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Prediction of fatty acids content in pig adipose tissue by near infrared spectroscopy: At-line versus *in-situ* analysis

E. Zamora-Rojas *, A. Garrido-Varo, E. De Pedro-Sanz, J.E. Guerrero-Ginel, D. Pérez-Marín

Department of Animal Production, Non-Destructive Spectral Sensor Unit, Faculty of Agricultural and Forestry Engineering, University of Córdoba, Agrifood Campus of International Excellence (ceiA3), Campus Rabanales, N-IV, km 396, Córdoba, 14014, Spain

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ABSTRACT

A handheld micro-electro-mechanical system (MEMS) based spectrometer working in the near infrared region (NIR) (1600–2400 nm) was evaluated for *in-situ* and non-destructive prediction of main fatty acids in Iberian pig (IP) carcasses. 110 IP carcasses were measured. Performance of the instrument was compared with at-line high-resolution NIRS monochromators working in two analysis modes: melted fat samples (transflectance cups) and intact adipose tissues (interactance fiber optic). Standard Error of Prediction (SEP) values obtained on the MEMS-NIRS device were: 0.68% (stearic), 1.30% (oleic), 0.55% (linoleic) and 1% (palmitic), explaining a variability of 83%, 84%, 81% and 78%, respectively. As expected, this represented a loss of predictive capability in comparison to at-line models, even with the same spectral characteristics as on the handheld device. However, the estimated total errors were at the same level for gas chromatography and NIRS analysis. This indicates that the MEMS-NIRS *in-situ* analysis of each individual carcass provides a cost-effective and real-time quality control system with suitable accuracy.

1. Introduction

The determination of the fatty acid (FA) profile has a high relevance for the quality control of raw or/and dry-cured Iberian pig (IP) meat products. FA profile of the subcutaneous adipose tissue performed by gas chromatography (GC) has been traditionally used for classifying or/and authenticating animals in different commercial categories. Furthermore, nowadays, due to the consumer demands and legal requirements (EU, 2011), the information displayed in the food label for local, traditional and added value meat products, as for IP, is very important. Mandatory and voluntary food traceability and labeling programs are, therefore, crucial to inform consumers and get a differentiated position on the market from other similar products since this compositional information can be used for marketing strategies related to nutritional and health aspects (Garrido-Varo & De Pedro, 2007).

Near Infrared Reflectance Spectroscopy (NIRS) has shown its potential for predicting FA composition in IP melted fats, adipose and meat tissues with a variable grade of precision and accuracy (De Pedro, Garrido, Bares, Casillas, & Murray, 1992; Fernández, De Pedro, Núñez, Silió, García-Casco, & Rodríguez, 2003; Fernández-Cabanás, Garrido-Varo, García-Olmo, De Pedro-Sanz, & Dardenne, 2007; Fernández-Cabanás, Polvillo, Rodríguez-Acuña, Botella, & Horcada, 2011; García-Olmo, Garrido-Varo, & De Pedro, 2001; González-Martín, González-Pérez, Álvarez-García, & González-Cabrera, 2005; González-Martín, González-Pérez, Hernández-Méndez, & Álvarez-García, 2003; González-Martín, González-Pérez, Hernández-Méndez, Álvarez-García, & Lázaro, 2002, 2005; Pérez-Marín, De Pedro, Guerrero-Ginel, & Garrido-Varo, 2009; Pérez-Marín, Fearn, Guerrero, & Garrido-Varo, 2010; Pérez-Marín, Garrido-Varo, De Pedro, & Guerrero-Ginel, 2007, 2010). Garrido-Varo, García-Olmo, and Pérez-Marín (2004) indicated that the differences found in the estimation of the main fatty acids and its overall statistical performance depend on the sample presentation, analysis mode and instrument characteristics. NIRS can be a fast, environmentally friendly, objective, cost-efficient analysis, although it requires a calibration stage based on well-characterized samples analyzed both spectrally and by wet chemistry (i.e. GC as reference analysis). In fact, melted fat analysis by NIRS can be useful as a substitute for GC analysis, to predict the main fatty acid composition, specially, stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and palmitic acid (C16:0) (Fernández-Cabanás et al., 2007; García-Olmo et al., 2001; Garrido-Varo et al., 2004), although some care should be taken for routine analysis such as deviations due to large time elapse between calibration development and NIRS analysis of unknown samples (Pérez-Marín et al., 2007, 2010). Moreover, the industry is interested in simplification of the sampling/sample presentation to perform individual analysis at the speed of the slaughter process and in the use of new low-cost NIRS instrumentation. NIRS has been studied for these purposes based on fiber-optic instrumentations showing potential for the prediction of oleic, linoleic, palmitic and stearic fatty acids (González-Martín et al., 2003; Pérez-Juan, Afseth, González, Díaz, Gispert, Furnols, Oliver, & Realini, 2010; Pérez-Marín et al., 2009). As in other food products, the variability between campaigns/seasons/years is a complex task that can affect the suitability of the

