Food Research International 52 (2013) 185–197

Contents lists available at SciVerse ScienceDirect

Food Research International

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

Classification of pig fat samples from different subcutaneous layers by means of fast and non-destructive analytical techniques

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ARTICLE INFO

Article history:
Received 23 January 2013
Accepted 11 March 2013

Keywords:
Pig fat
Multivariate classification
Variable selection
Tristimulus colorimetry
FT-NIR spectroscopy
Hyperspectral imaging

ABSTRACT

In the meat industry the fat portions coming from two different subcutaneous layers, i.e., inner and outer, are destined to the manufacturing of different products, hence the availability of cheap, rapid and affordable methods for the characterization of the overall fat quality is desirable. In this work the potential usefulness of three techniques, i.e. tristimulus colorimetry, FT-NIR spectroscopy and NIR hyperspectral imaging, were tested to rapidly discriminate fat samples coming from the two different layers. To this aim, various multivariate classification methods were used, also including signal processing and feature selection techniques. The classification efficiency in prediction obtained using colorimetric data did not reach excellent results (78.1%); conversely, the NIR-based spectroscopic methods gave much more satisfactory models, since they allowed to reach a prediction efficiency higher than 95%. In general, the samples of the outer layer showed a high degree of variability with respect to the samples of the inner layer. This is probably due to a greater variability of the outer samples in terms of fatty acid composition and water amount.

1. Introduction

The technological quality of the subcutaneous adipose tissue of pigs is mainly represented by its consistency and its resistance against oxidative processes, that render it suitable for processing and storage. The consistency is closely correlated to the lipid and water content, to the texture of the connective tissue and to the nature of the fatty acids which constitute the lipids. A low lipid content associated with a high water content leads to a poor consistency of the adipose tissue (Lebret & Mourot, 1988). Also the fatty acid composition exerts a key role in determining the consistency of the adipose tissue: a higher degree of unsaturation corresponds to a lower melting point of the fat and, consequently, to a lower consistency (Enser, 1983).

Furthermore, an adipose tissue excessively rich in unsaturated fatty acids is certainly positive from the nutritional point of view, but it can create serious problems from the technological point of view, since it can easily undergo hydrolytic and oxidative phenomena during manufacturing (Lo Fiego, 1996; Wood et al., 2008). As pointed out by Lebret and Mourot (1988), a high organoleptic and technologic quality is not generally associated to a high content of polyunsaturated fatty acids.

A very peculiar aspect of the pig fat covering tissue is that it is constituted by two or more layers having different composition and coming from different metabolic pathways (Mersmann & Leymaster, 1984). The outer layer, close to the rind, presents a greater consistency and is more rich in unsaturated fatty acids with respect to the inner layer (Malmfors, Lundstrom, & Hansson, 1978). The greater consistency of the outer layer, despite the higher degree of unsaturation, is probably due to a greater collagen content, i.e. of connective tissue, and to a greater organization of the connective structure surrounding the adipocytes (Lo Fiego, Tedeschi, Santoro, & Nanni Costa, 1987).

Abbreviations: coif: wavelet of the "coiflets" family; CV: Cross validation; d1: First order derivative pretreatment; d2: Second order derivative pretreatment; db: wavelet of the "daubechies" family; det1: Linear detrend pretreatment; det2: Quadratic detrend pretreatment; EFF: Efficiency %; FOP: Fiber Optic Probe; FT-NIR: Fourier Transform-Near InfraRed; HSI: HyperSpectral Imaging; *In*: Class corresponding to the inner layer samples; *In_low*: Samples of class *In* measured on the lower face of the disk; *In_up*: Samples of class *In* measured on the upper face of the disk; iPLS-DA: Interval Partial Least Squares-Discriminant Analysis; IS: Integrating Sphere; LV: Latent Variable; m: Meancentering pretreatment; MSC: Multiplicative Scatter Correction pretreatment; N: None pretreatment (raw spectra); NIR: Near InfraRed; *Out*: Class corresponding to the outer layer samples; *Out_low*: Samples of class *Out* measured on the lower face of the disk; *Out_up*: Samples of class *Out* measured on the upper face of the disk; PC: Principal Component; PCA: Principal Component Analysis; PLS-DA: Partial Least Squares-Discriminant Analysis; Q–T^2: Q residuals versus Hotelling's T^2; RMSECV: Root Mean Square Error in Cross Validation; ROI: Region Of Interest; S: Smoothing pretreatment; SNV: Standard Normal Variate pretreatment; S/N: Signal to noise; sym: wavelet of the "symlets" family; TRN: Training set (including only *Out_up* and *In_low* measurements); TST1: First test set (including only *Out_up* and *In_low* measurements); TST2: Second test set (including only *Out_low* and *In_up* measurements); VIP: Variable Importance in Projection; WPT: Wavelet Packet Transform; WPTER: Wavelet Packet Transform for Efficient pattern Recognition.

