model was obtained with 2 components (core consistency of 100% and explained variance of 99.2%). Whereas the loading profile of component 1 showed a maximum excitation at about 495 nm and an optimal offset of 60 nm, the loading profile of component 2 was characterized by a maximum excitation at 367 nm and an optimal offset of 90 nm. The results obtained using N-PLS regression showed good correlation between the spectral and analytical data: average recovery of 104 % for 4,8-DiMelQx, 102 % for both MelQx and IQx, and 103 % for PhIP were obtained. In conclusion this study indicates that SFS along with chemometrics could be used as a rapid technique for the determination of HAA in meat.

Key words: Heterocyclic aromatic amines; meat, synchronous fluorescence spectroscopy; parallel factor analysis; n-way partial least square regression.

1. Introduction

Heterocyclic aromatic amines (HAA) are small molecules which formed in trace amounts when food proteins and creatine/creatinine are exposed to heat. HAA in our food come from meat that have been grilled/barbecued, broiled or pan-fried at high temperatures. The amount of HAA formed in meat are dependant on several parameters including type of meat, method, time and temperature of cooking, etc. [1]. HAA are generally formed at temperatures above 150 °C [2] and their concentration increase by increasing cooking time and temperature [3]. However after attaining maximum concentration by prolonged cooking time, HAA may be subject to degradation or react with other compounds [4].The most commonly identified compounds are IQ, IQx, MelQ, MeIQx, 7,8-DiMeIQx, 4,8-DiMeIQx, PhIP and Trp-P-2 [5, 6] (see Abbreviations[1]).

Over twenty years ago, HAA were found to be potent mutagens and about 80 % of all mutagens are also carcinogens [5] in laboratory animals (rats, mice and hamster). HAA in high quantity cause tumors of liver, colorectum, prostate and mammery glands in rodents [7] when they were fed for long period of time. Mutagenic activity of HAA increases when meat temperature is increased up to 300 °C because pyrolysis of amino acids lead to the formation of other HAA [8]. International Agency for Research on Cancer classified HAA as possible human carcinogens [9]. Thus quantitative measurements of HAA are very essential in cooked foods to minimize the risks of cancer in human beings [10]. Estimated daily intake of HAA based on the concentration of HAA in cooked foods is about 0.4 µg/person/day (for IQ, MelQ, MelQx, 4,8-DiMelQx, 7,8-DiMelQx, PhIP) [6].

Identification and quantification of HAA were done initially by isolating these HAA from large quantity of cooked meat, using multiple chromatography steps. The mutagens were monitored by Ames bacterial mutagenesis assay and characterized by NMR and mass spectroscopy [7]. Then tandem-solid phase method was developed for HAA extraction and HPLC with UV diode array was used for detection [11]. Recently, liquid chromatography-electrospray ionization/tandem mass spectrometry (LC-ESI-MS/MS) has been used to identify and characterized many undiscovered HAA in cooked meat [12-14].

[1] Abbreviations : IQ : 2-amino-3-methylimidazo[4,5-f]-quinoline ; IQx : 2-amino-3-methylimidazo[4,5-f]-quinoxaline ; MelQ : 2-amino-3,4-methylimidazo[4,5-f]quinoline ; MelQx : 2-amino-3,8-dimethyllimidazo[4,5-f]quinoxaline; 7,8-DiMelQx : 2-amino-3,7,8-trimethylimidazo[4,5-f]quinoxalino ; 4,8-DiMelQx : 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline ; PhIP : 2-amino-I-methyl-6-phenylimidazo[4,5-b]pyridine ; Trp-P-2 : 3-amino-I-methyl-5H-pyrido[4,3-b]indole.

These methods were extremely time consuming and labour intensive. The growing needs for controlling the nutritional quality and safety of food products in minimum possible time led to replace the time consuming and expensive conventional methods with some reliable, rapid and less expensive techniques to evaluate Maillard reaction and lipid oxidation progress in food.

Among other techniques, fluorescence spectroscopy is a good candidate because of its excellent sensitivity, selectivity and rapidity. This techniques had already been used to determine the thermal changes on various food products including milk [15], infant formula model [16], cookies [17] and seeds [18]. Fluorescence fingerprint of food changes globally by heating, as a result of the degradation/modification of some native fluorophores and development of neoformed fluorophores derived from Maillard reaction [19] or lipid oxidation [20]. Nowadays, synchronous front-face fluorescence spectroscopy (SFS) have been used more and more instead of traditional front-face fluorescence spectroscopic methods. In SFS, excitation and emission monochromators are scanned simultaneously, keeping a constant wavelength difference ($\Delta\lambda$) between them. Enormous information about fluorophores could derived from SFS using multivariate analysis. SFS data could be decomposed into scores and loadings using three way parallel factor analysis (PARAFAC), enabling a thorough interpretation of spectral data. This chemometric technique had already been used in many food systems such as sugar [21], meat [22], fish oil [23], yoghurt [24], cheese [25], beer [26], red wine [27] and edible oils [28-31].

Among other regression methods, N-PLS algorithm developed by Bro [32] had the potential to maintain the three dimensional structures of synchronous data; and the capability to extract lot of information about fluorescent compounds present in samples and to predict the yields of food components.

Interestingly, HAA exhibiting conjugated double bonds have fluorescence properties that have been used to detect these molecules following HPLC analysis [33]. The aim of this research work was to show the potential of SFS to monitor the fluorescence changes taking place in meat samples, cooked for 5, 7 and 10 minutes at 237 °C, using PARAFAC to determine intrinsic fluorophores formed during cooking and also to quantify four HAA present in cooked meat using N-PLS regression method.

2. Materials and methods

2.1. Cooking of meat samples

Longissimus thoracis muscle was taken from the carcass of an 18-month-old heifer immediately after slaughter. Meat samples (thin slices (1-2 mm) of 5 cm diameter) were cooked between 218 and 245 °C (average = 237 °C) for 5, 7 and 10 minutes under control conditions. Jets of superheated steam mixed with air were applied on very thin slices of meat. Kondjoyan and Portanguen [34] gave a detailed description of the experimental apparatus used for cooking, with functional analysis. The samples were vacuum packed and stored at 4 °C for further analysis.

2.2. Identification and quantification of HAA

An LC-APCI-MS/MS method previously developed by Chevolleau and other [35] for the determination of HAA was adapted and optimized to quantify four HAA (4,8-DiMeIQx, MeIQx, IQx, and PhIP) in beef meat extracts. Kondjoyan and others [36] gave detailed description of methodology concerning the determination of HAA.

2.3. Synchronous fluorescence spectra

Fluorescence spectra were recorded using a FluoroMax-2 spectrofluorimeter (Spex-Jobin Yvon, Longjumeau, France) mounted with a front-surface sample-holder and the incidence angle of the excitation radiation was set at 56° to ensure that reflected light, scattered radiation, and depolarisation phenomena were minimized. Synchronous fluorescence spectra were collected in the 250–550 nm excitation wavelength range using offsets of 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150 and 160 nm between excitation and emission monochromators. The spectra were collected at room temperature and the experiment was repeated for eight times for each cooking time, so a total of 360 synchronous fluorescence spectra (3 different times of cooking x 8 repetitions x 15 offsets) were recorded.

2.4. Chemometrics

Data analysis were performed by MatLab Software (The Mathworks Inc., Natic, MA, USA) using N-way Toolbox (http://www.models.kvl.dk).

2.4.1. Pre-treatment of the spectra

In order to reduce scattering effects, the synchronous fluorescence spectra were normalized by reducing the area under each spectrum to a value of 1 according to Bertrand and Scotter [37].

2.4.2. Parallel Factor Analysis (PARAFAC)

PARAFAC models were developed (using N-way Toolbox) for each cooking time (data array with 8 samples, 301 excitation wavelengths and 15 offsets). In order to determine the appropriate number of PARAFAC components, core consistency diagnostic was used. It is often used as a measure of percentage of agreement of the PARAFAC models with ideal trilinearity and guide the choice of the number of components to be considered [38]. When the core consistency drops from a high value (above about 60%) to a low value (below about 50%), it indicates that an appropriate number of components has been attained [39]. In addition, the non-negativity was applied in three modes since both the spectral intensities and fluorophore concentrations are known to be positive.

2.4.3. N-PLS

N-PLS, an extension of two way PLS regression for multi-way data, was used on the three-way fluorescence array to predict the quantity of HAA in cooked meat samples. For the determination of each HAA, 24 samples (8 repetitions for each cooking time) along with 15 offsets and an excitation wavelength ranging from 250 to 550 nm were used. So, three-array data set was constructed (24 x 15 x 301) for each HAA in such a way that in the sampling mode, samples were arranged in increasing cooking time, i.e., sample number 1 to 8, 9 to 16 and 17 to 24 represented 5, 7 and 10 minutes of cooking time respectively. Root mean square of prediction (RMSEP) or cross-validation and coefficient of determination (R^2) were used as parameters to determine appropriate number of latent variables (LV). In addition to cross-validated prediction of HAA, N-PLS construct a structured array, holding the N-PLS model, which could be used to plot the profiles (most discriminant wavelengths) associated with each

compound to be predicted for each LV, to determine the optimal $\Delta\lambda$ of each compound to be predicted and to get the concentration mode of each LV.

3. Results and discussion

3.1. Concentration of HAA determined by analytical technique

Concentrations of four HAA were measured in meat samples cooked at 237 °C for 5, 7 and 10 minutes (table 1). Results showed that concentration of MelQx increased by increasing the time of cooking, however concentrations of 4,8-DiMelQx, IQx and PhIP decreased in the meat samples between 5 and 7 minutes of cooking and then increased for cooking time of 10 minutes. Smaller amounts of 4,8-DiMelQx and IQx were formed in meat compared to MelQx and PhIP [40]. It was observed that standard deviation increased by increasing cooking time: it was explained by the fact that it is very difficult to obtain a perfectly similar combination of time and temperature conditions using highly superheated steam jets [36].

3.2. Synchronous fluorescence spectra of cooked meat sample

The two dimensional synchronous florescence spectra presented in figure 1 showed visual differences for the meat samples cooked for 5, 7 and 10 minutes. Two prominent peaks could be observed at about 350 and 460 nm in synchronous spectra, however the peak at about 350 nm was more prominent in the spectra of meat samples cooked for 7 minutes. These peaks were not present in uncooked meat (data not shown) and appeared gradually during the kinetics of cooking. In fact, raw meat is characterized by a band at about 290 nm corresponding to protein tryptophan residues which are rapidly degraded during cooking at 237 °C. The peaks (corresponding to a $\Delta\lambda$ = 120 nm) observed in figure 1 mainly corresponded to the fluorescence of neo-formed compounds [41-43]. It showed the potential of SFS to differentiate among the samples cooked for different periods of time, on the basis of the fluorophores present in the samples, without using time consuming separation techniques.
As shown in figure 2, the spectra recorded at different offsets (20, 60, 100 and 140 nm) of the sample cooked for 7 minutes showed different peaks. It was noted that fluorescent bands appeared and disappeared in different offsets. For example at $\Delta\lambda$ = 20 and 60 nm, fluorescence spectra of meat sample showed a small peak at about 295 nm, which minimized/disappeared in higher offsets ($\Delta\lambda$ > 70 nm). This peak might originate from the fluorescence of residual protein tryptophan residues [18, 44] and was not detected at higher offsets because of its maximum emission at about 350 nm (i.e., $\Delta\lambda$ = 60 nm). Or this peak may be attributed to HAA since the literature reports the used of fluorescence excitation at

307 nm and emission at 370 nm to detect HAA following HPLC analysis [33]. Thus, lot of different fluorophores could be identified on a given spectrum using different Δλ. These differences in spectra showed the capability of SFS to explore the sample more efficiently than the traditional fluorescence spectroscopy by narrowing the spectral bands and to collect information in a given spectrum about several intrinsic fluorophores present in the sample.

3.3. PARAFAC models for cooked meat samples

PARAFAC analysis was used to decompose the three dimensional spectral data set (Δλ x samples x excitation wavelengths) to determine the contribution of each fluorophore present in cooked meat samples. For this purpose, the first step was to convert the synchronous fluorescence data set to a three-array (15 x 24 x 301) using all offsets (15), all samples cooked for 5, 7 and 10 minutes (3 times x 8 repetitions) and all wavelengths (250 to 550 nm range). In order to develop relevant PARAFAC models, core consistency diagnostic [44, 45] was applied using 1 to 4 components (table 2). Results of core consistency diagnostic showed 100 % robustness of the model using two components of PARAFAC, and this model with 2 components took into account 99.2 % of variance. But core consistency dropped to 0 % (explained variance 99.5 %) when a third component was added to PARAFAC model, showing that the model with more than two components is unstable or over fitted. So, PARAFAC model with two components was used to the investigated data set.

Loading profile of the first component of PARAFAC model showed maximum excitation band at about 460-530 nm (figure 3A) with an optimal Δλ of 60 nm (figure 3B). It was difficult to assign this fluorescence band (λexc = 460-530, λem = 520-590 nm) with a prominent peak at 468 nm (λem = 528 nm) to some particular fluorophores of cooked meat, since not much references in the literature were found about this fluorescence band. However Ait Ameur [41] found a similar peak in this fluorescence range in cookies (λexc = 470, λem = 532-536 nm) and named this fluorophores as 530 nm neoformed fluorescence or 530NF. Author correlated this peak to hydroxymethylfurfural (HMF) content, however knowing the fact that HMF is non-fluorescent, the correlation was made indirect.

Loading profile of the second component of PARAFAC model (figure 3A) showed an excitation band at about 325-410 nm with an optimal Δλ of 90 nm (λem = 415-500 nm) (figure 3B) corresponding to various fluorophores presented in cooked meat sample. Excitation wavelengths in this region might corresponds to the fluorophores formed during Maillard reaction [41, 42] and were named as Maillard reaction fluorescence [43]. Yaacoub

and others [18] found a fluorescence zone with maximum excitation/emission wavelengths of 360/450 nm in roasted nuts and sesame seeds and associated these fluorescent molecules to neoformed compounds arising during thermal process. This fluorescent region could also represent fluorophores formed by protein oxidation. Estevez and others [46] determined the increase in fluorescent protein oxidation products in oil-in-water emulsions using excitation wavelength of 350 nm and found maximum emission at about 410-445 nm region.

Loading profile in the concentration mode of the two component of PARAFAC model showed an increase in Maillard reaction products by increasing cooking time from 5 to 7 minutes. While from 7 to 10 minutes, minute changes in concentration mode of PARAFAC model could be observed (figure 3C): the concentration mode of factor 1 slightly decreased and the one of factor 2 remained constant.

These results showed that SFS coupled with PARAFAC decomposition models could be used to detect the changes occurred in meat during cooking.

3.4. Prediction of HAA using N-PLS regression

For prediction of HAA, N-PLS regression method was applied between three dimensional spectral data of SFS and HAA determined by analytical method. Results of N-PLS with cross validation showed that more than 99 % of response variance (Y) (i.e. HAA) could be explained by 99.6 % of the variance of explanatory variables (X) using 9 LV (table 3). RMSEP seemed to be quite higher for MelQx (59.6) and PhIP (39.9) in N-PLS models because these HAA contained higher standard deviations (SD) when they were determined by analytical methods, specially for the meat samples cooked for 7 and 10 minutes. This happened due to the difficulty to obtain a perfectly similar time and temperature history for the meat samples [36]. On whole RMSEP was similar to SD values obtained by analytical measurement of HAA.

Good correlations between the actual concentrations determined using LC-APCI-MS/MS of the four HAA and their concentrations predicted from SFS data using N-PLS were demonstrated in table 4. It can be noted that very good percentages of average recovery were found for the four studied HAA, i.e., 104 % for 4,8-DiMelQx, 102 % for MelQx and IQx, and 103 % for PhIP.

N-PLS model gave a structured array that could be used to get profiles (one for each HAA to be predicted) with the most discriminant wavelengths associated to each LV along with optimal $\Delta\lambda$. Although the results of N-PLS were obtained using 9 LV, here we describe in

detail, the first two LV of each response, in order to derive wavelengths used by the N-PLS model to predict each HAA [16].

