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Monitoring of thermal changes in meat by synchronous fluorescence spectroscopy

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ABSTRACT

Here we report the potential of synchronous front-face fluorescence spectroscopy in combination with chemometric tools to detect thermal changes in cooked meat. Samples of bovine meat (*Longissimus dorsi* muscle) were cooked at 66, 90 & 237 °C for 0, 1, 2, 5, 7, and 10 min. Synchronous front-face fluorescence spectra were collected on meat samples in the excitation wavelength range of 250–550 nm using offsets ($\Delta\lambda$) of 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, and 160 nm between excitation and emission wavelengths. The data from the synchronous fluorescence landscape containing 1080 spectra were analyzed using PCA & PARAFAC models. PCAs were applied to determine the potential of synchronous spectra to differentiate these samples as a function of cooking time and to retrieve additional information from different offset values. These results confirmed that the synchronous fluorescence spectra provide information related to the molecular structure of meat, allowing classification of samples as a function of cooking time & temperature. The best PARAFAC model was obtained with 3 components, having 55% core consistency values and 98% of the explained variance. The loading profiles of 1st, 2nd and 3rd components had an optimal $\Delta\lambda$ of 60, 30 and 80 nm, respectively, allowing the determination of excitation (exc.) and emission (em.) maxima wavelengths of 1st (fluorescence band at about exc.: 291 & em.: 351), 2nd (exc.: 297 nm & em.: 327 nm) and 3rd (exc.: 468 nm & em.: 548 nm) components. Loadings and scores of the PARAFAC model developed from the synchronous front-face fluorescence spectra enabled us to get information regarding the changes occurring in meat fluorophores during cooking of meat at 66, 90 & 237 °C from 0 to 10 min.

1. Introduction

Public interest in good quality meat and meat products has increased in recent decades due to an increase in the awareness of high quality and safety of food. Meat protein is susceptible to denaturation and production of Maillard-reaction compounds during heat treatment. Several neo-formed compounds are formed during the Maillard reaction and oxidation in the food induced by heat treatments. Some of these compounds are believed to be mutagenic and/or carcinogenic in nature. These molecules are also linked with the increase in the risk of cancers and cardiovascular diseases. The free amino groups of proteins that are present in meat can react with aldehydes either from lipid peroxidation or reducing sugars to give Schiff bases that ultimately undergo the Amadori rearrangement (Gatellier et al., 2009). After the formation of Schiff bases, proteins that are modified by aldehydes or sugars, have the ability to emit fluorescent light with spectral characteristics, dependant on the type of protein and adduct (Kagan, 1988).

In order to maintain and improve the quality of meat in industrial processing, there is a need to optimize the heat treatment to ensure the safety of the final product. Presence of fluorophores in meat makes fluorescence spectroscopy an ideal candidate to monitor the changes in meat. This method is rapid, reagent free, cost effective and can be used for on-line inspection of food processing in industries. Fluorescence spectroscopy is an analytical method; it has been used extensively in chemistry and biochemistry to explore the molecular structure and function of different compounds. Front-face fluorescence spectroscopy has been used in food science since decades. It provides very useful information on the presence of fluorescent molecules & their environment in

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food samples (Sahar et al., 2009a,b). For example, fluorescence properties of aromatic amino acids of proteins (Dufour et al., 1994; Lakowicz et al., 1983), heterocyclic aromatic amines (Sahar et al., 2010) and retinol (Dufour et al., 1990) have been reported. Recently quality of cereal & cereal products (Zekovic et al., 2012), milk (Yazdi and Corredig, 2012), eggs, olive oil (Tena et al., 2012), fish (Olsen et al., 2006; Hassoun and Karoui, 2015), fruit spirits (Tomkova et al., 2015), cheese (Dankowska et al., 2015) and meat (Sahar et al., 2009a,b; Sahar and Dufour, 2015) has been assessed using fluorescence spectroscopy. Similarly this techniques has been used to detect the adulteration in extra virgin olive oil (Guzman et al., 2015), authentication of walnut oil (Li et al., 2015), classification of honey (Lenhardt et al., 2015) screening of fruits and vegetable and identification of microbes (Yoshimura et al., 2014) to ensure food safety. This spectroscopic method had already been used to determine the protein denaturation in pasteurized and UHT milk (Leclère and Birlouez-Aragon, 2001). In addition, research was also conducted to follow the thermal oxidation of heated oils, oxidative changes in oatmeal, monitoring of Maillard reaction in sugar processing (Christensen, 2005), milk (Birlouez-Aragon et al., 2002), infant formulas (Birlouez-Aragon et al., 2005) and seeds (Yaacoub et al., 2009).