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http://dx.doi.org/10.1016/j.foodres.2013.03.022

The stratification of the pig fat in different layers plays an important role in the Italian industry, in fact the processing industry makes use of the outer fat, considered more hard, in the form of cubes for the production of salami and sausages, while the inner layer tissue, classified as soft fat, is mainly destined to the melting (Santoro, 1983).

From the analytical point of view, the fat quality is generally estimated by means of chemical analyses, i.e. redox titrations to determine the iodine value, that is used to assess the amount of unsaturation in fatty acids, and gas chromatography for the determination of the fatty acid composition. However, these common methods are expensive, time-consuming, detrimental to the environment because of the use of chemical reagents, and they are not suitable to be used to follow an industrial process in real-time.

Therefore, the aim of the present work is to verify the suitability of three fast and non-destructive methods, i.e. tristimulus colorimetry, Fourier Transform-Near InfraRed (FT-NIR) spectroscopy and NIR HyperSpectral Imaging (HSI) to discriminate between fat samples coming from two different subcutaneous layers. The choice to test these methods is due to the fact that they are particularly flexible, in fact they can supply qualitative and, in some cases, quantitative information with minimal or no sample preparation, which makes them suitable for on-line applications.

Colorimetric measurements have been recently used in the literature to investigate possible relationships between fat color and fatty acid composition (Wood et al., 2003) which is often variable, depending on genotype and sex of the swine and on the rearing system. Carrapiso and Garcia (2005) proved that CIE L*a*b* variables of subcutaneous fat were closely related to fatty acid composition. In particular, the largest co-relationships involve L* which is negatively related to most unsaturated fatty acids and positively to the most abundant saturated fatty acids, while Gandemer (2002) found larger whiteness and pinkness in firm fat than in low consistency fat.

The potential of NIR spectroscopy for predicting the fatty acid composition of fat samples is well known, in fact a number of papers have been published about this topic. Among them, some works are based on NIR measurements acquired by means of a fiber optic probe (Gonzalez-Martin, Gonzalez-Perez, Hernandez-Mendez, & Alvarez-Garcia, 2003; Pérez-Marín, De Pedro Sanz, Guerrero-Ginel, & Garrido-Varo, 2009), while other works made use of diffuse reflectance, transmission and transflectance measurements (Gjerlaug-Enger, Kongsro, Aass, Ødegard, & Vangen, 2011; Müller & Scheeder, 2008; Ripoche & Guillard, 2001), all of them generally reporting good results. The paper by Pérez-Juan et al. (2010) merits a particular mention for our purposes since they used NIR spectroscopy to analyze the fatty acid composition at two different locations of the subcutaneous fat.