Graphical representation of first and second loadings of LV were shown in figure 4A and B. It could be noted that the profile associated with the first LV is similar for all responses (figure 4A), because similar profiles and optimal Δλ were found for the first LV. These results are in agreement with Diez and others [16] who found that the first LV were similar for all responses in heat treated infant formula models. So, First LV represents an average fluorescence spectrum of all fluorophores formed during the cooking of meat samples. However, second LV showed different profiles for each predicted HAA (figure 4B) [16]. Second LV of N-PLS regression model showed that most discriminant wavelengths used to predict 4,8-DiMelQx and PhIP were grossly opposite to MelQx and IQx and also that optimal Δλ was 40 nm for all four HAA but PhIP (Δλ = 30 nm).

Considering figure 4B, a less prominent peak can be observed on the loadings for the second LV at about 295 nm with corresponding emission wavelengths (λem) at 335 nm for 4,8-DiMelQx, MelQx and IQx. Whereas, a first peak at about 289 nm (λem = 319 nm) was observed for PhIP profile. However, the literature reports the used of fluorescence excitation at 307 nm (λem = 370 nm) to detect HAA following HPLC analysis [33]. The differences between N-PLS results and the data from the literature can be explained by the fact that the locations of excitation and emission wavelengths of fluorophores largely depend on their environments [47]: indeed, the environments of HAA in meat or in HPLC solvent are different.

The profiles associated with the 2nd LV also showed 2 prominent peaks. First prominent peak was observed at 380 nm for 4,8-DiMelQx and PhIP (λem = 420 nm and 410 nm for 4,8-DiMelQx and PhIP, respectively), whereas the spectral loading of MelQx and IQx showed a less intense but prominent peak at 397 nm (λem = 437 nm) and 378 nm (λem = 418 nm), respectively. Another intense peak was located at about 468 nm (λem = 508 for 4,8-DiMelQx, MelQx and IQx, and λem = 498 nm for PhIP). The spectral loading of DiMelQx, MelQx and IQx also showed a fluorescent band at 530-550 nm (λem = 570-590 nm), this band was not observed in the fluorescence profile associated with PhIP.

Apart from some differences in excitation spectra of HAA, the fluorescence emission spectrum of PhIP would exhibit differences from the ones of the 3 others HAA, because the N-PLS model indicated an optimal Δλ equal to 30 nm for PhIP. Thus, SFS showed that the fluorescence properties of the IQx compounds should be different from PhIP. These two types of compounds are formed from different precursors and have different chemical structures.

Precursors for IQx compounds include free amino acids (threonine for MelQx and 4,8-DiMelQx), hexoses and creatine already present in raw meat. It was thought that creatine formed the amino-imidazo part of the molecule, while the remaining part came from Strecker degradation products that were formed during Maillard reaction. However, formation of PhIP was favoured by glucose, phenylalanine and creatine [6]. The obtained results ensure the stability and robustness of N-PLS model to predict the response variable accurately.

Figure 5 showed the concentration mode of N-PLS models corresponding to the concentration of each HAA determined by 2nd LV of N-PLS model. It showed that all HAA but PhIP increased by increasing the time of cooking. Indeed, PhIP concentration was decreased when the cooking time of the meat samples were increased from 5 to 7 minutes: this hold true if we looked at the amount of PhIP measured by analytical method (table 1) which decreased between 5 and 7 min cooking. Thus, 2nd LV of N-PLS contained precise information which is related to the fluorescence properties of HAA produced in meat during cooking. Again it confirmed the accuracy of the model developed from SFS data using N-PLS.

Conclusions

In this study we demonstrated the potential of SFS and chemometrics for the analysis of HAA in cooked meat samples. Synchronous fluorescence spectra of the meat samples cooked for different periods of time reveal information at molecular level about the fluorescent changes occurring during cooking of meat, in general, and the fluorescence signals of four HAA, in particular, following PARAFAC and N-PLS analysis. From the two components of PARAFAC, lot of information could be derived about the fluorophores present in meat. In addition, N-PLS regression method was used to quantify four HAA formed during cooking and to derive information from profiles associated to LV on the fluorescence characteristics of HAA. Good correlations were found between the actual and predicted HAA. Thus SFS along with chemometrics could be used as a non-destructive and innovative method to monitoring the neo-formed compounds such as HAA during thermal processing of meat.

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Legends of the figures

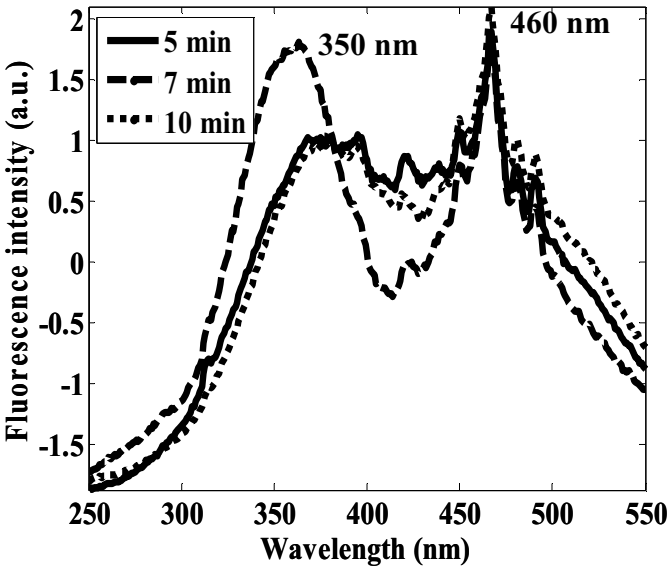
Figure 1. Normalized synchronous fluorescence spectra ($\Delta\lambda$ = 120 nm) of meat recorded after 5 , 7 and 10 minutes of cooking.

Figure 2. Normalized synchronous fluorescence spectra of meat cooked for 7 minutes recorded for different $\Delta\lambda$ ($\Delta\lambda$ = 20, 60 and 100 nm).

Figure 3. Loading profiles of the two components of PARAFAC model derived from synchronous fluorescence spectra recorded on meat samples cooked for 5, 7 and 10 minutes at 237 °C. (A) excitation profiles, (B) $\Delta\lambda$ profile, (C) Concentration mode.

Figure 4. Loading profiles of first (A) and second (B) latent variables of N-PLS model used for the prediction of heterocyclic amines (4,8-DiMelQx, IQx, MelQx and PhIP).

Figure 5. Concentration mode of N-PLS model constructed using synchronous fluorescence spectra of meat samples cooked for 5 (black dots), 7 (white dots) and 10 minutes (parallel lines).



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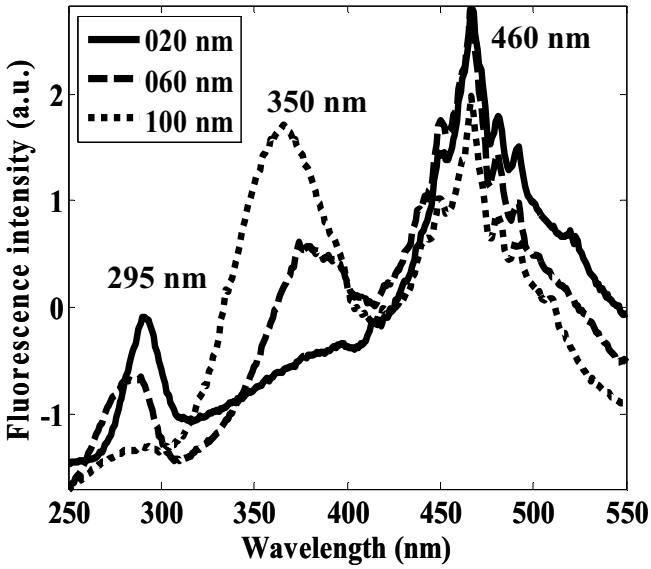
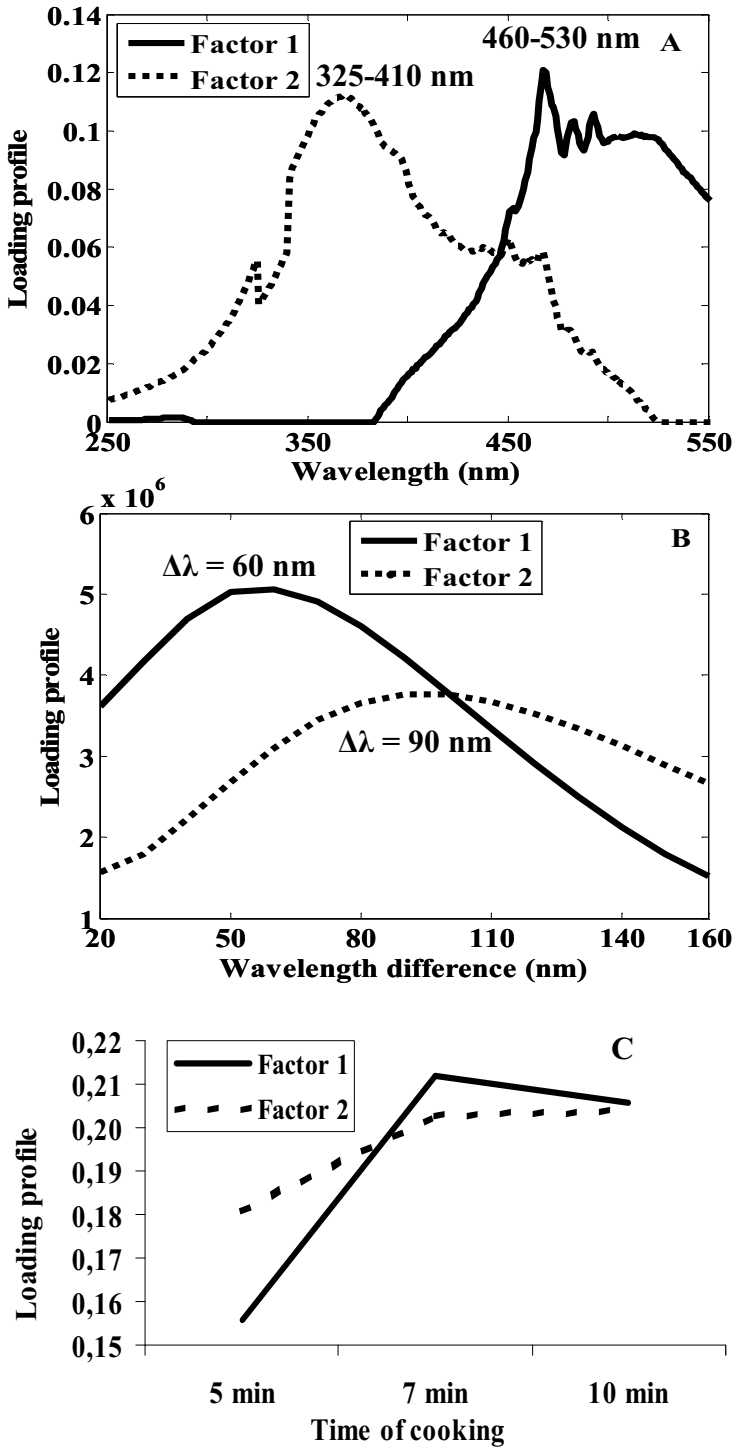


Figure 2. Normalized synchronous fluorescence spectra of meat cooked for 7 minutes recorded for different $\Delta\lambda$ ($\Delta\lambda$ = 20, 60 and 100 nm).



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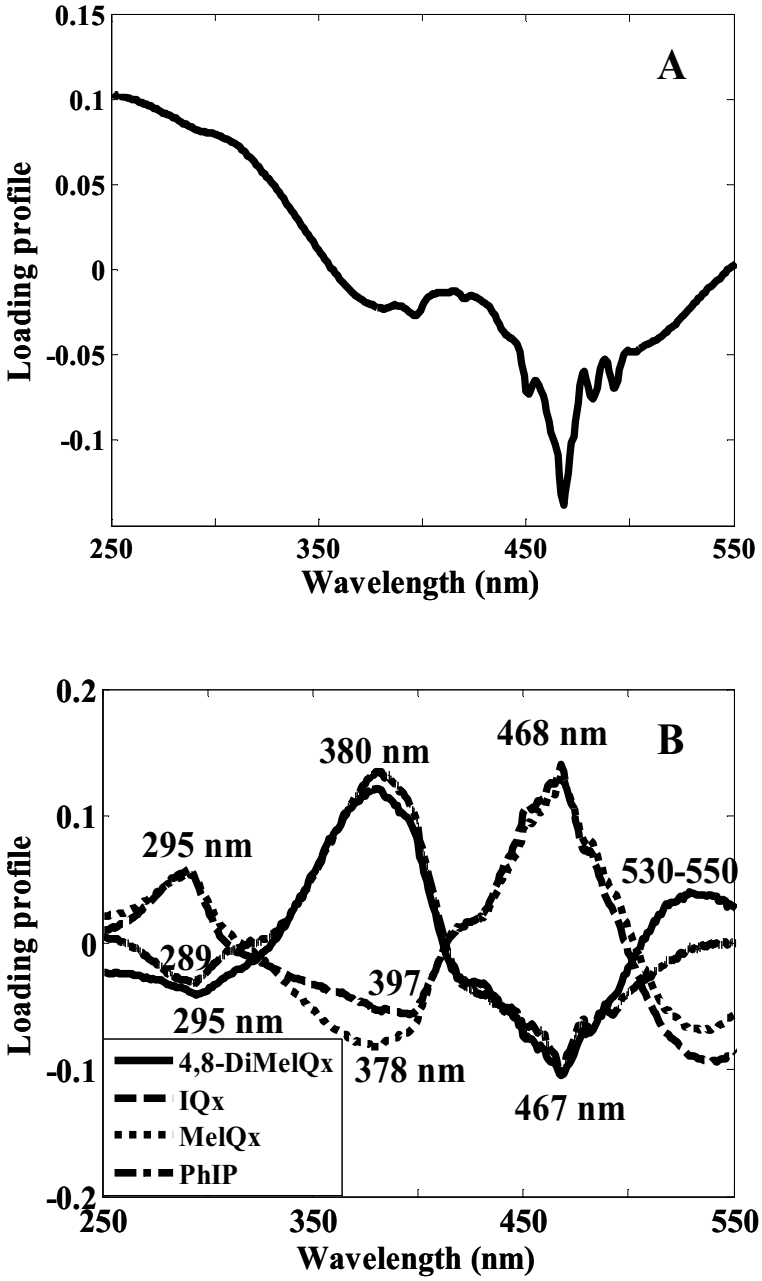

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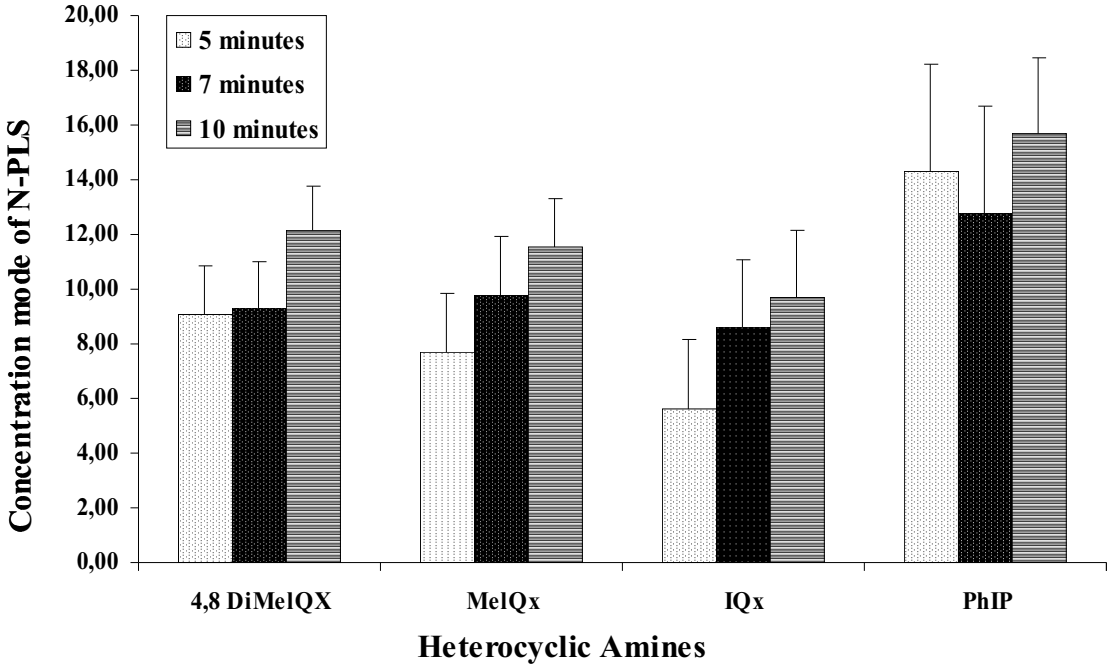

Figure 5. Concentration mode of N-PLS model constructed using synchronous fluorescence spectra of meat samples cooked for 5 (black dots), 7 (white dots) and 10 minutes (parallel lines).

