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# Performance of fluorescence spectroscopy for beef meat authentication: Effect of excitation mode and discriminant algorithms

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ABSTRACT

This study evaluated the performance of classical front face (FFFS) and synchronous (SFS) fluorescence spectroscopy combined with Partial Least Square Discriminant Analysis (PLSDA), Support Vector Machine associated with PLS (PLS-SVM) and Principal Components Analysis (PCA-SVM) to discriminate three beef muscles (*Longissimus thoracis, Rectus abdominis* and *Semitendinosus*). For the FFFS, 5 excitation wavelengths were investigated, while 6 offsets were studied for SFS.

Globally, the results showed a good discrimination between muscles with Recall and Precision between 47.82 and 94.34% and Error ranging from 6.03 to 32.39%. For the FFFS, the PLS-SVM with the 382 nm excitation wavelength gave the best discrimination results (Recall, Precision and Error of 94.34%, 89.53% and 6.03% respectively). For SFS, when performing discrimination of the three muscles, the 120 nm offset gave the highest Recall and Precision (from 57.66% to 94.99%) and the lowest Error values (from 6.78 to 8.66%) whatever the algorithm (PLSDA, PLS-SVM and PCA-SVM).

## 1. Introduction

During the past 50 years, considerable effort has been made on the safety of food and especially meat, in the areas of production processes and relationship between the quality and production. More recently, analytical methods have been implemented to measure different parameters of meat quality (e.g. composition, safety, tenderness). These methods, generally invasive and/or destructive, limit their use in- or on-line. To date, one of the food industry issues is to obtain reliable information on the meat quality throughout the production process to ensure the final product quality. This challenge requires rapid detection, precise and non-destructive tools, able to be installed in- or on-line, and adapted to a difficult environment. These tools should enable continuous assessment of each step of the process. The techniques that meet these conditions and that could be used for the rapid analysis of meat quality were presented in different review and research papers (Damez & Clerjon, 2013; Mourot et al., 2014). The most promising methods identified to date are based on the use of spectroscopy (e.g. Infrared, Near Infrared, and Fluorescence), multispectral and hyperspectral image analysis (Aït-Kaddour, Jacquot, Micol, & Listrat, 2016; Ropodi, Panagou, & Nychas, 2017; Rodopi, Pavlidis, Mohareb, Panagou, & Nychas, 2015; Sanz et al., 2016; Xiong et al., 2015). One of the advantages of these techniques is not only to investigate the chemical composition of the samples but also to assess a characteristic spectrum or spectral image that represents a real fingerprint of the sample.

Fluorescence spectroscopy has been mainly used to investigate quality properties of meat and meat products, such as sensory qualities (Dufour & Frencia, 2001; Olsen et al., 2005; Swatland, Gullett, Hore, & Buttenham, 1995). Fluorescence spectroscopy associated with chemometrics were widely used also to authenticate and classify meat products (Sahar, Boubellouta, Lepetit, & Dufour, 2009; Sahar, Rahman, Kondjoyan, Portanguen, & Dufour, 2016) as a function of their breeding conditions (Gatellier et al., 2007), manufacturing process, storage and cooking conditions (Gatellier et al., 2009; Hassoun & Karoui, 2015; Møller, Parolari, Gabba, Christensen, & Skibsted, 2003) or microbial spoilage (Aït-Kaddour, Boubellouta, & Chevallier, 2011). Quantitative evaluations of some meat components (e.g. fat and fatty acids) were developed using multivariate regression techniques (Aït-Kaddour, Jacquot, et al., 2016; Egelandsdal, Dingstad, Tøgersen, Lundby, & Langsrud, 2005; Sahar, Portanguen, Kondjoyan, & Dufour, 2010; Wold, Kvaal, & Egelandsdal, 1999). As previously noted by Sanz et al. (2016),

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few proposal exist in scientific literature to tackle the problem of separating muscles types by classical spectroscopy and more specifically by fluorescence spectroscopy. This is especially important because in recent years, the retail cost of beef having increased significantly, the substitution of high value raw meat with cheaper meat has also increased (Cozzolino & Murray, 2004). Moreover, the intensification of agriculture and urbanization over the last decades has created concern of many consumers about authentication and the safety of meat (e.g. Horse gate, Bovine spongiform encephalopathy). In this context, it is therefore necessary to provide to beef manufacturers a tool to achieve rapid and precise discrimination of raw pieces. Thus, the objective of this paper is to evaluate the relevance of fluorescence spectroscopy (i.e. classical Front Face Fluorescence spectroscopy and Synchronous Front Face Fluorescence Spectroscopy noted FFFS and SFS respectively in the text) to discriminate directly three beef muscles: *Longissimus thoracis* (LT), *Rectus abdominis* (RA) and *Semitendinosus* (ST), based on their fluorescence fingerprints. We also investigated the performance of three multivariate algorithms, Partial Least Square Discriminant Analysis (PLSDA) and Support Vector Machine (SVM) coupled with Principal Components Analysis (PCA) and Partial Least Square Analysis (PLS) on the discrimination accuracy to propose the best one.