As for hyperspectral imaging, that is an emerging technique, at present only a limited number of works have been published concerning the analysis of fat (for instance, Kobayashi, Matsui, Maebuchi, Toyota, & Nakauchi, 2010 on beef samples and Kamruzzaman, ElMasry, Sun, & Allen, 2011 on lamb samples). In the context of swine products a single paper does exist (O'Farrell, Wold, Høy, Tschudi, & Schulerud, 2010), aimed to the on-line quantification of the fat amount in inhomogeneous pork trimmings. HSI simultaneously collects the spectral and spatial information on a sample to compose a visual image of components distribution. This kind of representation enables the characterization of complex heterogeneous samples by looking at the spatial features and allows the identification of a wide range of surface constituents by looking at the spectral features (Gowen, O' Donnell, Cullen, Downey, & Frias, 2007). In particular, HSI is useful not only to define what chemical species are present in the sample and how much of each is present, but also principally to indicate where they are located. Moreover, it is useful whenever a fast and non-destructive technique is needed to characterize a sample and to obtain a visual representation of the analysis.

The datasets acquired with the different analytical techniques have been initially processed by means of Principal Component Analysis (PCA), that was used as an explorative tool to detect possible outlier measurements, then they were subjected to classification analysis. Firstly the classification has been performed on the whole original data using the Partial Least Squares-Discriminant Analysis (PLS-DA) algorithm, and then the NIR-based datasets were also subjected to variable selection before building the classification models. Two variable selection methods were used, i.e. iPLS-DA, a local modeling procedure based on PLS-DA, and WPTER (Wavelet Packet Transform for Efficient pattern Recognition), a wavelet-based feature selection algorithm (Antonelli et al., 2004; Cocchi et al., 2004, 2005). The obtained classification models have been compared, both in order to evaluate the predictive ability of the models obtained using different analytical techniques, and to interpret the chemical meaning of the choices made by the tested algorithms. Particular attention has been paid to the samples that were incorrectly classified in order to understand the reasons for their misclassification.

2. Experimental

2.1. Samples

Sixty-six pigs from *Italian Landrace* × *Large White* crossbreeds provided 205 samples of fat tissue by means of the following sampling procedure. The subcutaneous adipose tissue was hand-slashed by an expert operator at the last rib level in a way to obtain disks of fat tissue having diameter of about 3 cm and thickness ranging from 3 mm to 2 cm. These fat samples consisted in two adjacent layers, lying at different depths with respect to the rind. The layer close to the rind (that was previously removed) was labeled as Outer (*Out*) and the layer far from the rind as Inner (*In*). The two layers were then separated by means of a manual cut, after a visual assessment of the line of demarcation of the layers, to gain the corresponding *Out* and *In* samples. It has to be noticed that for each pig specimen from 1 to 4 *Out* and *In* samples have been collected; this means that for each original fat disk the *Out* and *In* parts were not necessarily all kept. To keep track of the sampling point where the following analytical measurements were carried out on the fat disk, an additional labeling was introduced: *Out_up* for the upper face of the *Out* layer, *Out_low* for the lower face of the *Out* layer, *In_up* for the upper face of the *In* layer and *In_low* for the lower face of the *In* layer. The procedure adopted to delimit and label the fat samples is represented in Scheme 1.

All the collected samples were stored in dark conditions at −20 °C. Before analysis, the samples were slowly defrosted at 4 °C for 1 h and then at room temperature for other 30 min. All the measurements were performed at room temperature. Each day, only the samples to be analyzed were defrosted and analyzed following a random order. Then, this order was shuffled, repeated measurements were performed on each sample and, at the end of the daily measurement session, the samples were stored again at −20 °C.

2.2. Sample preparation and analysis

2.2.1. Tristimulus colorimetry

Colorimetric measurements were accomplished using a Chroma Meter CR-400 Konica Minolta (CIE standard illuminant D65) tristimulus colorimeter. The standard instrumental procedure for calibration was applied before use.

For each fat disk, four measurements were performed. In particular, a total of 820 measurements (=205 samples × 2 acquisitions on each disk face × 2 repeated measurements) has been acquired.

Each recorded tern of values, consisting in CIE L*, a* and b* colorimetric parameters, is the result of the instrumental mean of three measurements. In addition, starting from the recorded L*, a* and b* values, two further colorimetric parameters, i.e. Hue and Chroma, have been calculated using the following equations:

$$\text{Hue} = \arctan(b*/a*) \quad (1)$$