Table 1 – Concentrations of four HAA (ng/g of freeze-dried product) determined by analytical method in meat samples after 5, 7 and 10 minutes of cooking at 237 °C.

Heterocyclic Amines	5 min.	7 min.	10 min.
4,8-DiMelQx	11.7 ± 1.4	10.1 ± 2.9	20.1 ± 5.8
MelQx	292.8 ± 20.0	316.9 ± 81.2	473.1 ± 89.1
IQx	17.2 ± 0.4	18.7 ± 5.2	25.5 ± 2.7
PhIP	205.0 ± 8.2	160.7 ± 50.8	292.3 ± 37.3

Table 2. Core consistency values and explained variance resulting from PARAFAC models.

	Number of components of PARAFAC models			
	1	2	3	4
Core consistency (%)	100	100	0	0
Explained Variance (%)	96.1	99.2	99.5	99.7

Table 3. Results of N-PLS regression between heterocyclic amines and synchronous fluorescence spectra of cooked meat samples.

Heterocyclic Amines	Percent Variance Captured by N-PLS Model				
	[a]LV	[b]X-Block	[c]Y-Block	[d]RMSEP	[e]R^2
4,8-DiMelQx	9	99.6	99.0	2.9	0.8
MelQx	9	99.6	99.4	59.6	0.7
IQx	9	99.6	99.3	3.5	0.5
PhIP	9	99.6	99.3	39.9	0.7

[a]LV: Number of latent variable.

[b]X-Block: Synchronous fluorescence spectra.

[c]Y-Block: Heterocyclic amines determined by analytical technique.

[d]RMSEP: Root mean square error of prediction.

[e]R^2: Coefficient of determination.

Table 4. Actual (determined by analytical method in ng/g of freeze-dried product) and predicted concentrations of four heterocyclic amines and recovery (%) using N-PLS models between synchronous fluorescence spectra and heterocyclic amines determined by analytical method.

Sample	4,8-DiMelQx			MelQx			IQx			PhIP		
No.	Actual	Predicted	Recovery	Actual	Predicted	Recovery	Actual	Predicted	Recovery	Actual	Predicted	Recovery
1	12	13	105	310	287	93	17	17	99	207	198	96
2	13	12	99	298	289	97	18	17	94	212	188	89
3	10	10	101	271	270	100	17	16	98	196	194	99
4	12	11	92	293	285	97	17	18	104	205	222	108
5	12	11	90	310	276	89	17	17	101	207	206	99
6	13	12	96	298	301	101	18	17	99	212	211	99
7	10	10	99	271	280	103	17	17	100	196	184	94
8	12	11	94	293	291	99	17	17	101	205	195	95
9	8	14	177	260	385	148	15	22	147	125	212	170
10	12	12	95	374	314	84	22	17	78	197	171	87
11	10	8	82	317	260	82	19	15	82	161	134	83
12	8	11	137	260	320	123	15	19	129	125	175	140
13	12	9	78	374	308	82	22	18	82	197	154	78
14	10	12	119	317	378	119	19	23	121	161	197	122
15	8	12	154	260	381	147	15	22	148	125	193	154
16	12	8	69	374	256	68	22	15	67	197	117	60
17	25	24	96	538	566	105	26	28	106	309	325	105
18	28	23	85	597	541	91	28	28	100	349	286	82
19	18	15	83	426	404	95	21	24	113	257	245	95
20	15	17	109	405	416	103	27	22	82	277	239	86
21	15	20	139	399	475	119	25	26	106	269	308	115
22	20	15	76	473	418	88	25	25	98	292	286	98
23	25	29	116	538	616	114	26	28	105	309	373	120
24	28	27	98	597	575	96	28	26	93	349	343	98
Average recovery (%)			**104**			**102**			**102**			**103**

Rapid determination of spoilage bacteria on chicken breast fillet using synchronous front-face fluorescence spectroscopy coupled with chemometrics

A. SAHAR, T. BOUBELLOUTA, É. DUFOUR*

U.R. "Typicité des Produits Alimentaires", ENITA Clermont, Clermont Université,

BP 35, F-63370 Lempdes, France

Running title : SFS for spoilage bacteria evaluation of chicken fillets

* Corresponding author: Pr DUFOUR É, Ministère de l'alimentation, de l'agriculture et de la pêche, DGER, 1ter avenue de Lowendal, 75700, Paris 07 SP, France

E-mail address: eric.dufour@agriculture.gouv.fr

Tel.: +33.1.49.55.85.53

Abstract

In this work potential of synchronous front-face fluorescence spectroscopy along wioth chemometric methods was investigated for the determination of microbial load on chicken breast fillets stored aerobically at 5°C during 0, 1, 2, 3, 5 and 8 days, and 15 °C for 0, 0.5, 1, 2, 3 and 5 days. Total viable count (TVC), *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta* were determined on chicken breast fillets at each step of the 2 kinetics using culture dependent methods. Initial TVC was 3.4 log cfu/cm² and TVC reached 8.4 log cfu/cm² and 8.3 log cfu/cm² following 8 days at 5°C and 2 days at 15°C, respectively. In parallel, synchronous fluorescence spectra were recorded in an excitation wavelength range of 250 – 500 nm using offsets of 20, 40, 60, ….., 180 nm between excitation and emission monochromators. PARAFAC (parallel factor) analysis allowed to capture the changes occurring in the synchronous fluorescence spectral data. The best PARAFAC models showed 3 and 2 components for kinetics recorded at 5 and 15°C, respectively. PLSDA (partial least square discriminant analysis) was carried out to test the reallocation of the spectra of the individual samples within the six groups corresponding to the six investigated storage times and the results showed that 100 % of good classifications were obtained using 4 PLS factors. Finally, N-PLS regression was used to predict the microbial counts for TVC, *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta* from the spectral data. Good average recoveries of 100 to 102 %, excellent correlations (R^2 = 0.99) and very small root mean squares (between 0.1 and 0.2 log cfu/cm²) of prediction were obtained from the spectral data sets recorded on samples stored at 5°C and 15°C for TVC, *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*.

Key words: synchronous fluorescence spectroscopy; aerobic storage; chicken; microbes; chemometrics

1. Introduction

Muscle foods including both poultry and meat are an important part of human diet since several thousands years. These foods are susceptible to spoilage arising from physical damage, chemical changes and microbial spoilage, the later being the most significant to cause meat deterioration. Spoilage mainly affect organoleptic characteristics including changes in appearance (discoloration), the development of off-odors, slime formation and any other changes in meat that makes it undesirable for human consumption (Ellis and Goodacre, 2001). Microbial load and composition of micro flora are very important parameters to determine the quality and shelf life of meat and meat products. Depending on the temperature after slaughtering, microorganisms can spoil the chicken from 4 to 10 days under refrigeration. There are different types of microorganisms that can cause spoilage even in chilled meat including Pseudomonas, *Acinetobactor, Moraxella, Aeromonas, Brochothrix thermosphacta* and family of *Enterobacteriacae*. Total viable count, *Pseudomonas* and *Enterobacteriaceae* were generally high for chicken samples stored aerobically and *Brochothrix thermosphacta* found to be prominent in modified atmosphere packaging. It is generally accepted that sensory spoilage of chicken fillets stored under aerobic conditions becomes detectable when the total viable count reaches 8 $\log_{10}$ cfu g^{-1} (Newton and Gill, 1978; Nychas and Tassou, 1997).

Meat spoilage depends on the initial load of micro flora, as well as product composition and growth environment. However, microbial growth and colonization occurs in few steps. The first step is the attachment of bacterial cells with the surface of meat (Marshall et al., 1971) and the second one involves the formation of an adhesive extracellular polysacchride layer (Costerton et al., 1981) on meat surface (slim formation). The slim layer that is responsible

for the surface spoilage of stored meat corresponds to bacteria forming biofilm and consists of a mixed-species/strains population of microorganisms (Jay et al., 2003).

The conventional microbiological methods for detection and quantification of microbial load in meat are time consuming and take several hours to several days to provide results. In recent years some new techniques were introduced to replace the culture dependent techniques. Several methods have been used since last two decades including some enumeration techniques based on microscopy (Pyle et al., 1999), ATP bioluminescence (Champiat et al., 2001) and measurement of electrical phenomenon (Betts, 1999) or some detection methods including immunological (Jabbar and Joishy, 1999) and molecular-based techniques (ELISA, PCR, etc.) (Mullis and Faloona, 1987). It is clear that there is a wide range of methods to determine the presence, type and enumeration of microorganisms in meat, but the major drawback is that these methods are still laborious and slow.

Among other techniques, spectroscopy is a good candidate as they are rapid and non-destructive analytical techniques. Mid infrared spectroscopy has been used since last few years for discrimination and detection of adulteration in meats (Al-Jowder et al., 1999; Al-Jowder et al., 1997; Al-Jowder et al., 2002). Authors have also demonstrated that a mid infrared spectrum of a bacteria is the fingerprint of this bacterial strain allowing its identification (Naumann et al., 1988). Infrared spectroscopy has been presented as an alternative to the above mentioned methods for the determination of meat spoilage. Indeed, near infrared and mid-infrared spectroscopies have also been used for rapid and quantitative detection of microbial spoilage in chicken meat (Lin et al., 2004) and beef meat (Ammor et al., 2009), respectively.

Considering spectroscopic techniques, fluorescence spectroscopy have also recently been used for identification of bacteria (Ammor et al., 2004; Leblanc and Dufour, 2002), separation of bacteria from yeast (Bhatta et al., 2006) and differentiation among microorganisms

belonging to different taxonomical families (Giana et al., 2003; Leblanc and Dufour, 2002; Sorrell et al., 1994; Tourkya et al., 2009). A lot of endogenous molecules such as proteins, vitamins, coenzymes, … of bacteria are fluorescent thus making fluorescence spectroscopy a valuable technique for the determination of microbial spoilage in meat. This technique is also well known for its excellent sensitivity, selectivity and rapidity. Front-face fluorescence spectroscopy has the potential to detect and quantify the microbial spoilage present at the surface of meat. Indeed, fluorescence characteristics of molecules are very sensitive to their environment which leads to the advantage to report a lot of information related to the changes occurring at the surface of meat sample such as the development of biofilms.

Synchronous front-face fluorescence spectroscopy (SFS) can be helpful to explore the fluorophores present in sample in more detail. In, SFS excitation and emission monochromators are scanned simultaneously, keeping a constant wavelength difference ($\Delta\lambda$) between them, which makes it possible to narrow the spectral bands and get information on several fluorophores in a given spectrum.

Parallel factor (PARAFAC) analysis, a multidimensional statistical method corresponding to the generalization of principal components analysis to higher-order arrays (Burdick, 1995), is capable for a thorough interpretation of three-way fluorescence spectral data (Bosco et al., 2006) and has already been used in many food systems including meat (Moller et al., 2003), fish oil (Pedersen et al., 2002), milk (Boubellouta and Dufour, 2008), cheese (Christensen et al., 2003), sugar (Bro, 1999), beer (Sikorska et al., 2008), red wine (Airado-Rodriguez et al., 2009) and oils (Dupuy et al., 2005; Guimet et al., 2005a; Guimet et al., 2004; Guimet et al., 2005b; Sikorska et al., 2004).

In order to quantify the microbial loads from three-way fluorescence spectral data, N-PLS regression method (Bro, 1996) can be used. This predictive method is able to extract lot of

information about fluorescent compounds present in synchronous spectra and allows to predict the counts of bacteria.

The aim of present research work was to explore the potential of SFS coupled with chemometrics to detect the changes in microbial flora, i.e., total viable count (TVC), *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*, occurring in chicken breast fillets during aerobic storage kinetics at 5 and 15 °C, and to predict the microbial counts, i.e., TVC, *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*, from synchronous fluorescence spectra.

2. Materials and methods

2.1. Sampling and storage conditions

21 chicken breast fillets without skin were purchased from a local super-market (CORA, Lempdes) in packed form and transported to the laboratory within 20 minutes. One sample was analyzed immediately and it was considered as the starting point of the storage kinetics. Samples were stored aerobically by enclosing them into permeable polyethylene bags. The samples were divided into two lots (10 fillets/lot) and packed individually (1fillet/pouch). One lot (10 fillets) of chicken breast samples was stored at 5 °C and analyzed on 1, 2, 3, 5 and 8 days of storage, while the other lot (10 fillets) was stored at 15 °C for analysis on 0.5, 1, 2, 3 and 5 days of storage. For analysis, one pouch was used for microbial determination and an other for collection of fluorescence spectra.

2.2. Microbiological analysis

Samples were taken aseptically from the pouch and placed on a clean and aseptic place at room temperature. Then the chicken fillet was sampled by swab test using sterile cotton wool

swabs held by a stick (ISO, 18593). These swabs were moistened with 0.1 % peptone water. An area of 50 cm^2 was marked with a sterile frame of 5 cm x 10 cm. The swabs were rubbed on the marked sites continuously for about 30 seconds and then put into distilled screw-capped test tube containing 10 ml of peptone water (BK018). For uniform distribution of microorganisms, these test tubes were shaken well using vortex mixer for about 30 seconds. Tenfold serial dilutions up to 10^{-8} (increased with increasing storage time) were prepared for all samples. For microbial enumeration, 50 µl of serial decimal dilutions of poultry homogenates was plated on the surface respective media plate using spiral plate technique (NF, V 08-100).

TVC were determined by incubating 50 µl of appropriate dilution on plate count agar for 72 hours at 25 °C (ISO, 2293). Pseudomonas was enumerated on Cetrimide, Fucidine and Cephaloridine (CFC) agar and incubating 50 µl of dilution at 25 °C for 48 hours (ISO, 13720). Members of *Enterobacteriaceae* family were determined by inoculated 50 µl of the suitable dilution on Violet Red Bile Dextrose Agar (VRBDA) for 24 hours at 30°C (ISO, 5552). Streptomycin Tallium Actidione Agar (STAA) was used to determine *Brochothrix thermosphacta* by incubation of the plates at 20 °C for 72 hours (ISO, 13722). Two replicates of at least five appropriate dilutions (depending on the day of sampling) were enumerated. All plates were visually analysed for morphology and typical colony types associated with each growth medium. Actual colony counts (cfu/cm^2) of microbiological data were transformed to logarithmic values.

2.3. Collection of synchronous fluorescence spectra

Each sampling day, a chicken breast fillet was taken out aseptically from one pouch and a sample of approximately 4 cm x 1 cm x 0.3 cm were excised in parallel to the microbiological analysis. The sample was placed between 2 quartz slides and mounted in the sample holder of

the spectrofluorimeter. Fluorescence spectra were recorded using a FluoroMax-2 spectrofluorimeter (Spex-Jobin Yvon, Longjumeau, France) mounted with a front-surface sample-holder and the incidence angle of the excitation radiation was set at 56° to ensure that reflected light, scattered radiation, and depolarisation phenomena were minimized. Synchronous fluorescence spectra were collected in the 250–550 nm excitation wavelength range using offsets of 20, 40, 60, 80, 100, 120, 140, 160 and 180 nm between excitation and emission monochromators. The spectra were collected at room temperature and the experiment was repeated for two times for each sample, so a total of 216 synchronous fluorescence spectra (2 different temperature of storage x 6 different time of storage x 2 repetitions x 9 offsets) were recorded.

