Food Chemistry 131 (2012) 1030–1036

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Potential of a custom-designed fluorescence imager combined with multivariate statistics for the study of chemical and mechanical characteristics of beef meat

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ARTICLE INFO

Article history:
Received 20 July 2009
Received in revised form 7 January 2011
Accepted 19 September 2011
Available online 25 September 2011

Keywords:
Bovine muscle
Fluorescence imaging
Image analysis
Multivariate statistics
Biological properties
Chemical composition
Mechanical properties

ABSTRACT

The potential of fluorescence imaging to discriminate different bovine muscles in relation with animal age, muscle type, chemical and mechanical properties was examined. Twenty-four muscles of three types (*Gluteus medius*, *Longissimus dorsi*, and *Semitendinosus*) and two animal age groups (10–13-years old and 12–24-months old) were obtained from the carcasses of Limousin breed cows. One hundred and forty-four images were collected at three illuminating conditions (exc 320 nm, exc 380 nm, and white light) using a custom-designed imager. The image cubes were processed using "regionprops" algorithm developed earlier in order to extract image shape features (*number of shapes*, *area*, *major-axis-length*, *eccentricity*, and *solidity*). Extracted image shape features were processed using custom-designed programs. The results of the PLSDA performed on image shape features showed 100% good discrimination for the three types of muscles. Muscle samples were also subjected to chemical analysis (dry matter, fat, pyridinoline, total, insoluble and soluble collagen) and mechanical tests (shear stress and breaking energy). PLSR models indicated relations between extracted image shape features and mechanical properties, i.e., $R^2 = 0.69$ and RMSEV = 0.514 were observed for breaking energy for adult-animal muscles. Regarding chemical composition, image shape features allowed to predict total collagen of *L. dorsi* with $R^2 = 0.61$ and RMSEV = 0.756. This study has demonstrated a promising potential of the custom-designed fluorescence imager combined with multivariate statistical tools in the study of beef meat.

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1. Introduction

There is a continuously growing demand for the high-speed systems able to measure quality and safety characteristics of meat, e.g., defects, composition and tenderness (Monin, 1998). These systems are supposed to replace labour-intensive and time-consuming analytical techniques and visual and manual inspections yet practiced in the meat and poultry industry at present.

New commercially available instruments including spectrometers and imaging devices allow measuring quality attributes of foods in rapid and non-destructive manner. Moreover, these instruments combined with data processing tools allow collecting large amount of experimental data and extracting useful information related to food properties (Faucitano, Huff, Teuscher, Gariepy, & Wegner, 2005; Irie, Izumo, & Mohri, 1996; Li, Tan, Martz, & Heymann, 1999).

Front-face fluorescence spectroscopy has being investigated extensively as an alternative technique to analyse food properties and their constituents (Kulmyrzaev, Karoui, De Baerdemaeker, & Dufour, 2007). Interesting results have been reported on the use of front-face fluorescence spectroscopy in examination of connective tissue and fat in meat (Egelandsdal, Wold, Sponnich, Neegard, & Hildrum, 2002; Skjervold et al., 2003; Wold, Lundby, & Egelandsdal, 1999), meat tenderness (Dufour & Frencia, 2001; Frencia, Thomas, & Dufour, 2003), texture (Allais, Viaud, Pierre, & Dufour, 2004; Lebecque, Laguet, Chanonat, Lardon, & Dufour, 2003), and fish freshness (Dufour, Frencia, & Kane, 2003; Karoui, Thomas, & Dufour, 2005). Fluorescence spectroscopy was also applied to characterise different chicken diseases and was proposed as a rapid technique to detect unwholesome chicken carcass (Kang, Kim, Chao, Kim, & Chen, 2002).

Classical spectroscopy (fluorescence, visible, and infrared) traditionally yield information on a small region of a sample at a time. Therefore, the product samples studied using classical spectroscopy

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doi:10.1016/j.foodchem.2011.09.066

must be relatively homogeneous in order to extract characteristics reflecting properties of the whole product. The drawbacks of conventional spectroscopy can be easily eliminated by use of spectroscopic imaging techniques referred to as hyperspectral imaging and multispectral imaging (Chao, Mehl, & Chen, 2002; Jun et al., 2007). When dealing with highly heterogeneous products such as meat, characteristics obtained by imaging a relatively large region do truly represent the non-uniform distribution of components in the heterogeneous products, e.g., collagen and fat in meat (Basset, Buquet, Abouelkaram, Delachartre, & Culioli, 2000; Wold et al., 1999). The hyperspectral imaging techniques combine spectroscopic and imaging systems to collect spectral and spatial information simultaneously. Therefore, the amount of information obtained by hyperspectral and multispectral imaging techniques is incomparably large and may really reflect the total variance of the product properties.

Collagen, the major fraction of connective tissue, contributes significantly to toughness of meat (Foegeding, Lanier, & Hultin, 1996). Over 90% of the intramuscular collagen is located in the perimysium and is comprised of the collagen of types I and III depending upon animal age, muscle type, and gender (Jeremiah, Dugan, Aalhus, & Gibson, 2003). Collagen solubility is also shown to be influenced by gender, breed, and animal age (Bailey, 1985; Cross, Schaunbacher, & Crouse, 1984; Lepetit, 2007) as well as collagen cross-links (Bailey, 1985; Bosselmann, Moller, Steinhart, Kirhgessner, & Schwarz, 1995; Lepetit, 2007; Mairano, McCormick, Field, & Snowder, 1993; McCormick, 1999). Thus, collagen characteristics can be used as markers to discriminate muscles depending on its type and animal age and gender. Intramuscular fat or marbling affects juiciness and sensory properties of meat (flavour and mouth feel) (Foegeding et al., 1996). It accounts for 12–14% of the variation in all palatability trait (Jeremiah et al., 2003). However, excessive amounts of intramuscular fat decrease the usable lean portion and meat quality grades. Consequently, accurate measurement of collagen and intramuscular fat in meat provides adequate information about meat quality. Moisture content also influences juiciness of meat directly and its tenderness indirectly (Jeremiah et al., 2003).