## 2. Materials and methods

### 2.1. Muscle samples

Two hundred and sixty one muscles, 139 *Longissimus thoracis* (LT), 58 *Rectus abdominis* (RA) and 64 *Semitendinosus* (ST) were withdraw from 36 bulls of 3 genotypes with varying lipogenesis capacities (Angus > Limousin > Blond d'Aquitaine). The three muscles were chosen due to their difference in their glycolytic metabolism, because it has been reported that oxidative metabolism can be in favor of meat quality, particularly in terms of tenderness (Renand, Picard, Touraille, Berge, & Lepetit, 2001). RA muscle has a slow oxidative metabolism property, whereas ST muscle has a fast glycolytic metabolism property, the LT muscle having an intermediate metabolism (Hocquette et al., 2012). Samples ( ~ 100 g) of the LT, RA and ST muscles were collected at 24 h post mortem, divided into small cubes (1 $cm^3$), immediately frozen in liquid nitrogen and stored at − 80 °C. All the details on the experimental design and diets were previously described by Gruffat et al. (2013).

### 2.2. Fluorescence spectroscopy

Just before analysis, the frozen samples of muscles were ground into fine and homogeneous powders in liquid nitrogen with a mixer mill (Retch MM301, Hann Germany) and thawed during 1 h00 at 20 °C. Then a proportion of 3 g of each meat powder was placed successively between a powder sample holder and a quartz cell and mounted in a solid sample holder. Before fluorescence acquisition samples were visually controlled to ensure that the entire measurement window was totally covered with sample.

Fluorescence spectra were recorded with a FluoroMax-4 spectrofluorometer (Jobin–Yvon, Longjumeau, signal-to-noise ratio: 3000:1, France) equipped with a solid sample holder with an incidence angle of the excitation radiation set at 60° to minimize reflected light, scattered radiation and depolarization phenomena. Two excitation modes were successively applied on the same sample, classical excitation one (FFFS: Front Face Fluorescence Spectra) and synchronous excitation one (SFS: Synchronous Fluorescence Spectra). SFS and FFFS were performed in duplicates for the 261 muscle samples. For FFFS, emission spectra were recorded at 305–400, 340–540, 360–570, 400–650 and 410–700 nm after excitation at 290, 322, 335, 350 and 382 nm, respectively. Those excitation spectra mainly addressed the fluorescence of tryptophan, vitamin A, collagen/pyridinolin/riboflavin, NADH, and vitamin E, respectively. At the end of the experiment, a total of 2610 emission spectra were recorded (261 muscles × 2 repetitions × 5 excitation wavelengths).

For SFS, the excitation wavelength (λex) and emission wavelength (λem) were scanned simultaneously, usually maintaining a constant wavelength interval, named offset or Δλ, between λex and λem. In this study, six Δλ (i.e. 20, 40, 60, 80, 100, and 120 nm) were used. All the excitation fluorescence spectra were recorded between 250 and 550 nm, giving a total of 3132 spectra (261 muscles × 2 repetitions × 6 offsets).

Before performing discrimination analysis, FFFS and SFS were subjected to a preprocessing procedure, smoothing by Savisky-Golay (polynomial order: 2; number of data: 11), Standard Normal Variate and mean-centering using MATLAB R2013b (The MathWorks Inc., Natic, MA, USA) with the PLS-Toolbox v.7.5 2013 (Eigenvector Research, Manson, Washington, USA).

### 2.3. Chemometrics analysis

Partial Least Square Discriminant Analysis (PLSDA) derived from the PLS regression is one of the most well-known classification procedures in chemometrics. Its aim is to predict the membership of an individual to a qualitative group preliminary defined. This method derives from the PLS regression. PLSDA let the Y matrix be a class response taking values in {1 … K}, therefore, given a new observation of X, prediction for Y is made in the classical way (Wang et al., 2016). The optimum number of PLSDA factors was estimated by analyzing the percentage of validation error and the variance captured error.

Super Vector Machine (SVM) is also a well spread supervised method. The aim of SVM is to find an optimal hyperplane that correctly separates objects belonging to different group. This is done by leaving the largest possible fraction of points of the same class on the same side while maximizing the distance of either class from the hyperplane. The kernel function of SVM was chosen as radial basis function, and the parameters of SVM were optimized by a grid search procedure and 5 fold cross-validation. The SVM was coupled with PCA and PLS algorithms to compress the X block data. The goal of PCA is to express the main variations contained in the initial data in a lower number of variables, the principal components. PLS as the advantage to take into account the correlation between the X and Y matrix, while extracting the latent variables from the X matrix, thus, the latent variables directly refer to the given component (Preda, Jodal, Sixt, Stokland, & Hansson, 2007). For PCA-SVM and PLS-SVM models, the number of principal components and latent variables used respectively in the models were chosen based on the minimum prediction error.

Before performing discrimination analyses, meat samples were divided into three classes related to muscles (LT, RA, and ST). The PLSDA, PCA-SVM and PLS-SVM were performed in MATLAB R2013b (The MathWorks Inc., Natic, MA, USA) using the PLS-Toolbox v.7.5 (Eigenvector Research, Manson, Washington, USA). To facilitate comparison between the three model performances (i.e. PCA-SVM, PLS-SVM, and PLSDA), the database was divided into two sets. Two-thirds of the samples (n = 174) were used for calibration and 1/3 (n = 87) for validation or prediction of the models. The selection of the two sets of data was automatically identified by using the Nearest Neighbor Thinning method (PLS-Toolbox v.7.5, Eigenvector Research) on the initial data matrix containing all the data sets. This method enabled to select validation samples which best fill out all covariance space. Moreover, the performance of the discrimination models were evaluated by Recall, Precision, and Error values in percentage (%), using the Eqs. (1) to (3) respectively.

$$\text{Recall} = \frac{\text{True positives}}{\text{True positives} + \text{False negatives}} \times 100 \quad (1)$$

$$\text{Precision} = \frac{\text{True negatives}}{\text{True negatives} + \text{False positives}} \times 100 \quad (2)$$