2.4. Chemometrics

2.4.1. Pre-treatment of spectra

In order to reduce scattering effects, the synchronous fluorescence spectra were normalized by reducing the area under each spectrum to a value of 1 (Bertrand and Scotter, 1992).

2.4.2. Analysis of variance (ANOVA)

Statistical analysis of microbial data was carried out using STATISTICA software. Significance differences of the growth of each microbe during storage was determined by analysis of variance (ANOVA) using the least square difference method (Fisher test). Differences were considered significant at $P < 0.01$ level.

2.4.3. Discriminant analysis (PLSDA)

Partial least square discriminant analysis (PLSDA) with cross validation were performed on normalized spectral data in MATLAB (The Mathworks Inc., Natic, MA, USA) using ‘Saisir’

package available at the website address: http://easy-chemometrics.fr. The aim of this technique is to predict the membership of an individual to a qualitative group defined as a preliminary (Vigneau et al., 2006). Synchronous fluorescence spectra obtained using various offsets (20, 40, 60, ……., 180) were combined into one file (concatenation). Chicken breast samples were classified into six groups, according to their storage time, i.e., 0, 1, 2, 3, 5 and 8 days for 5°C, and 0, 0.5, 1, 2, 3 and 5 days for 15 °C. The PLSDA assesses new synthetic variables called loading factors, which are linear combinations of the variables and allows a better separation of the centre of gravity of the considered groups. The method allows the individual samples to be reallocated within the various groups. Comparison of the predicted groups to the real group is an indicator of the quality of the discrimination and it is valued as the percentage of correct classification. The spectral collection was divided into two groups: half of the investigated chicken breast samples were used for the calibration set and half as validation set.

2.4.4. Parallel Factor (PARAFAC) analysis

PARAFAC model of meat samples were developed (using N-way Toolbox) for each storage temperature (3-way fluorescence array with 12 samples (6 storage times x 2 repetitions, for a given temperature), 301 excitation wavelengths and 9 offsets). In order to determine the appropriate number of PARAFAC components, core consistency diagnostic was used as a measure of percentage of agreement of the PARAFAC models with ideal trilinearity and guide the choice of the number of components to be considered (Pyle et al., 1999). When the core consistency drops from a high value to a low value (below 50%), it indicates that an appropriate number of components has been attained (Moberg et al., 2001). In addition, the non-negativity had applied in three modes since both the spectral intensities and fluorophore concentrations are known to be positive.

Data analysis were preformed by MatLab Software (The Mathworks Inc., Natic, MA, USA) using N-way Toolbox (http://www.models.kvl.dk).

2.4.5. N-PLS regression method

N-PLS (extension of two way PLS regression for multi-way data) was used on the three-way fluorescence array to predict the load of microbes (TVC*, Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta)* of chicken samples stored at 5 °C and 15 °C.

For each storage temperature, N-PLS model (Bro, 1996) was constructed on 12 samples (2 repetitions x 6 storage times) along with 9 offsets and an excitation wavelength ranging from 250 to 550 nm. So, three-way data array was constructed (12 x 9 x 301) for each storage temperature. Similarly N-PLS regression was also tested on the 2 data sets merged together (two storage temperature), and for this purpose three-array was constructed on 24 samples (24 x 9 x 301).

Root mean square of prediction (RMSEP), recovery percentage and coefficient of determination (R^2) were used as parameters to determine appropriate number of latent variables (LV) (Durante et al., 2006; Rezaei et al., 2009). In addition to cross-validated prediction of microbial load, N-PLS construct a structured array holding the N-PLS model, which could be used to plot the excitation spectra associated with each LV, to determine the optimal $\Delta\lambda$ and to get the concentration mode associated with each LV (Diez et al., 2008).

3. Results

3.1. Changes in microbial flora of fresh chicken breast samples stored aerobically at 5 and 15 °C

In this study we monitored the changes in microbial flora (TVC, *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*) of chicken breast fillets, which were stored aerobically at 5 and 15 °C for several days (table 1). Microbial growths were significantly different for the storage kinetics at 5°C and 15 °C. As TVC increased significantly from day 1 to day 8 for storage at 5°C reaching 8.4 log cfu/cm^2, the microbial load reached a plateau after day 2 in the sample stored at 15 °C.

Pseudomonas grew significantly from 0 to 8 day of storage at 5°C and became the dominant population on day 3 (7.4 log cfu/cm^2) increasing up to 8.3 log cfu/cm^2 on 8th day of storage. In case of samples stored at 15 °C, *Pseudomonas* counts reached a maximum (8.1 log cfu/cm^2) after 2 days of storage.

Initial (0 day) counts of *Enterobacteriaceae* were 3.4 log cfu/cm^2, which increased significantly from 1 to 8 days of storage at 5°C. However these bacteria reached a plateau after 2nd day of storage at 15°C .

Brochothrix thermosphacta also showed increased of the counts during kinetics of storage: starting with an initial load of 2.9 log cfu/cm^2, the final counts reached 8.0 log cfu/cm^2 and 7.4 log cfu/cm^2 for storage at 5°C and 15°C, respectively. However, unlike other bacteria, *Brochothrix thermosphacta* population significantly kept on increasing until 5th day of storage at 15 °C.

3.2. Synchronous fluorescence spectra of meat samples stored at 5 and 15 °C

Two dimensional synchronous fluorescence spectra of chicken fillet samples stored at 5°C for 1, 3 and 5 days were shown in figure 1A. Visual differences were observed among the three spectra. The spectra obtained on the first day of storage showed two prominent peaks at about 290 nm (λem = 410 nm) and 350 nm (λem = 470 nm) along with a fluorescent band at 445 – 470 nm (λem = 565 – 590 nm). The intensity of peak at about 290 nm decreased by increasing days of storage, and, in the same time, the intensity of the peak at about 350 nm (λem = 470 nm) increased and became a fluorescent band at 325 – 350 nm (λem = 445 – 470 nm).

Synchronous fluorescence spectra recorded at different offsets (40, 120 and 160 nm) on chicken fillet stored for 2 days at 5°C were shown in figure 1B. It appeared that each offset showed a particular set of peaks. The spectrum collected using an offset of 40 nm showed one prominent peak at 300 nm (λem = 340 nm), whereas the spectrum collected for an offset of 120 nm showed a fluorescent band at 280 – 300 nm (λem = 400 – 420 nm), one peak at about 350 nm (λem = 470 nm) and an other fluorescent band at 445 – 470 nm. Finally, the spectrum recorded with an offset of 160 nm contained a prominent peak at 350 nm (λem = 510 nm) with a fluorescent region at 445 – 470 nm, but the peak at 300 nm has disappeared.

3.3. Classification of chicken fillet samples according to their storage time from SFS spectra

The synchronous fluorescence data collected using different offsets during the storage kinetic at 5°C were combined (concatenation) into one data set . Another data set was built for spectral data collected during the storage kinetic at 15°C. PLSDA was applied on each data set in order to test the ability of the spectra to discriminate between each of the six storage times.

Calibration and validation results of the discriminant model using four factors made it possible to obtain 100 % of good classification at 5 °C (table 2). Similar results were obtained for the samples stored at 15 °C using a PLSDA model with 4 factors (data not shown).

3.4. Decomposition of three way spectral data using PARAFAC

Synchronous fluorescence data set were converted into 2 three–way fluorescence arrays (Δλ x samples x excitation spectra), i.e., one for each storage kinetic, and PARAFAC analysis was used to decompose these 2 matrices into the pure spectra of contributing fluorophores. For PARAFAC analysis performed on samples stored at 5°C for 0, 1, 2, 3, 5 and 8 days, the results of the core consistency diagnostic (table 3) showed 95 % robustness (and 99.7 % of explained variance) using 3 components. Adding a fourth component dropped core consistency values to 40 %, showing that the model was unstable or over fitted. Thus a PARAFAC model with 3 components was used to describe the matrix formed by spectra recorded at 5 °C. Loading profile of the first component of PARAFAC model showed maximum excitation at 292 nm (figure 2A), with an optimal Δλ of 60 nm. Similarly, the second component of PARAFAC model showed a maximum excitation peak at 302 nm (figure 2A) and an optimal Δλ of 20 nm (figure 2B). Finally, the loading profile of the third component of PARAFAC model was characterized by two excitation maxima at 268 nm and 350 nm along with a fluorescent band at about 445 – 470 nm, and an optimal Δλ of 100 nm.

Considering the loading profiles of the concentration mode (figure 2C) for the three components of PARAFAC model, an increase in fluorophore concentrations from 0 to 3 days of storage was observed, afterward they changed minutely until day 8 of storage.

Core consistency diagnostic test applied to the spectral data of chicken samples stored at 15 °C showed that, in this case, a model with two components was robust with 100 % core consistency value and 96.3 % of explained variance (table 3). If an additional component was

added, the core consistency values dropped to 35 %. Considering this PARAFAC model, the loading profile of first component showed an excitation maximum at 301 nm with an optimal Δλ of 40 nm, while the second component showed an excitation maximum at 280 nm (Δλ = 80 nm) along a band at about 325 – 395 nm.

We also constructed a matrix combining the two data sets obtained at 5 and 15 °C. In this case, a four component PARAFAC model was obtained with 55 % of core consistency and 99.6 % of explained variance. The loading profiles of the four components (excitation and emission maxima) were given in table 4. The loading profile of the first component showed an excitation maximum at 295 (Δλ = 40 nm), the second component was characterized by two prominent peaks at 265 and 303 nm (Δλ = 20 nm), the third component showed a peak at 272 nm (Δλ = 60 nm) and the fourth components showed two fluorescent peaks at 288 and 352 nm (Δλ = 100 nm).

3.5. Prediction of bacterial counts using N-PLS

N-PLS regression method was applied on the three dimensional synchronous fluorescence spectral data in order to predict bacterial counts (TVC*, Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*) found on chicken breast samples and the results of the prediction are presented in table 5.

The results of N-PLS regression with cross validation applied for data recorded on chicken fillet samples stored at 5 °C showed that 99.9 % of the response variance (Y) (i.e. microbes) were explained by using more than 92 % of the explanatory variable (X). In addition, coefficient of determination of 0.99 was obtained using N-PLS models with 8, 7, 4 and 8 LV to predict TVC, *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*, respectively. The obtained results showed excellent recoveries (101 to 102 %) and small

errors of cross validation, i.e., 0.14, 0.2, 0.25 and 0.15 log cfu/cm^2 for TVC, *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*, respectively.

Graphical representations of loading profiles associated with first, second, third and fourth LV are shown in figure 3. It was observed that the 4 loading profiles, corresponding to TVC, *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta* prediction, of the first LV of N-PLS model were similar for all responses, with a maximum peak at 300 nm and two fluorescence regions in 335 – 365 nm and 445 – 475 nm ranges (figure 3A), with an optimal $\Delta\lambda$ of 40 nm. Figure 3B shows the 4 loading profiles associated with 2nd LV corresponding to the 4 bacteria to be predicted. In this case, the loading profiles presented some differences, but they all exhibited an optimal $\Delta\lambda$ of 120 nm. First prominent positive fluorescent peak was observed at 270 – 300 nm in the spectral loading of TVC, *Pseudomonas* and *Enterobacteriaceae*, whereas loading profile of *Brochothrix thermosphacta* showed a peak slightly shifted to higher wavelengths with a maximum at 296 nm. An other prominent negative peak was seen in the spectral loading of TVC, *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta* at 323 nm. This band was followed by a positive fluorescent band at about 400 – 435 nm and a negative one at 450 – 470 nm. Interestingly, the 2nd LV loading associated to *Brochothrix thermosphacta* showed a peak slightly shifted to higher wavelengths with a maximum at 428 nm compared with other considered bacteria.

Figure 3C showed the loading profiles of the 3rd LV. Four positive fluorescence peaks were observed at about 263, 310, 370 and 437 nm, along a negative peak at 290 nm, a fluorescent band at about 335 – 350 nm and another negative peak at 405 nm. Loading profile corresponding to the four bacteria to be predicted showed fluorescent differences, they all hadan optimal $\Delta\lambda$ of 120 nm except TVC, which contained an optimal $\Delta\lambda$ of 140 nm. Finally, loading profiles associated to the 4th LV are shown in figure 3D. Negative fluorescent peaks were observed at about 263 nm, 310 nm and 370 nm and positive fluorescent peaks could be

seen at about 292 nm, 325 nm, 450 nm with a shoulder at about 420 nm for TVC, *Pseudomonas, Enterobacteriaceae*. Whereas, *Brochothrix thermosphacta* showed bands at similar wavelengths, but mostly in the opposite direction compared to the other investigated bacteria. In fact the profiles associated with 4^{th} LV exhibited differences from one bacteria to another allowing to predict the counts of investigated bacteria. An optimal $\Delta\lambda$ of 120 nm was observed for all loading profiles of bacteria.

N-PLS regression was also carried out using data recorded on chicken breast samples stored at 15 °C for 0, 0.5, 1, 2, 3 and 5 days. From the results, it could be noted that 99.9 % of response variance was explained by about 91 % of explanatory variance using 6 LV. In addition, N-PLS model gave low RMSEP values, coefficients of determination of 0.99 and percentages of recovery in the 100 to 101 % range (table 5).

N-PLS regression was also carried out on the matrix gathering all the spectra collected during the kinetics at 5 and 15 °C. As shown table 5, the results showed good correlations (0.95 for TVC and *Brochothrix thermosphacta*, 0.94 for *Pseudomonas* and *Enterobacteriaceae*) and high percentages of recovery (100 % for TVC and *Enterobacteriaceae*, and 101 % for *Pseudomonas* and *Brochothrix thermosphacta*).

4. Discussion

Determination of microbial spoilage is traditionally performed by culture dependant methods which are no doubt reliable but are time consuming, costly and destructive, which make them irrelevant for rapid and on-line analysis. Sensory analysis is also often used to evaluate the freshness/spoilage of meat and meat products. The disadvantages of sensory analysis are its reliance on highly trained panelists, which makes it costly and unattractive for routine application. Today there is a need to establish rapid and innovative methods based on biochemical changes occurring in the meat (microbial metabolic products etc.), as a tool to assess meat spoilage (Ammor et al., 2009), or on the direct determinations of the different bacteria present at the surface of spoiled meat.

Knowing the fact that microorganisms grow on the surface of the chicken muscles and form biofilms, the most popular way to sample them on the meat surface is swab test (Martinez et al., 2009). In this study we determined microbial loads of chicken breast samples using traditional methods following sampling using the swab test. Low initial load of microbes of chicken fillet samples determined by traditional methods, suggested overall good hygienic conditions and safety practices applied at the poultry slaughterhouse (Balamatsia et al., 2006). In this experiment, initial loads of *Enterobacteriaceae* and TVC (3.4 log cfu/cm^2) were higher than *Pseudomonas* and *Brochothrix thermosphacta* (2.9 log cfu/cm^2). Although *Pseudomonas* had low initial load, it became the most prominent microbe (7.4 log cfu/cm^2) on third day of storage at 5°C. It has already been cited that *Pseudomonas* grow faster in aerobic storage and at low temperature than other bacteria, and remain dominant (Gill and Newton, 1977). Considering meat samples stored at 15 °C, *Enterobacteriaceae* were the dominant bacteria. This is in agreement with the results reported by (Huis In't Veld, 1996; Liu et al., 2006).

It has been reported that the first sign of product deterioration (off-odor) started when TVC reached 7 log cfu/cm^2 (Balamatsia et al., 2006). When surface population of bacteria reached 8 log cfu/cm^2, simple carbohydrates were almost exhausted leading to sensory spoilage (Ellis and Goodacre, 2001). Our results showed TVC of 8.4 and 8.3 log cfu/cm^2 for the samples stored at 5 °C (8th day) and 15 °C (2nd day), respectively, showing upper acceptability limits for fresh poultry meat (Patsias et al., 2006).