The present study is a part of a greater research project where the primary goal is to study how spectroscopy (Kulmyrzaev & Dufour, 2002) and multispectral imaging (Kulmyrzaev, Bertrand, & Dufour, 2008) can be used to measure food quality. The specific objective of this study was intended to assess the potential of a custom-designed imager based mainly on autofluorescence imaging to discriminate different bovine muscles in relation with biological factors (muscle type and animal age). The potential of the image shape features extracted from the recorded image cubes to discriminate the three different muscles was investigated. The correlations between the features extracted from the images using chemometrics with the chemical and mechanical parameters measured on the muscle samples were also examined.

2. Materials and methods

2.1. Experimental muscles and sample preparation for further analysis

The experimental muscles were supplied by "Charal Egletons" (Egletons, France) and "Charal La Chataigneraie" (La Chataigneraie, France). Four young (12–24 months, $n = 4$) and four adult (10–13 years, $n = 4$) female animals of *Limousin* breed cows were selected to consider the variation in muscle properties related to the animal age factor. *Gluteus medius* (GM), *Longissimus dorsi* (LD), and *Semitendinosus* (ST) muscles were removed from carcasses 24 h after slaughter. Each muscle was then cut into three pieces to conduct three kinds of experiments, imaging, chemical composition measurement, and mechanical measurement. The muscle pieces for imaging were identified, vacuum-packaged in polyethylene bags and stored at 4 °C. Imaging of the muscles was conducted 48 h after slaughter. The epimysium of the muscle pieces intended for chemical analysis was carefully dissected and a sample of about 200 g was taken from each muscle. Samples were cut into pieces of 4–5 cm cross-section. The muscle samples were sealed under vacuum in plastic bags and stored at −20 °C until preparation for analysis. Frozen muscles were homogenised in a household cutter, lyophilised for 48 h, then pulverised in a horizontal blade mill and stored at −4 °C in stoppered plastic flasks until analysed. All the analyses described below were carried out on representative subsamples of each pulverised muscle. The muscle samples for mechanical measurements were cut into pieces (approximately $10 \times 10 \times 4$ cm), vacuum packed and stored 14 days at 4 ± 2 °C and then were frozen. The samples were thawed in water bath at 10 °C for 1 h just before measurements.

A sample holder was designed allowing cutting the muscle samples with a smooth surface appropriate for high-quality imaging. The sample holder consisted of two sections to accommodate two different muscles simultaneously. Each cell section was built of three removable rectangular frames with the internal size of 4×4 cm and height of 1 cm. The muscles were placed in the sample holder and were cut perpendicular to the muscle fibre orientation using a sharp cutter. After cutting, the frames containing muscles were removed from the sample holder and placed in the imaging system. Two cuts were made per experimental muscle providing two surfaces for imaging.

The first figure in the image codes denoted the number assigned to animals, that is 1–4 were adult animals and 5–8 were young animals. Next two characters denoted muscle type (GM, LD, and ST). The last figure in the codes was the number of a cut (1 and 2). For instance, code 1GM2 was assigned to the image of the second cut (2) of the *G. medius* (GM) muscle removed from the carcass of the adult animal number 1.

2.2. Chemical analysis

To estimate total collagen content, hydroxyproline content was measured on lyophilised muscle powder after acid hydrolysis of 500 mg lyophilised meat in 20 ml of 6 M HCl heated at 110 °C overnight in a screw-capped glass tube as described by Listrat et al. (1999). Collagen solubility was determined according to a procedure adapted from Hill (1966). Briefly, lyophilised muscle powder was rehydrated for 1 h and heated in a water bath at 75 °C for 1 h. After centrifugation at 3300*g* for 20 min at room temperature, the pellet was submitted to acid hydrolysis (same conditions than for total collagen) and its collagen content was determined, i.e., the heat-insoluble collagen. The soluble collagen was determined as following:

$$\text{Soluble Collagen} = \frac{\text{Total Collagen} - \text{Insoluble Collagen}}{\text{Total Collagen}} \cdot 100\%.$$

Pyridinoline cross-linking was assessed after an acid hydrolysis. The resulting hydrolysate, in fact a sample of this used for total collagen, was centrifuged at 16,000*g* for 5 min and 300 μl of the supernatants were added with 300 μl of 6 M NaOH and 300 μl of 1 M Tris. Final pH was adjusted between 7 and 8 with a few microlitres of 6 M HCl or NaOH. Pyridinoline (PYD) was determined using the enzyme linked immunoassay Metra PYD EIA kit (Quidel Corporation, San Diego, CA, USA). Previous assays had evidenced no interfering effect of the 1/10 diluted muscle extract on the PYD determination (results not shown).

Dry matter and free fat contents of the experimental muscles were determined following the standard methods (ISO1442:1997).

Each chemical analysis was carried out in triplicate.

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