In the traditional fluorescence spectroscopic technique, only excitation or emission wavelength has been used to determine the quality of food products. This approach limits the use of fluorescence spectroscopy. In order to retrieve more information from food matrices, both excitation and emission wavelengths can be used simultaneously. Synchronous fluorescence spectroscopy can be assumed as more powerful method for analysing complex food matrices, as excitation and emission wavelengths are scanned simultaneously keeping a constant wavelength interval between these two wavelengths. It makes it possible to narrow down the spectral bands and retrieve information on several fluorophores instead of a single fluorophore at the same time. Three dimensional data is received by synchronous fluorescence spectroscopy (samples $\times$ offsets $\times$ excitation wavelengths) that can be analyzed by decomposition models like parallel factors analysis (PARAFAC) (Bro, 1997). PARAFAC has already been used for the decomposition of the three way data matrices (Ni et al., 2008) of different foods like sugar (Bro, 1999), meat (Møller et al., 2003), milk (Boubellouta and Dufour, 2008), fish oil (Pedersen et al., 2002), yogurt (Christensen, 2005), edible oils (Guimet et al.,, 2004; Sikorska et al., 2004), wine (Airado-Rodriguez et al., 2009) and cheese (Christensen et al., 2003).

The objective of this study was to investigate the potential of synchronous front-face fluorescence spectroscopy in combination with chemometric tools, to detect the changes occurring in meat by the application of heat treatments 66, 90 and 237 °C for 0–10 min.

2. Materials & methods

2.1. Sample preparation

2.1.1. Cooking of meat samples

The *Longissimus thoracis* muscles were obtained from charolais-breed cows (1–4 years old). These muscles were vacuum-packed at 3 days post-slaughter and aged at 4 °C for up to 12 days post-mortem. After aging, the muscles were frozen and stored at −20 °C. The muscles were frozen in a non-ventilated −20 °C chamber and can therefore be considered slow-frozen. The big pieces of meat selected for heat treatment were thawed in a water bath at 15 °C, and, a 5 cm-diameter cylinder was cut from the center. This cylinder was then sliced to obtain 1–2 mm-thick disk-shaped slices. These meat samples in slices were cooked at 66, 90 and 237 °C for 1, 2, 5, 7, and 10 min. Jets of superheated steam mixed with air were applied on these thin slices of meat. Samples 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 alongside with functional analysis.

2.1.2. Synchronous fluorescence spectra

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. 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 the reflected light, scattered radiation, and depolarization phenomena were minimized (Sahar et al., 2009a,b). The spectra were collected at room temperature. The experiment was repeated for 4 times for each cooking time and temperature, so a total of 1080 synchronous fluorescence spectra (6 different times of cooking $\times$ 4 repetitions $\times$ 15 offsets $\times$ 3 different temperatures) were recorded.

2.2. Chemometrics

2.2.1. Principal component analysis (PCA)

PCA was applied to the synchronous spectra collected in each offset to investigate the differences in the spectra (Sahar et al., 2009a,b). 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, 1997; Herbert et al., 2000, 1999).

2.2.2. Parallel factor analysis (PARAFAC)

A decomposition method that is known as PARAFAC is used in this study. It is a generalized form of principal component analysis (PCA) to higher-order arrays. 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 ($N \geqslant 3$) into a sum of the outer products of N loading components (Rutledge and Jouan-Rimbaud 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 Jouan-Rimbaud Bouveresse, 2007). Several methods can be used to determine the appropriate number of PARAFAC components. 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 50%) 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 has been applied in 3 modes to build the components of the models. Imposing non-negativity constraint on decomposition model parameters of fluorescence 3-way spectral data is a common practice since both the spectral intensities and fluorophores concentrations are known to be positive (Bro et al., 2002). Chemometric analyses were performed using MATLAB (The Mathworks Inc., Natick, Mass., U.S.A.).