Fourier transform infrared spectroscopy has already been used for the monitoring of the meat spoilage kinetics (Ammor et al., 2009; Ellis et al., 2004; Ellis and Goodacre, 2001). Recently fluorescence spectroscopy emerged as a rapid, easy to use and non-destructive analytical technique for the prediction of cheese texture (Karoui et al., 2003), the authentication of food products (Karoui et al., 2004) and the identification of bacteria (Leblanc and Dufour, 2002; Tourkya et al., 2009). As fluorescence spectroscopy is very specific, sensitive and allows measurements in ppm range of compounds in food products (Karoui et al., 2004; Kulmyrzaev and Dufour, 2002; Kulmyrzaev et al., 2007), it might be a good candidate for the determination of spoilage on the meat surface.

Indeed, fluorescence spectroscopy offers several inherent advantages. First, it is 100-1000 times more sensitive than other spectrophotometric techniques. Second, fluorescent compounds extensively represented in muscle and bacteria cells are extremely sensitive to their environment. This environmental sensitivity enables characterization of conformational changes such as those attributable to the thermal, solvent or surface denaturation of proteins, as well as the interactions of proteins with other food components. Third, most fluorescence methods are relatively rapid and a spectrum is recorded in less than 1 second with a CCD detector. Moreover, front-face or surface fluorescence spectroscopy developed at the end of the sixties (Parker, 1968) allows the investigation of the fluorescence properties of powdered, turbid and concentrated samples. In this case, the surface of the samples is simply illuminated

by excitation light, and the emitted fluorescence from the same surface is measured. Due to the high absorbance of these samples, all the photons are absorbed by the first 100th microns of the sample (Genot et al., 1992).

In this study front-face synchronous fluorescence spectroscopy was used to monitor the microbial spoilage on the surface of chicken fillets stored at 5 and 15 °C. Visual differences could be noted among various spectra collected on the samples which were stored at 5 and 15 °C, showing the potential of SFS to differentiate among different samples stored in different storage conditions. Appearance and disappearance of certain peaks as a function of offset values show the ability of SFS to explore the sample in an efficient way and that, in a given spectrum, information can be gathered on different fluorophores present in the sample. This suggests the potential of synchronous fluorescence spectra to discriminate between times and temperatures of storage of chicken fillets. PLSDA has been used to investigate the ability to discriminate the six storage times from the fluorescence spectra. Considering PLSDA results, 100% of good classification of the samples have been obtained for the kinetics performed either at 5°C or 15°C. These results confirm the capability of SFS spectra to capture the changes occurring at the surface of chicken muscles during storage kinetics.

As mentioned above, a synchronous fluorescence spectrum exhibits peaks corresponding to several different fluorophores that are present at the surface of the sample. In order to identify these fluorophores, PARAFAC analysis has been applied on the three-way fluorescence array. This method decomposes the fluorescence landscapes into trilinear components according to the number of fluorophores present in the sample and the loading profiles could be related to the fluorescence characteristics of fluorophores. Thus, excitation and emission wavelengths of loading profiles could be used for the interpretation and identification of the fluorophores (Christensen et al., 2005).

The main intrinsic fluorophores retained by bacteria (Leblanc and Dufour, 2002), as well as by muscular cells (Skjervold et al., 2003), include aromatic amino acids, nucleic acids, vitamins and some co-enzymes. Among aromatic amino acids, tryptophan in proteins is fluorescent at an excitation of 295 nm, whereas at an excitation range of 280 – 295 nm both tyrosine and tryptophan are fluorescent. In addition, phenylalanine shows fluorescence along with tyrosine and tryptophan from 250 to 260 nm (Tourkya et al., 2009). Nucleic acids also exhibit fluorescence, but their quantum yields are lower than aromatic amino acids (Leblanc and Dufour, 2002). In order to discriminate bacteria or investigate the metabolic activities of bacterial cells, NADH could be used as a marker (Leblanc and Dufour, 2002). NADH is highly fluorescent following excitation at about 340 nm (emission: 440 nm). Similarly, Skjervold et al (2003) have identified several fluorophores in meat, i.e., tryptophan (exc.: 290 nm/em.: 332 nm) in proteins of meat myofibrils, vitamin A (exc.: 322 nm/em.: 440 nm) in fats and pyroindoline (exc.: 380 nm/em.: 440 nm) in connective tissue.

Considering the PARAFAC model with 3 components describing the matrix formed by spectra recorded at 5 °C, the loading profile of the first component with a maximum excitation at 292 nm (optimal $\Delta\lambda$ of 60 nm) corresponds well with the fluorescence characteristics of tryptophan residues in proteins (from meat and bacteria) reported in the literature. Similarly, the loading profile of the third component characterized by two excitation maxima at 268 nm and 350 nm (optimal $\Delta\lambda$ of 100 nm) can be assigned to NADH of bacteria as reported by Leblanc and Dufour (2002). Regarding the loading profile of the second component, fluorophore with a maximum excitation peak at 302 nm and an optimal $\Delta\lambda$ of 20 nm has never been reported in the literature from our knowledge.

The loading in sample mode (Fig. 2C) represents the concentration mode for each fluorophore. For the spectral data recorded during the storage kinetic at 5°C, the concentrations of the 3 components increase between 0 and 3 days, then the concentrations of

components 1 and 2 remain constant till 8 days, and the one of component 3 slightly decreases. These changes in concentration mode of the 3 components parallel well with microbial growth at 5 °C (table 1), i.e., TVC counts drastically increase from day 0 to day 3 and then they slightly increase till day 8. The results of PARAFAC analysis in concentration mode suggest that synchronous fluorescence spectra retain information that is correlated to the bacterial counts determined during the storage kinetics of chicken fillets. It has also been shown that part of the information retains in the synchronous fluorescence spectra may originate from fluorophores found in bacteria such as NADH. For the other identified fluorophore, i.e., tryptophan, the recorded signal may come from bacteria, as well as from muscle cells.

It indicates that synchronous fluorescence spectra can be used to predict the loads of the different bacteria investigated (Total Viable Count*, Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*). N-PLS regression method has been used to correlate the results of microbial data and three dimensional synchronous fluorescence data. For Total Viable Count*, Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta,* good average recoveries (101-102%), excellent correlations (R^2 = 0.99) and very small root mean squares (between 0.1 and 0.2 log cfu/cm²) of prediction were obtained from the data sets obtained at 5°C and 15°C. These results confirm that the synchronous fluorescence spectra contain information that is related to bacteria found on the surface of meat and, in addition, there is information that is specific to a given genus allowing to predict its count. Indeed, it has been shown that an emission spectrum recorded on a given bacteria is a fingerprint allowing its identification (Leblanc and Dufour, 2002; Tourkya et al., 2009). Synchronous fluorescence spectra show that it is also possible to identify and to quantify a given bacteria in a mixture, without any prior isolation. This is made possible because the loading profiles associated with LVs of the N-PLS models exhibit specific profiles. If the first LV is considered (Figure 3A),

all the four loading profiles superimpose. But it has already been referred that loading profile of the first LV of N-PLS model remained similar for all responses (Diez et al., 2008; Guimet et al., 2005c). Considering the second LV, the loading profiles (Figure 3B) associated to TVC, *Pseudomonas, Enterobacteriaceae* are similar, whereas the one corresponding to *Brochothrix thermosphacta* present large differences. These differences in the discriminant wavelengths forming the 2nd LV allow to discriminate *Brochothrix thermosphacta,* a gram-positive bacteria, from other ones. The 3rd LV also shows spectral differences among the four studied bacteria: it separated TVC from the other bacteria because loading profile of TVC had an optimal $\Delta\lambda$ of 140 nm, while loading profiles of all other bacteria had an optimal $\Delta\lambda$ of 120 nm. The loading profiles associated with 4th LV corresponding to the 4 bacteria clearly separated *Brochothrix thermosphacta* from all others as fluorescent peaks corresponded to *Brochothrix thermosphacta* are always in opposition to the other bacteria, thus separating a gram positive from the rest of the bacteria present on the surface of chicken fillet. In addition, the profiles associated to TVC, *Pseudomonas,* and *Enterobacteriaceae* show differences in the shapes of the bands. It is concluded that synchronous fluorescence spectra retain information which is specific to each of the investigated bacteria and that can be taken out using chemometric methods.

5. Conclusions

In this research study we presented the potential of SFS along with chemometrics for the determination of microbial load on the surface of chicken fillets stored at 5 and 15 °C. SFS is capable to reveal changes in fluorescence of meat surface during storage kinetics and PLSDA performed on SFS spectra allow to classify the samples correctly (100 % of good

classification) according to their storage time. We have also shown that PARAFAC allow to derive lot of information about 3-way fluorescence matrices and results strongly suggest that the spectral information is originating from bacteria present at the surface of chicken fillets. N-PLS regression makes it possible to predict each studied bacteria count from SFS data with a good accuracy. The loading profiles associated with LVs confirm that the three dimensional synchronous fluorescence spectral data recorded directly at the surface of chicken fillets retain specific information on the investigated bacteria (Total Viable Count*, Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta)*. Thus SFS coupled with chemometrics could be used as an innovative method to monitor the microbial changes taking place in meat during storage.

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Legends of figures

Figure 1. Synchronous fluorescence spectra of chicken fillets (A) stored for 1, 3 and 5 days at 5 °C ($\Delta\lambda$ = 120 nm), and (B) stored for 2 days at 5 °C with $\Delta\lambda$ = 40, 120 and 160 nm.

Figure 2. Three components non-negativity constraint PARAFAC model constructed on synchronous fluorescence spectra of chicken breast samples stored at 5 °C for 0, 1, 2, 3 and 5 days. (A) Loading profiles, (B) $\Delta\lambda$ profiles and (C) concentration modes.

Figure 3. Loading profiles associated to the first 4 LVs of N-PLS model for the chicken samples stored at 5 °C for 0, 1, 2, 3 and 5 days. (A) 1st LV, (B) 2nd LV, (C) 3rd LV, (D) 4th LV.

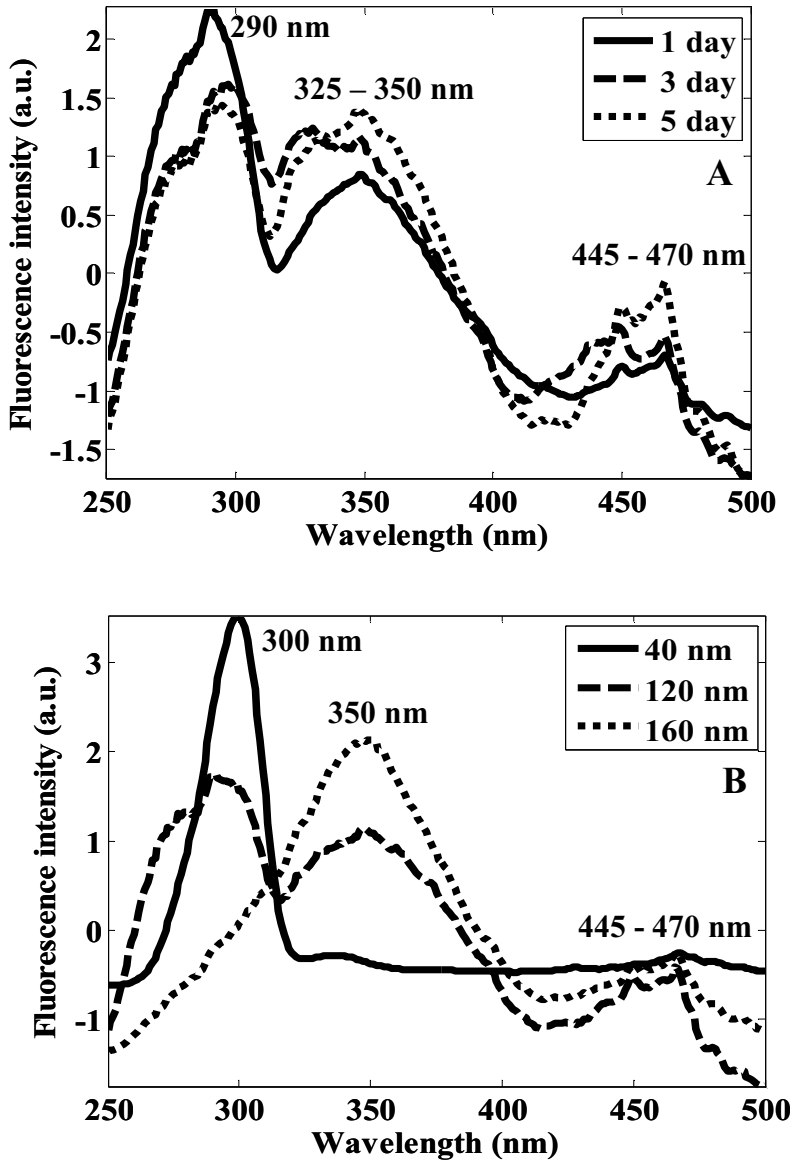

Figure 1. Synchronous fluorescence spectra of meat (A). stored for 1, 3 and 5 days at 5 °C ($\Delta\lambda$ = 120 nm), and (B) stored for 2 days at 5 °C ($\Delta\lambda$ = 40, 120 and 160 nm).

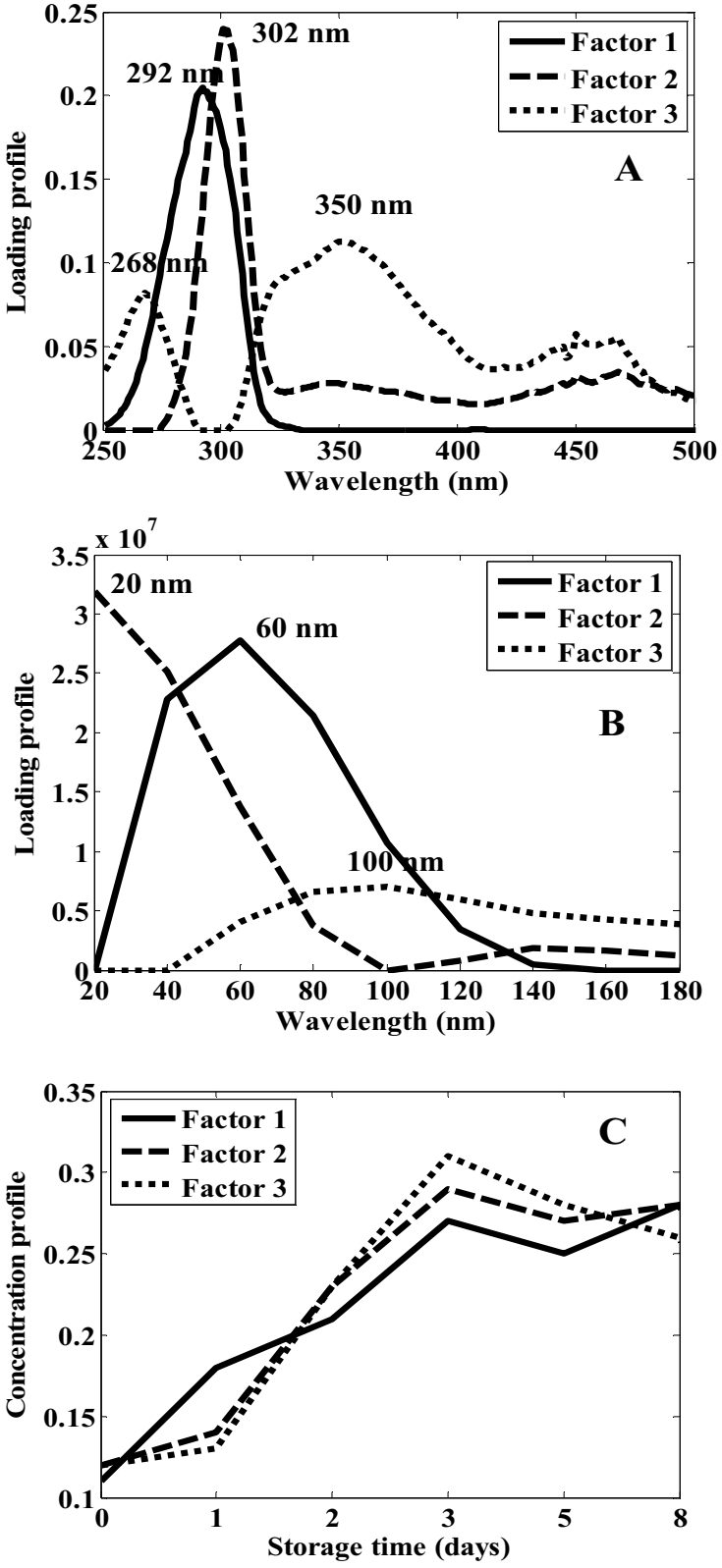

Figure 2. Three components non-negativity constraint PARAFAC model constructed on synchronous fluorescence spectra of chicken breast samples stored at 5 °C for 0, 1, 2, 3 and 5 days. (A) Loading profile, (B) Δλ profile and (C) concentration mode.