* Corresponding author. Tel./fax: +34 957 21 85 55.
E-mail address: e.zamora@uco.es (E. Zamora-Rojas).

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technology. In extensive IP productions, the lack of uniformity in the chemical composition between individual animals and different slaughtering campaigns, due probably to variability in the diet based on natural resources of the field (De Pedro, Núnez, García, Aparicio, Campos, & Pérez, 2007; Garrido-Varo et al., 2004; Tejerina, García-Torres, de Vaca, Vázquez, & Cava, 2012), has been shown.

A further step, rather than take the sample from the process and present it to the instrument, is to move the instrument to the process line, making non-destructive *in-situ* NIRS analysis a reality. Preliminary work published the first on-site NIRS application for the prediction of FA profiles in the slaughter process line with a fiber optic post-dispersive spectro-radiometer (Pérez-Marín et al., 2009). They measured live animals, subcutaneous adipose tissues in the carcass process line and in the laboratory for the prediction of FA profile. However, they concluded that the instrumentation used is not easy to adapt in the industry due to its weight and other portability characteristics.

New innovative handheld devices based on micro-electro-mechanical systems (MEMS) are now on the market, combining small size, light weight, robustness, cost-effectiveness and ease of use. Zamora-Rojas, Pérez-Marín, De Pedro-Sanz, Guerrero-Ginel, and Garrido-Varo (2012) showed the suitability of this kind of instrumentation for the *in-situ* classification/authentication of IP carcasses in different commercial categories based on the feeding regime of the animals during the two months prior to slaughter. However, there is no information about its applicability for the prediction of FAs analysis. Moreover, all the studies performed using other instruments are difficult to compare since they used different sample sets showing independent results that do not enable us to know the effect of instrumentation and/or characteristics of the sample presentation/analysis mode in the predictive capacity of the models.

In this paper, different NIRS analysis modes and instrumentations are evaluated for the quantitative at-line and *in-situ* fatty acid profile prediction (C16:0, C18:0, C18:1 and C18:2) in IP subcutaneous adipose tissues using the same data set. The study compares the performance and accuracy of two NIRS at-line applications (melted fat and intact adipose tissue analysis) versus an *in-situ* application (non-destructive measurements of subcutaneous adipose tissue in the carcass) based on a handheld MEMS-NIRS device in the carcass process line.

2. Materials and methods

2.1. Sample set

One hundred and ten Iberian pigs (pure Iberian and Iberian-duroc crossbreds), from two slaughtering seasons (2008/2009 and 2009/2010) and different feeding regimes ("Acorn", "*Recebo*" and "Feed"), as described in Spanish legislation (BOE, 2007) and monitored by trained personnel, were slaughtered at several commercial Spanish slaughterhouses at the age of 12–14 months and with an average weight of 160 kg.