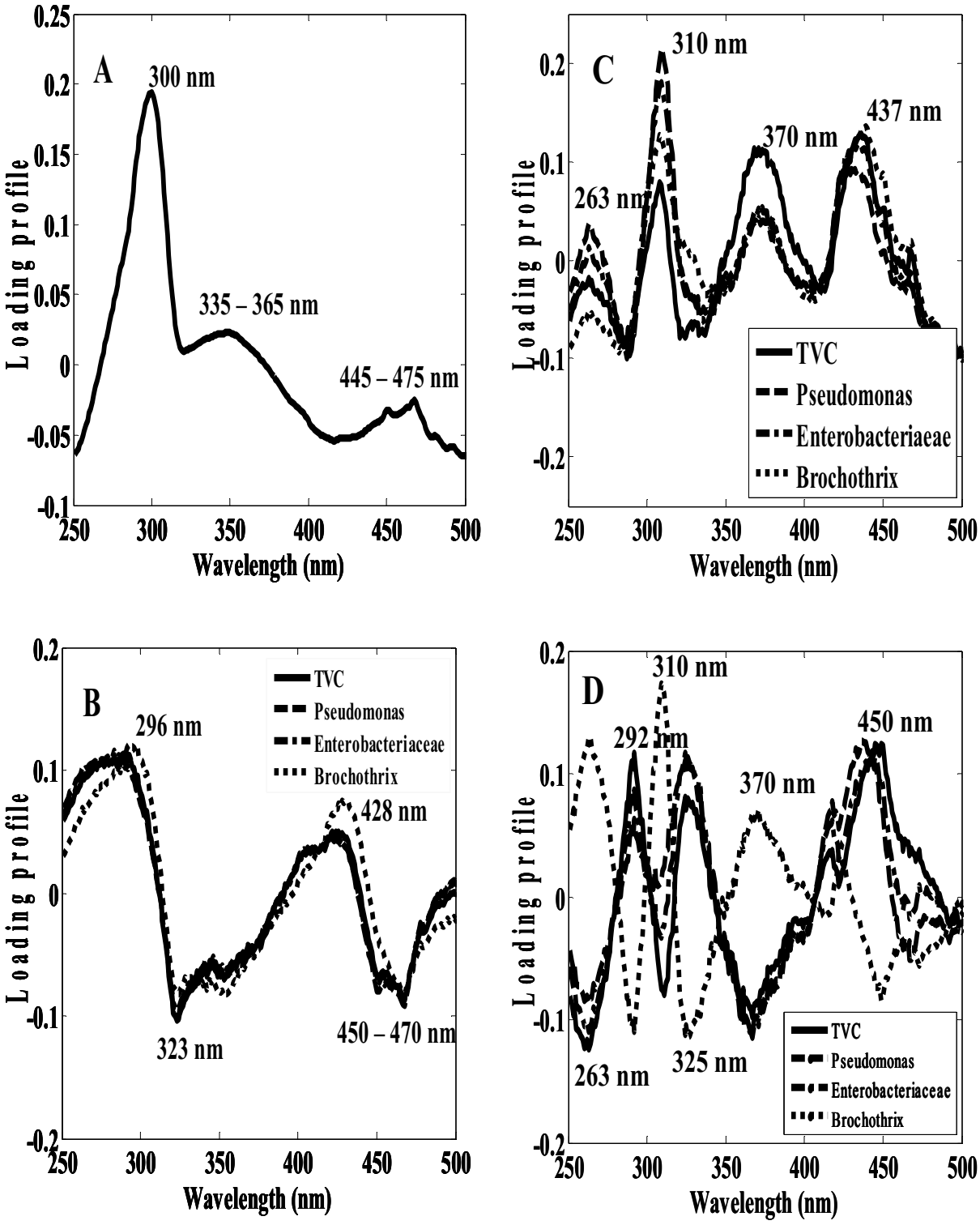

Figure 3. Loading profile of N-PLS model for the meat sample stored at 5 °C for 0, 1, 2, 3 and 5 days (A) 1st LV, (B) 2nd LV, (C) 3rd LV, (D) 4th LV.

Table 1. Changes in microbial flora of chicken breast samples stored at 5 and 15 °C under aerobic conditions.

Storage Time (days)	Microbial analysis (log cfu/cm2)			
	Total Viable Counts	Pseudomonas	Enterobacteriaceae	Brochothrix thermosphacta
	Storage temperature 5 °C			
0	$3.4^{a} \pm 0.01$	$2.9^{a} \pm 0.08$	$3.4^{a} \pm 0.05$	$2.9^{a} \pm 0.05$
1	$5.6^{b} \pm 0.02$	$5.6^{b} \pm 0.02$	$5.3^{b} \pm 0.01$	$4.9^{b} \pm 0.02$
2	$5.9^{c} \pm 0.02$	$6.2^{c} \pm 0.04$	$6.4^{c} \pm 0.06$	$5.4^{c} \pm 0.05$
3	$7.2^{d} \pm 0.06$	$7.4^{d} \pm 0.03$	$7.2^{d} \pm 0.08$	$5.6^{d} \pm 0.01$
5	$7.9^{e} \pm 0.03$	$7.6^{e} \pm 0.01$	$7.7^{e} \pm 0.02$	$7.7^{e} \pm 0.04$
8	$8.4^{f} \pm 0.02$	$8.3^{f} \pm 0.14$	$8.4^{f} \pm 0.05$	$8.0^{f} \pm 0.03$
	Storage temperature 15 °C			
0	$3.4^{A} \pm 0.01$	$2.9^{A} \pm 0.08$	$3.4^{A} \pm 0.05$	$2.9^{A} \pm 0.05$
0.5	$4.1^{B} \pm 0.02$	$4.2^{B} \pm 0.02$	$4.4^{B} \pm 0.01$	$3.5^{B} \pm 0.01$
1	$5.5^{C} \pm 0.08$	$5.4^{C} \pm 0.01$	$5.6^{C} \pm 0.01$	$4.4^{C} \pm 0.01$
2	$8.3^{D} \pm 0.06$	$8.1^{D} \pm 0.03$	$8.3^{D} \pm 0.06$	$6.9^{D} \pm 0.01$
3	$8.5^{E} \pm 0.06$	$8.1^{D} \pm 0.01$	$8.4^{D} \pm 0.02$	$7.2^{E} \pm 0.01$
5	$8.5^{E} \pm 0.04$	$8.0^{D} \pm 0.04$	$8.3^{D} \pm 0.06$	$7.4^{F} \pm 0.03$

Mean values $\pm$ standard error

$^{abcdef \text{ and } ABCDEF}$ Means microbial flora within a column with the same superscripts are not significantly different ($P < 0.01$).

Table 2. Results of calibration and validation sets of partial least square discriminant analysis carried out on concatenation synchronous fluorescence spectra of the chicken breast samples stored at 5 °C for 0, 1, 2, 3, 5 and 8 days, using 4 factors (rows: observed classification; columns: predicted classification).

Groups	Calibration						
	0	1	2	3	5	8	% of good classification
0	1	0	0	0	0	0	100
1	0	1	0	0	0	0	100
2	0	0	1	0	0	0	100
3	0	0	0	1	0	0	100
5	0	0	0	0	1	0	100
8	0	0	0	0	0	1	100
	Validation						
	0	1	2	3	5	8	% of good classification
0	1	0	0	0	0	0	100
1	0	1	0	0	0	0	100
2	0	0	1	0	0	0	100
3	0	0	0	1	0	0	100
5	0	0	0	0	1	0	100
8	0	0	0	0	0	1	100

Table 3. Core consistency and explained variance percentage resulting from PARAFAC models.

	5 °C		15 °C		All samples (5 °C and 15 °C)	
No. of Factors	Core consistency (%)	Explained variance (%)	Core consistency (%)	Explained variance (%)	Core consistency (%)	Explained variance (%)
1	100	94.2	100	92.4	100	93.3
2	100	97.6	100	96.3	100	96.8
3	95	99.7	35	99.2	65	99.4
4	40	99.8	30	99.5	55	99.6
5	0	99.9	0	99.7	5	99.8

Table 4. Excitation and emission maxima wavelengths of PARAFAC components derived from synchronous fluorescence spectra recorded on chicken breast samples.

PARAFAC factor number	[a]λmax.exc./λmax.emi. (nm)	
	15°C	All samples (5°C and 15°C)
1	301/341	295/335
2	280/360, 325-395/405-475	265/285, 303/323
3	-	272/332
4	-	288/388, 352/452

Table 5. Results of N-PLS regression between microbial data determined by culture dependent method and synchronous florescence spectra of chicken breast samples.

Microbes	Results obtained by N-PLS models					
	[a]LV	[b]X-Block	[c]Y-Block	[d]RMSEP	[e]R^2	[f]Rec (%)
	5 °C					
Total Viable Counts	8	92.5	99.9	0.14	0.99	101
Pseudomonas	7	93.4	99.9	0.2	0.99	102
Enterobacteriaceae	4	92.3	99.9	0.25	0.99	102
Brochothrix thermosphacta	7	93.2	99.9	0.15	0.99	101
	15 °C					
Total Viable Counts	6	91.6	99.9	0.18	0.99	100
Pseudomonas	6	90.8	99.9	0.21	0.99	101
Enterobacteriaceae	6	91	99.9	0.17	0.99	100
Brochothrix thermosphacta	6	91.2	99.9	0.11	0.99	100
	All samples (5 and 15 °C)					
Total Viable Counts	10	98.5	99.9	0.37	0.95	100
Pseudomonas	10	99.1	99.9	0.44	0.94	101
Enterobacteriaceae	10	98.5	99.9	0.43	0.94	100
Brochothrix thermosphacta	10	99.2	99.9	0.38	0.95	101

FOURIER TRANSFORM-INFRARED SPECTROSCOPY FOR RAPID DETERMINATION OF SPOILAGE BACTERIA ON CHICKEN BREAST FILLET STORED AT 5 AND 15 °C UNDER AEROBIC CONDITIONS

A. SAHAR, T. BOUBELLOUTA, É. DUFOUR*

U.R. "Typicité des Produits Alimentaires", ENITA Clermont, Clermont Université,

BP 35, F-63370 Lempdes, France

Running title : MIR spectroscopy for spoilage bacteria evaluation in chicken fillets

* Corresponding author: Pr DUFOUR É, Ministère de l'alimentation, de l'agriculture et de la pêche, DGER, 1ter avenue de Lowendal, 75700, Paris 07 SP, France

E-mail address: eric.dufour@agriculture.gouv.fr

Tel.: +33.1.49.55.85.53

Abstract

In this work potential of mid infrared (MIR) spectroscopy was investigated for the determination of microbial load on chicken breast fillets stored aerobically at 5 °C during 0, 1, 2, 3, 5 and 8 days, and 15 °C for 0, 0.5, 1, 2, 3 and 5 days. We showed that MIR spectroscopy can be used directly on the surface of meat samples to produce interpretable fingerprints related to the microbial biofilms developing at their surface. Total viable count (TVC), *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta* were determined on chicken breast fillets at each step of the 2 kinetics using culture dependent methods. Initial TVC was 3.4 log cfu/cm² and TVC reached 8.4 log cfu/cm² and 8.3 log cfu/cm² following 8 days at 5°C and 2 days at 15°C, respectively. In parallel, MIR spectra were recorded (4000 – 700 cm^{-1} range). PLSDA (partial least square discriminant analysis) was carried out to test the reallocation of the spectra of the individual samples within the six groups corresponding to the six investigated storage times and the results showed that 100 % of good classifications were obtained using 4 PLS factors. PLS regression was used to predict the microbial counts for TVC, *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta* from the MIR spectral data. An excellent correlations (R^2 = 0.99) and very small root mean squares error (between 0.01 and 0.97 log cfu/cm²) of validation were obtained from the spectral data sets recorded on samples stored at 5°C and 15°C for TVC, *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*. Thus MIR spectroscopy is capable to acquire rapidly a microbial snapshot present at the surface of meat and to quantify the microbial loads of meat samples accurately.

Key words: mid infrared spectroscopy; aerobic storage; chicken; microbes;

1. Introduction

Meat is one of the most perishable foods, and its composition is ideal for the growth of a wide range of spoilage bacteria. Meat is believed to be spoiled if organoleptic changes make them unacceptable to the consumer. These changes include discoloration, development of off-odors, slime formation, changes in taste etc. The initial mesophilic bacterial count on meat is about 10^2 – 10^3 cfu/cm^2 or gram, consisting of a large variety of species (Borch, Kant-Muermans & Blixt, 1996). At a levels of 10^7 cfu/cm^2 off-odors become evident and once the population of bacteria has reached 10^8 cfu/cm^2, the supply of simple carbohydrates has been exhausted and it reached to sensory spoilage (Ellis & Goodacre, 2001).

Spoilage organisms primarily belong to the genus *Pseudomonas*, as they attach more rapidly to meat surfaces compared to other spoilage bacteria. Other major members of the spoilage flora of meat stored aerobically include *Enterobacteriaceae, Moraxella spp., Psychrobacter spp.* and *Acinetobacter spp*. (Otero, Garcia-Lopez & Moreno, 1998). Whilst the dominant spoilage microflora are generally Gram-negative, the initial population also contain Gram-positive bacteria such as lactic acid bacteria and *Brochothrix thermosphacta* (Ellis & Goodacre, 2001).

Spoilage of raw meat accounts for major annual losses to processors and retailers. Current methods used for microbial detection are time-consuming and labor intensive. For example, classical method used for determining the status of meat, with respect to spoilage, is the analysis of the total viable counts (TVC) and/or specific spoilage bacteria. An obvious drawback with this method is the incubation period of 1 to 3 days that is required for colony

formation. There is a need to replace these classical methods with some rapid, reagentless, relatively inexpensive and non destructive innovative methods.

The application of various vibrational spectroscopic techniques has been used to identify and characterize microorganisms (Al-Holy, Lin, Al-Qadiri, Cavinato & Rasco, 2006; Lin, et al., 2004). Fourier transform infrared spectroscopy in mid infrared (MIR) region has been presented as a rapid and non invasive technique in clinical applications (Naumann, Fijala, Labischinski & Giesbrecht, 1988) and in food industries (Amiel, Mariey, Curk-Daubié, Pichon & Travert, 2000; Lefier, Lamprell & Mazerolles, 2000). It involves the vibrations of molecules that are excited by an infrared beam, and an infrared absorbance spectrum is considered as a fingerprint, which is characteristic of any chemical or biochemical substance (Ellis, Broadhurst, Kell, Rowland & Goodacre, 2002; Gillie, Hochlowski & Arbuckle-Keil, 2000). Vibrational spectroscopy is capable to measure the biochemical changes within the meat substrate and it can be used to exploit the information about the decomposition of the meat and formation of metabolites by microorganisms. This technique has already been used to discriminate and identify the pathogenic (Beattie, Holt, Hirst, Sutherland & MacDonald, 1998) and spoilage microorganisms in food (Amiel, et al., 2000). Recently this technique has been used to detect the bacterial spoilage of meat under aerobic (Ellis, Broadhurst & Goodacre, 2004) and different storage conditions (Ammor, Argyri & Nychas, 2009). MIR represents the spectrum of the absorption of all the chemical bonds having an infrared activity between 4000 and 400 cm^{-1}. Differences in the structure and quantity of cell wall polysaccharide, lipids and protein are reflected in the MIR spectra, which can be used for the differentiation among different bacteria. The acyl-chain is mainly responsible for the absorption observed between 3000 and 2800 cm^{-1}, whereas the peptidic bound CO-NH is mainly responsible of the absorption occurring between 1700 and 1500 cm^{-1}. But water also

strongly absorbs in the Amide I region. Finally, the 1500 and 900 cm^{-1} region is known to exhibit bands attributed to polysaccharides, peptidic bonds and phosphate.

In order to interpret the molecular structural information present in complex spectra and to correlate it to the microbial composition, various multivariate statistical tools have been used (Burgula, et al., 2007). The use of analytical technique couple with chemometrics has been used for various foods (Ammor, et al., 2009; Dupuy, et al., 2005).

The aim of present research work was to explore the potential of MIR spectroscopy coupled with chemometrics to detect the changes in microbial flora, i.e., TVC*, Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*, occurring in chicken breast fillets during aerobic storage kinetics at 5 (0, 1, 2, 3, 5 and 8 days) and 15 °C (0, 0.5, 1, 2, 3 and 5 days), and to predict the microbial counts, i.e., TVC*, Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*, from MIR spectra.

2. Materials and methods

2.1. Sampling and storage conditions

21 chicken breast fillets without skin were purchased from a local super-market (CORA, Lempdes) in packed form and transported to the laboratory within 20 minutes. One sample was analyzed immediately and it was considered as the starting points of the storage kinetics. Samples were stored aerobically by enclosing them into permeable polyethylene bags. The samples were divided into two lots (10 fillets/lot) and packed individually (1fillet/pouch). One lot (10 fillets) of chicken breast samples was stored at 5 °C and analyzed on 1, 2, 3, 5 and 8 days of storage, while the other lot (10 fillets) was stored at 15 °C for analysis on 0.5, 1, 2, 3 and 5 days of storage. For analysis, one pouch was used for microbial determination and an other for collection of mid infrared spectra.

2.2. Microbiological analysis

Samples were taken aseptically from the pouch and placed on a clean and aseptic place at room temperature. The chicken fillet was sampled by swab test using sterile cotton wool swabs held by a stick (ISO, 18593). These swabs were moistened with 0.1 % peptone water. An area of 50 cm^2 was marked with a sterile frame of 5 cm x 10 cm. The swabs were rubbed on the marked sites continuously for about 30 seconds and then put into distilled screw-capped test tube containing 10 ml of peptone water (BK018). For uniform distribution of microorganisms, these test tubes were shaken well using vortex mixer for about 30 seconds. Tenfold serial dilutions up to 10^{-5} (increased with increasing storage time) were prepared for all samples. For microbial enumeration, 50 µl of serial decimal dilutions of poultry

homogenates was plated on the surface of respective media plate using spiral plate technique (NF, V 08-100).

TVC were determined by incubating 50 µl of appropriate dilution on plate count agar for 72 hours at 25 °C (ISO, 2293). Pseudomonas was enumerated on Cetrimide, Fucidine and Cephaloridine (CFC) agar and incubating 50 µl of dilution at 25 °C for 48 hours (ISO, 13720). Members of *Enterobacteriaceae* family were determined by inoculated 50 µl of the suitable dilution on Violet Red Bile Dextrose Agar (VRBDA) for 24 hours at 30°C (ISO, 5552). Streptomycin Tallium Actidione Agar (STAA) was used to determine *Brochothrix thermosphacta* by incubation of the plates at 20 °C for 72 hours (ISO, 13722). Two replicates of at least five appropriate dilutions (depending on the day of sampling) were enumerated. All plates were visually analyzed for morphology and typical colony types associated with each growth medium. Actual colony counts (cfu/cm^2) of microbiological data were transformed to logarithmic values.

2.3. Mid infrared spectra

MIR spectra were recorded between 4000 – 700 cm^{-1} at a resolution of 4 cm^{-1} on a Fourier transform spectrometer (Thermo Optek, Nicolet, Trappes, France) mounted with an attenuated total reflection (ATR) accessory equipped with a grip. The ATR cell was made of horizontal ZnSe crystal. A slice of meat sample (8 cm x 1 cm x 0.5 cm) was placed on the crystal, a pressure on the grip ensuring a good contact between the crystal and the sample was applied. Before each measurement, the spectrum of the ZnSe crystal was recorded and used as background. For each experimental sample, two spectra were recorded.

In this study, the MIR spectra were divided into three regions: 3000 – 2800 cm^{-1}, 1700 – 1500 cm^{-1} and 1500 – 1000 cm^{-1} (Karoui, et al., 2005).

2.4. Chemometrics

2.4.1. Pre-treatment of spectra

Baseline correction was applied to the spectra using MatLab software.

2.4.2. Analysis of variance (ANOVA)

Statistical analysis of microbial data was carried out using STATISTICA software. Significance differences of the growth of each microbe during storage was determined by analysis of variance (ANOVA) using the least square difference method (Fisher test). Differences were considered significant at $P < 0.01$ level.

2.4.3. Discriminant analysis (PLSDA)

Partial least square discriminant analysis (PLSDA) with cross validation were performed on MIR spectral data in MATLAB (The Mathworks Inc., Natic, MA, USA) using 'Saisir' package available on-line (http://easy-chemometrics.fr). The aim of this technique is to predict the membership of an individual to a qualitative group defined as a preliminary (Vigneau, Qannari, Jaillais, Mazerolles & Bertrand, 2006). Chicken breast samples were classified into six groups, according to their storage time, i.e., 0, 1, 2, 3, 5 and 8 days for 5°C, or 0, 0.5, 1, 2, 3 and 5 days for 15 °C. The PLSDA assesses new synthetic variables called

loading factors, which are linear combinations of the initial variables and allows a better separation of the centres of gravity of the considered groups. The method allows the individual samples to be reallocated within the various groups. Comparison of the predicted groups to the real group is an indicator of the quality of the discrimination and it is valued as the percentage of correct classification. The spectral collection was divided into two groups: two-thirds of the investigated chicken breast samples were used for the calibration set and one-third for validation set.

2.4.4. PLS regression method

PLS regression with cross validation (Vigneau, et al., 2006) was used to find the fundamental relations between two matrices i.e. MIR spectra and microbial load (TVC*, Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta)* on chicken breast samples stored at 5 °C and 15 °C. Root mean square error of calibration (RMSEC) and validation (RMSEV) and coefficient of determination (R^2) of calibration and validation, were determined.

3. Results and discussions

3.1. Changes in microbial flora of fresh chicken breast samples stored aerobically at 5 and 15 °C

Table 1 showed the changes in microbial flora (TVC, *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*) of chicken breast fillets, which were stored aerobically at 5 and 15 °C for several days.

Results obtained from traditional methods showed that the initial load of microbes is quite low on chicken samples, it suggested an overall good hygienic conditions and safety practices applied at the poultry slaughterhouse (Balamatsia, Paleologos, Kontominas & Savvaidis, 2006). Microbial growths were significantly different for the storage kinetics at 5°C and 15 °C. As TVC increased significantly from day 1 to day 8 for storage at 5°C reaching 8.4 log cfu/cm^2, the microbial load reached a plateau (8.3 log cfu/cm^2) after day 2 in the sample stored at 15 °C, showing upper acceptability limits for chicken meat (Patsias, Chouliara, Badeka, Savvaidis & Kontominas, 2006).

Pseudomonas grew significantly from 0 to 8 day of storage at 5°C and became the dominant population on day 3 (7.4 log cfu/cm^2) increasing up to 8.3 log cfu/cm^2 on 8th day of storage. In case of samples stored at 15 °C, *Pseudomonas* counts reached a maximum (8.1 log cfu/cm^2) after 2 days of storage. Although *Pseudomonas* had low initial load, it became the most prominent microbe (7.4 log cfu/cm^2) on third day of storage at 5°C which was in accordance to (Gill & Newton, 1977) who showed that *Pseudomonas* grow faster in aerobic storage and at low temperature than other bacteria, and remain dominant in microflora.

Initial (0 day) counts of *Enterobacteriaceae* were 3.4 log cfu/cm^2, which increased significantly from 1 to 8 days of storage at 5°C. However these bacteria reached a plateau after 2nd day of storage at 15°C (Huis in't Veld, 1996; Liu, Yang & Li, 2006).

Brochothrix thermosphacta also showed increased of the counts during kinetics of storage: starting with an initial load of 2.9 log cfu/cm^2, the final counts reached 8.0 log cfu/cm^2 and 7.4 log cfu/cm^2 for storage at 5°C and 15°C, respectively. However, unlike other bacteria, *Brochothrix thermosphacta* population significantly kept on increasing until 5th day of storage at 15 °C.

3.2. Mid infrared spectra of meat samples stored at 5 and 15 °C

Most of the spectral information useful for the analysis of MIR spectra is located in three regions (Karoui, Dufour & De Baerdemaeker, 2006).

The 3000 – 2800 cm^{-1} range correspond to the C-H bond of methyl and methylene groups of molecules and these bands are mainly attributed to fatty acids of lipids and phospholipids (Casal & Mantsch, 1984). Four bands at about 2961, 2925, 2870 and 2853 cm^{-1} can be seen in MIR spectra recorded on chicken breast sample at 5 °C on 0, 2 and 5 days of storage (figure 1C). The maximum absorbance of the 4 bands were decreasing from 0 day to 5 days.

The 1700 – 1500 cm^{-1} region is characterized by the presence of amide I and II bands related to proteins and by the absorbance of water at about 1640 cm^{-1}. Amide I band, which is

used to investigate the secondary structure of proteins (Dousseau & Pézolet, 1990), can be observed at 1638 cm^{-1} (figure 1B). The absorbance at 1638 cm^{-1} might also originate from the water (O-H) in meat samples (Ammor, et al., 2009). An other band at 1550 cm^{-1} was observed which is generally assigned to amide II vibrations of proteins. Interestingly, the absorbance at 1640 and 1550 cm^{-1} are increasing from 0 day to 5 days. As the absorbance of both Amide I and II bands were higher after 5 days storage it was suggested that it was resulted from proteins.

The 1500 – 1000 cm^{-1} region is called the fingerprint region and numerous chemical bonds are absorbed in the region. No apparent differences were found in bands present at about 1456 (asymmetric CH_3 and CH_2 bending mode (Al-Holy, et al., 2006)), 1396, 1310, and 1236 cm^{-1}. However most of the bands between 1280 and 1100 cm^{-1} showed clear differences in the MIR spectra of chicken samples taken on 0, 2 and 5 days of storage (figure 1A). From previous studies, it has been shown that bands at about 1100, 1150 and 1250 cm^{-1} were related to phosphate absorbance (Boubellouta, Galtier & E., 2009).

The absorbances in the 1280 and 1100 cm^{-1} region are decreasing, as it was observed for 3000 – 2800 cm^{-1} range. Visual differences among various spectral peaks of the samples which were stored at 5 and 15 °C, showed the potential of MIR to differentiate among different samples during aerobic storage.

As the evanescent wave is penetrating by several microns in the sample placed on the ZnSe crystal, it is hypothesized that the spectra mainly retain information from bacterial biofilms located on the surface of chicken filets.

3.3. Classification of chicken fillet samples according to their storage time from MIR spectra

Each region of MIR spectral data collected during the storage kinetic at 5 °C and 15 °C were evaluated using PLSDA in order to test the ability of MIR spectra to discriminate among each of the six storage times.

Considering the storage kinetic at 5°C, PLSDA using four factors applied on the 3 MIR data sets (3000-2800 cm^{-1}, 1700-1500 cm^{-1} and 1500-1000 cm^{-1}) made it possible to obtain 100 % of good classification for calibration and validation sets (table 2). Similar results were obtained for the samples stored at 15 °C using a PLSDA model with 4 factors (data not shown).

These results showed the potential of MIR spectroscopy to completely discriminate among the samples stored for different periods of time. The spectra retain information that is related to the changes of chicken filets during the kinetics of storage. During the time of storage, the bacterial loads increased from 3.4 log cfu/cm^2 to 8.5 log cfu/cm^2 and, considering the use of ATR sample holder, it is suggested that the spectra mainly retain information originating from the bacterial biofilms.

3.4. Prediction of bacterial counts using PLS regression

Although microbes have the same cellular constituents, namely, proteins, polysaccharides, phospholipids and nucleic acids, the quantity and distribution of these constituents are different (Al-Holy, et al., 2006), along with some differences in microbial

metabolites, providing a distinctive MIR spectrum for individual microbe (Naumann, et al., 1988).

N-PLS regressions were used to predict the microbial counts for TVC*, Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta* from the spectral data (3000-2800 cm^{-1}, 1700-1500 cm^{-1} and 1500-1000 cm^{-1}) recorded during the storage kinetics of chicken filets at 5°C and 15°C. Results of PLS regressions presented in Tables 3, 4 and 5 showed an excellent prediction of microbes i.e., TVC*, Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*, from the three regions of the MIR spectra.

Best results for the predictions of the chicken-filets microbial-loads at 5 °C were obtained from 1500 – 1000 cm^{-1} region , which was able to predict TVC*, Pseudomonas* and *Enterobacteriaceae* using 4 PLS factor models: values of R^2 equal to 0.99 were obtained for both, calibration and validation data sets. A value of R^2 equal to 0.97 (validation) was obtained for *Brochothrix thermosphacta* using 5 factors of PLS (table 3). Due to the small number of PLS factors used in the developed models, over-prediction is not supposed to occur.

This region is known as fingerprint region and refers to C-O and C-C stretching modes (1153 – 900 cm^{-1}) and those around 1474 – 1200 cm^{-1} are due to the bending modes of O-C-H, C-C-H and C-O-H (Sivakesava & Irudayaraj, 2001). Ellis, et al., (2002) found that the peaks present in this region (1088 and 1096 cm^{-1}) are the most significant peaks of the MIR spectra for the prediction of spoilage of chicken.

Regarding the MIR spectra in the range of 1700 – 1500 cm^{-1} recorded for samples stored at 5°C and 15°C, excellent predictions (R^2 = 0.99) were obtained, using 5 factors of PLS (Table 4), for the four microbial flora (TVC*, Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*). These results of predictions showed very small RMSEC and RMSEV (table 4), ranging between 0.13 and 0.24 log cfu/cm² for RMSEV for data recorded at 5°C.

The MIR spectra in 3000 – 2800 cm^{-1} range for the samples stored at 5 °C showed values of R^2 (validation) equal to 0.98, 0.99, 0.99 and 0.94 for TVC*, Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*, respectively, using 5 PLS factor models. However, for the samples of chicken breast stored at 15 °C, 5 PLS factor models gave R^2 (validation) value equal to 0.87, 0.90, 0.89, 0.87 for TVC*, Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*, respectively. Comparatively, higher values of RMSEC and RMSEV were obtained for this MIR spectral region (table 5) than for the two previous ones.

This study shows that MIR spectra coupled with PLS regression makes it possible to identify and to quantify a given bacteria from a mixture, without any prior isolation. The good predictions of individual bacteria strongly suggest that the MIR spectra retain information on individual bacteria and that PLS regression is able to extract specific information for individual bacteria. It has been shown in the past that MIR spectra recorded on bacteria isolates are fingerprints allowing their identification. Our results show that it is possible to predict individual bacterial load from MIR spectra recorded on bacterial community, i.e., bacterial biofilms present at the surface of chicken fillets.

5. Conclusions

In this study we showed the potential of MIR spectroscopy coupled with chemometrics as an economical, rapid and non invasive tool to monitor the microbial spoilage of chicken breast samples stored aerobically at 5 and 15 °C. It was evident that MIR spectra collected directly from the surface of chicken breast samples , contained information that could be correlated with the spoilage status of the samples.

A good classification (100 %) of meat samples according to their storage time was obtained using four factors PLSDA for the samples stored at 5 and 15 °C. Results of PLS regression performed on the MIR spectra showed, using a small number of PLS factors, an excellent prediction of microbes (TVC*, Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*) detected from classical methods.

Thus, a MIR spectrum seems to be a fingerprint and contained useful information related to the microbes present on the surface of chicken samples.

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Figure 1. Mid infrared spectra of chicken breast samples stored at 5 °C recorded between A). 1500 – 1000 cm^{-1}, B). 1700 – 1500 cm^{-1}, C). 3000 – 2800 cm^{-1}.