2.2. NIRS measurements and reference data

All samples were analyzed using three NIRS instruments:

- One handheld micro-electro-mechanical system (MEMS)-based NIR spectrometer (Phazir™ 1624, Polychromix Inc., USA) working in reflectance mode in the 1600–2400 nm range with a non-constant wavelength step of around 8 nm (pixel resolution 8 nm, optical resolution 12 nm). Sensor integration time was 600 ms. The device was equipped with quartz protection to prevent dirt accumulation. Transverse subcutaneous adipose tissue sections in the tail insertion area were measured directly on the carcasses about 2 h post-slaughter, chilled in a temperature controlled chamber. Four spectra per carcass were collected over the sample area, two per hypodermis sub-layer (Fig. 1) to cover the possible variability of the sample.
- Two Foss NIRSystems (FNS) 6500 spectrometers (Foss-NIRSystems Inc., Sylver Spring, MD, USA), one equipped with an interactance fiber optic for intact analysis of subcutaneous adipose tissues and other with a spinning module for the analysis in transflectance mode of melted fat samples. The instruments operate in the spectral range 400–2500 nm with a spectral interval of 2 nm. Duplicate spectra were collected. Skin-free subcutaneous adipose tissue samples were obtained from the same area as the MEMS-NIRS measurements and stored at −20 °C until 24 h before the at-line NIRS analysis. After intact measurements with the FNS instrument, the samples were melted in a microwave following De Pedro, Casillas, and Miranda (1997) for its NIRS analysis using folded-transmission gold reflector cups with a pathlength of 0.1 mm.

Gas chromatography (GC) was used to determine the fatty acid composition as reference method. The methyl esters of fatty acids were extracted with hexane using a PerkinElmer Sigma 3D chromatograph with FID detector and automatic injection system. Values are expressed as percentages (%) of the total FAs analyzed (BOE, 2004).

2.3. Data processing

Different software and chemometric packages were used for data processing. Spectra repeatability and population structuring analysis were performed using the WinISI™ software package (ver 1.50, Infrasoft International, LLC., USA). Interpolation of the data between instruments was performed on MATLAB® (ver 7.10, The Mathworks, Inc., USA). Chemometric PLS Toolbox software for MATLAB® was used for calibration development and evaluation (ver 5.8, Eingenvector Research, Inc., USA).

The selection of the optimized wavelength range for each application is a critical point apart from the selection of the sample library, analysis performance, spectral repeatability, etc. Based on previous papers, the data matrices proceeding from the interactance FNS analysis of intact adipose tissues were trimmed to the range 450–2208 nm and from MEMS-NIRS reflectance carcass analysis to the region 1600–2208 nm (Zamora-Rojas et al., 2012), prior to any other data analysis. This was done to avoid noisy regions.

2.3.1. Spectral repeatability

The Root Mean Squared (RMS) statistic (Shenk & Westerhaus, 1995, 1996) was used for spectrum repeatability since the collection of high-quality spectra is critical for a reliable calibration model. This statistic indicates the similarity between different spectra of a single sample. RMS cut-off values used for spectral quality assurance were

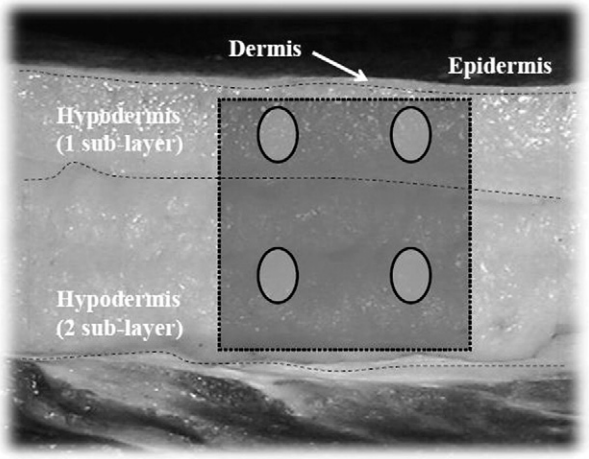

Fig. 1. Schematic drawing of a subcutaneous adipose tissue section. Spot areas (8×10 mm^2) scanned by the MEMS-NIRS instrument (light gray) and area (43×43 mm^2) analyzed by the fiber optic of the FNS monochromator (dark gray).