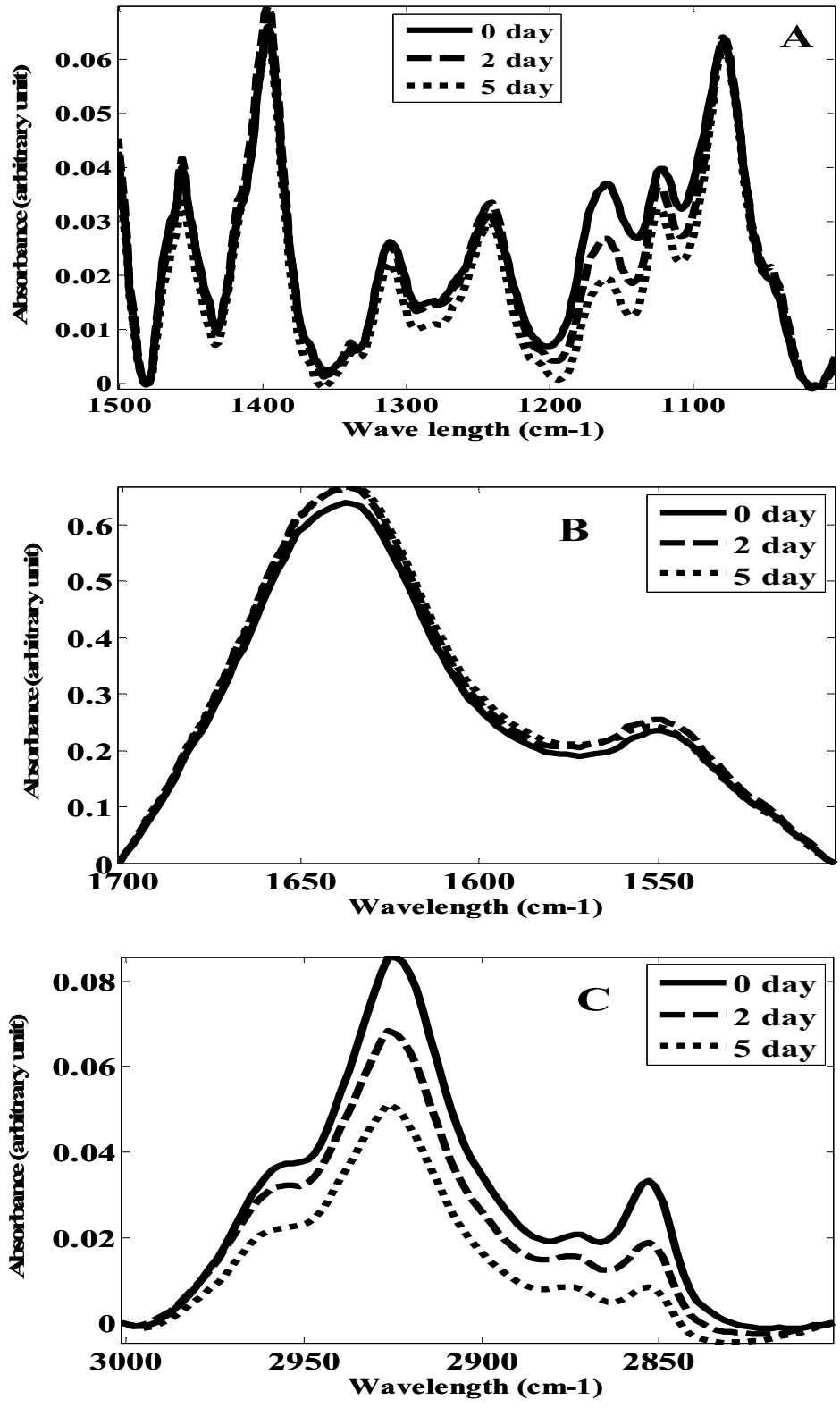

Table 1. Changes in microbial flora of chicken breast samples stored at 5 and 15 °C under aerobic conditions.

Storage Time (days)	Microbial analysis (log cfu/cm2)			
	Total Viable Counts	Pseudomonas	Enterobacteriaceae	Brochothrix thermosphacta
	Storage temperature 5 °C			
0	$3.4^{a} \pm 0.01$	$2.9^{a} \pm 0.08$	$3.4^{a} \pm 0.05$	$2.9^{a} \pm 0.05$
1	$5.6^{b} \pm 0.02$	$5.6^{b} \pm 0.02$	$5.3^{b} \pm 0.01$	$4.9^{b} \pm 0.02$
2	$5.9^{c} \pm 0.02$	$6.2^{c} \pm 0.04$	$6.4^{c} \pm 0.06$	$5.4^{c} \pm 0.05$
3	$7.2^{d} \pm 0.06$	$7.4^{d} \pm 0.03$	$7.2^{d} \pm 0.08$	$5.6^{d} \pm 0.01$
5	$7.9^{e} \pm 0.03$	$7.6^{e} \pm 0.01$	$7.7^{e} \pm 0.02$	$7.7^{e} \pm 0.04$
8	$8.4^{f} \pm 0.02$	$8.3^{f} \pm 0.14$	$8.4^{f} \pm 0.05$	$8.0^{f} \pm 0.03$
	Storage temperature 15 °C			
0	$3.4^{A} \pm 0.01$	$2.9^{A} \pm 0.08$	$3.4^{A} \pm 0.05$	$2.9^{A} \pm 0.05$
0.5	$4.1^{B} \pm 0.02$	$4.2^{B} \pm 0.02$	$4.4^{B} \pm 0.01$	$3.5^{B} \pm 0.01$
1	$5.5^{C} \pm 0.08$	$5.4^{C} \pm 0.01$	$5.6^{C} \pm 0.01$	$4.4^{C} \pm 0.01$
2	$8.3^{D} \pm 0.06$	$8.1^{D} \pm 0.03$	$8.3^{D} \pm 0.06$	$6.9^{D} \pm 0.01$
3	$8.5^{E} \pm 0.06$	$8.1^{D} \pm 0.01$	$8.4^{D} \pm 0.02$	$7.2^{E} \pm 0.01$
5	$8.5^{E} \pm 0.04$	$8.0^{D} \pm 0.04$	$8.3^{D} \pm 0.06$	$7.4^{F} \pm 0.03$

Mean values $\pm$ standard error

$^{abcdef \text{ and } ABCDEF}$ Means microbial flora within a column with the same superscripts are not significantly different ($P < 0.01$).

Table 2. Results of calibration and validation sets of partial least square discriminant analysis carried out on mid infrared spectra of the chicken breast samples stored at 5 °C for 0, 1, 2, 3, 5 and 8 days, using 4 factors (rows: observed classification; columns: predicted classification).

MIR region	Storage time (Days)	Calibration							Validation						
		0	1	2	3	5	8	% of good classification	0	1	2	3	5	8	% of good classification
3000 - 2800 cm^{-1}	0	2	0	0	0	0	0	100							
	1	0	1	0	0	0	0	100	0	1	0	0	0	0	100
	2	0	0	1	0	0	0	100	0	0	1	0	0	0	100
	3	0	0	0	1	0	0	100	0	0	0	1	0	0	100
	5	0	0	0	0	2	0	100							
	8	0	0	0	0	0	1	100	0	0	0	0	0	1	100
1700 - 1500 cm^{-1}	0	2	0	0	0	0	0	100							
	1	0	1	0	0	0	0	100	0	1	0	0	0	0	100
	2	0	0	1	0	0	0	100	0	0	1	0	0	0	100
	3	0	0	0	1	0	0	100	0	0	0	1	0	0	100
	5	0	0	0	0	2	0	100							
	8	0	0	0	0	0	1	100	0	0	0	0	0	1	100
1500 - 1000 cm^{-1}	0	2	0	0	0	0	0	100							
	1	0	1	0	0	0	0	100	0	1	0	0	0	0	100
	2	0	0	1	0	0	0	100	0	0	1	0	0	0	100
	3	0	0	0	1	0	0	100	0	0	0	1	0	0	100
	5	0	0	0	0	2	0	100							
	8	0	0	0	0	0	1	100	0	0	0	0	0	1	100

Table 3. Results of PLS regression carried out on mid infrared spectra (1500 –1000 cm^{-1}) and microbial data obtained by traditional technique on chicken breast samples at 5 °C.

PLS Results	Microbial Flora			
	TVC	Pseudomonas	Enterobacteriaceae	B. thermosphacta
	5 °C			
No. of PLS Factors	4	4	4	5
R^2C	0.99	0.99	0.99	0.99
RMSEC	0.14	0.20	0.18	0.07
R^2V	0.99	0.99	0.99	0.97
RMSEV	0.16	0.19	0.19	0.39
	15 °C			
No. of PLS Factors	5	5	5	5
R^2C	0.99	0.99	0.99	0.99
RMSEC	0.01	0.01	0.01	0.03
R^2V	0.98	0.98	0.99	0.99
RMSEV	0.28	0.30	0.21	0.19

R^2C: Coefficient of regression for calibration

RMSEC: Root mean square error of calibration

R^2V: Coefficient of regression for validation

RMSEV: Root mean square error of validation

Table 4. Results of PLS regression carried out on mid infrared spectra (1700 – 1500 cm^{-1}) and microbial data obtained by traditional technique on chicken breast samples at 5 °C.

PLS Results	Microbial Flora			
	TVC	Pseudomonas	Enterobacteriaceae	B. thermosphacta
	5 °C			
No. of PLS Factors	5	5	5	5
R^2C	0.99	0.99	0.99	0.99
RMSEC	0.03	0.06	0.05	0.13
R^2V	0.99	0.99	0.99	0.99
RMSEV	0.13	0.18	0.19	0.24
	15 °C			
No. of PLS Factors	5	5	5	5
R^2C	0.99	0.99	0.99	0.99
RMSEC	0.13	0.14	0.13	0.13
R^2V	0.99	0.99	0.99	0.99
RMSEV	0.22	0.12	0.15	0.21

R^2C: Coefficient of regression for calibration

RMSEC: Root mean square error of calibration

R^2V: Coefficient of regression for validation

RMSEV: Root mean square error of validation

Table 5. Results of PLS regression carried out on mid infrared spectra (3000 – 2800 cm^{-1}) and microbial data obtained by traditional technique on chicken breast samples at 5 °C.

PLS Results	Microbial Flora			
	TVC	Pseudomonas	Enterobacteriaceae	B. thermosphacta
	5 °C			
No. of PLS Factors	5	5	5	5
R^2C	0.99	0.99	0.99	0.99
RMSEC	0.06	0.55	0.07	0.13
R^2V	0.98	0.99	0.99	0.94
RMSEV	0.40	0.47	0.51	0.51
	15 °C			
No. of PLS Factors	5	5	5	5
R^2C	0.99	0.99	0.99	0.99
RMSEC	0.21	0.24	0.23	0.18
R^2V	0.87	0.90	0.89	0.87
RMSEV	0.96	0.97	0.89	0.86

R^2C: Coefficient of regression for calibration

RMSEC: Root mean square error of calibration

R^2V: Coefficient of regression for validation

RMSEV: Root mean square error of validation

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RÉSUMÉ

Il y a une nécessité à maîtriser la qualité des produits carnés au cours du processus de fabrication en un temps aussi court que possible, ce qui exige de remplacer les techniques classiques (longues et coûteuses) par des techniques rapides, sensibles et moins coûteuses. Les techniques spectroscopiques présentent les qualités requises. En particulier, les spectroscopies de fluorescence et moyen infrarouge couplées à la chimiométrie ont été considérées comme de bons candidats pour l'analyse rapide des produits alimentaires, en général, et de la viande et des produits carnés, en particulier.

La première partie de cette thèse porte sur la discrimination de sept muscles de bovins (*Semitendinosus*, *Semimembranosus,*, *Tensor fasciae latae*, *Rectus abdominis*, *Longissimus thoracis et lumborum*, *Triceps branchii* and *Infraspinatus)* et la prédiction de certains paramètres physico-chimiques de la viande à partir des spectres de fluorescence. Les méthodes d'analyse statistique multidimensionnelle (PLS, PLSDA) appliquées aux données spectrales ont permis de discriminer les sept muscles de bovins et de prédire certaines caractéristiques physico-chimiques de la viande.

Dans la deuxième partie, les effets de différentes températures (66, 90 et 237 °C) et temps (1, 2, 5, 7 et 10 minutes) de cuisson sur les caractéristiques des constituants de la viande ont été observés à l'aide des spectroscopies de fluorescence synchrone et moyen infrarouge couplées à la chimiométrie. Les méthodes ACP et PARAFAC ont été utilisées pour extraire les informations relatives à la formation de composés issus de la réaction de Maillard et à l'évolution des tryptophanes des protéines. La méthode de régression N-PLS a été utilisée avec succès pour prédire certaines amines hétérocycliques (4.8 DiMeIQx, MeIQx, IQx et PhIP) de la viande grillée.

Enfin, dans la troisième partie de la thèse, le potentiel des spectroscopies de fluorescence synchrone et moyen infrarouge couplées à la chimiométrie a été envisagé pour la détermination de la charge microbienne (flore totale, *Pseudomonas, Enterobacteriaceae* et *Brochothrix thermosphacta*) de filets de poulet durant les cinétiques de stockages (0, 1, 2, 3, 5 et 8 jours à 5 °C et 0, 0,5, 1, 2, 3, et 5 jours à 15 °C). Les méthodes de régression N-PLS et PLS ont permis de prédire avec une bonne précision les charges pour chaque microorganisme considéré présent à la surface des filets de poulet à partir des spectres enregistrés. Les résultats ont mis en évidence le potentiel des spectroscopies de fluorescence et moyen infrarouge couplées à la chimiométrie pour l'analyse non destructive de la qualité nutritionnelle et hygiénique des viandes.

Mots-clés : viande, spectroscopie de fluorescence, spectroscopie de fluorescence synchrone, spectroscopie moyen infrarouge, paramètres physico-chimiques, chimiométrie, amines aromatiques hétérocycliques, microorganismes

ABSTRACT

There is a growing need to control the quality of meat products throughout the manufacturing process in minimum possible time, which requires replacement of the conventional techniques (long and expensive) with some rapid, sensitive and less expensive techniques. Spectroscopic techniques appear to be reagentless, fast and non destructive techniques. Particularly, fluorescence and mid infrared spectroscopies coupled with chemometrics have been considered as good candidates for rapid analysis of food products, in general, and for meat and meat products, in particular.

The first part of this thesis work focuses on the discrimination of seven bovine muscles (*Semitendinosus*, *Semimembranosus,*, *Tensor fasciae latae*, *Rectus abdominis*, *Longissimus thoracis et lumborum*, *Triceps branchii* and *Infraspinatus)* and prediction of certain physico-chemical parameters of meat using front-face fluorescence spectroscopy. The statistical analysis (PLS, PLSDA) applied to the fluorescence spectral data discriminate well seven bovine muscles and good predictions of physico-chemical characteristics of meat were obtained.

In the second part, the effects of different temperatures (66, 90 and 237 °C) and cooking times (1, 2, 5, 7 and 10 minutes) on the characteristics of the constituents of meat were investigated using synchronous fluorescence and mid infrared spectroscopy coupled with chemometrics. PCA and PARAFAC methods were used to derive information about the evolution of Maillard reaction. N-PLS regression method was successfully used to predict the amounts of certain heterocyclic amines (4.8 DiMeIQx, MeIQx, IQx and PhIP) in grilled meat.

Finally, in the third part, the potential of synchronous fluorescence and mid infrared spectroscopies along with chemometrics was investigated for the determination of the microbial loads (total viable count, *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta*) on chicken fillets during the kinetics of storages (0, 1, 2, 3, 5, 8 days at 5 °C and 0, 0.5, 1, 2, 3, 5 days at 15 °C). N-PLS and PLS regression methods allowed to predict with a good accuracy the loads for each bacterium on the surface of chicken fillets from synchronous fluorescence and mid infra data.

The results showed the potential of fluorescence and mid infrared spectroscopies coupled with chemometrics for the non-destructive analysis of nutritional and hygienic quality of meat and meat products.

Keywords : Meat, fluorescence spectroscopy, synchronous fluorescence spectroscopy, mid infrared spectroscopy, physico-chemical parameters, chemometrics, heterocyclic aromatic amines, microorganisms.