Meat Science 90 (2012) 378–385

Contents lists available at SciVerse ScienceDirect

Meat Science

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

Identification of cattle, llama and horse meat by near infrared reflectance or transflectance spectroscopy

L.W. Mamani-Linares [a,b], C. Gallo [a], D. Alomar [c,*]

[a] Instituto de Ciencia Animal, Facultad Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile
[b] Programa de Doctorado en Ciencias Veterinarias, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Chile
[c] Instituto de Producción Animal, Facultad Ciencias Agrarias, Universidad Austral de Chile, Valdivia, Chile

ARTICLE INFO

Article history:
Received 27 April 2011
Received in revised form 5 August 2011
Accepted 5 August 2011

Keywords:
Meat or meat juice
Beef
Llama
Horses
NIRS

ABSTRACT

Visible and near infrared reflectance spectroscopy (VIS–NIRS) was used to discriminate meat and meat juices from three livestock species. In a first trial, samples of *Longissimus lumborum* muscle, corresponding to beef (31) llamas (21) and horses (27), were homogenised and their spectra collected in reflectance (NIRSystems 6500 scanning monochromator, in the range of 400–2500 nm). In the second trial, samples of meat juice (same muscle) from the same species (20 beef, 19 llama and 19 horse) were scanned in folded transmission (transflectance). Discriminating models (PLS regression) were developed against "dummy" variables, testing different mathematical treatments of the spectra. Best models indentified the species of almost all samples by their meat (reflectance) or meat juice (transflectance) spectra. A few (three of beef and one of llama, for meat samples; one of beef and one of horse, for juice samples) were classified as uncertain. It is concluded that NIRS is an effective tool to recognise meat and meat juice from beef, llama and horses.

1. Introduction

Meat and meat products are important components of the human food chain. Their quality and origin are important issues to consumers, government agencies and retailers. Consumer demands have changed in the last decades in terms of quality and safety traits (Andrée, Jira, Schwind, Wagner, & Schwägele, 2010) with several determinant factors involved. Lifestyle, religion, diet and health issues are some of the aspects that affect choice of some products over others (Ballin, 2010; Resurreccion, 2003). A matter of concern is that meat and meat products can be attractive targets for adulteration in many ways (Ballin & Lametsch, 2008) such as substitution of raw ingredients of high value by low cost species or materials, or by adding other proteins from several origins (Cozzolino & Murray, 2004). Minced meat production removes the morphological characteristics of muscle, making it difficult to identify one type of muscle from another. For this reason, meat substitution with other species of lower quality is one of the forms of economic adulteration in the minced meat industry, a fraud that could result in economic and health problems (Hargin, 1996; Meza-Márquez, Gallardo-Velázquez, & Osorio-Revilla, 2010). This is a concern for importation and the meat packers, but also at the restaurant and retail level, where the substitution is easier to conceal.

The identification of meat from different species has been addressed by a number of works involving different techniques, such as immunological (Patterson & Jones, 1990; Smith, 1991) enzymatic (Sharma, Srivatava, Gill, & Joshi, 1994), electrophoresis (Sieberte, Beneke, & Bentler, 1994) PCR and real-time PCR techniques (Fajardo et al., 2007; Fajardo et al., 2008; Kesmen, Gulluce, Sahin, & Yetim, 2009; Soares, Amaral, Mafra, Beatriz, & Oliveira, 2010). For a review on authentication methods for meat and meat products, the reader is referred to Ballin (2010). Most of these methods have the ability to detect low levels of adulteration and their reliability is high (Ahmed, 2002; Ballin, Vogensen, & Karlsson, 2009; Raamsdonk et al., 2007). However, most of these techniques are destructive, tedious and time-consuming, making them unsuitable for on-line applications (Ahmed, 2002; Liu, Lyon, Windham, Lyon, & Savage, 2004; Prieto, Andrés, Giráldez, Mantecón, & Lavín, 2008).

Rapid and reliable methods for detection of meat adulteration are crucial for implementation of food labelling regulations and product quality control. Methods for these purposes need to be specific, sensitive, rapid, economic and able to analyse cooked products as well as raw meats, and provide quantitative results (Cen & He, 2007; Meza-Márquez et al., 2010). Near infrared spectroscopy (NIRS) is an attractive technique for such applications, since it is fast, non-destructive, requires small samples, has a high-penetration radiation beam and no further preparation of the samples is needed (Alishahi, Farahmand, Prieto, & Cozzolino, 2010; Bosco, 2010; Cozzolino & Murray, 2004).

A typical NIR spectrum consists of several bands formed by absorption peaks, valleys and shoulders resulting from overlapping signals. Absorption bands are produced when NIR radiation of a specific frequency or wavelength vibrates at the same frequency as a

* Corresponding author. Tel.: +56 63 221653.
E-mail address: dalomar@uach.cl (D. Alomar).

0309-1740/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2011.08.002

specific molecular bond present in the sample in the form of X–H, where X is carbon, nitrogen or oxygen (Shenk, Workman, & Westerhaus, 2008).

In the meat sector, NIRS calibrations have been developed for the quantitative prediction of the chemical, physical and sensory quality of meat (Alomar, Gallo, Castañeda, & Fuchslocher, 2003; Prieto et al., 2009; Prieto, Roehe, Lavín, Batten, & Andrés, 2009; Ripoll, Albertí, Panea, Olleta, & Sañudo, 2008; Viljoen, Hoffman, & Brand, 2007).

NIRS can be also successfully employed in discriminant analysis to recognise a specimen without the need of any chemical analysis, e.g., to discriminate amongst different types of ground beef samples (Prieto et al., 2008) to differentiate breed in beef (Alomar et al., 2003), to identify suckling kid meat (Ripoll, Alcalde, Horcada, & Panea, 2011), to discriminate fresh and frozen-then-thawed beef and lamb (Downey & Beauchene, 1997; Thyoldt & Isaksson, 1997) and to discriminate between meat from different feeding systems in different species (Dian, Andueza, Jestin, Prado, & Prache, 2008; Osorio et al., 2007; Osorio et al., 2009; Pla, Hernández, Ariño, Ramírez, & Díaz, 2007; Tejerina, López-Parra, & García-Torres, 2009). The technique has been employed successfully to discriminate between beef and kangaroo meat (Ding & Xu, 1999), beef, pork, chicken and lamb meat (Arnalds, Fearn, & Downey, 2002; Cozzolino & Murray, 2004; Downey, 2000), lamb and veal meat (McElhinney & Downey, 1999), to detect and quantify adulterants in meat and minced beef (Meza-Márquez et al., 2010; Ortiz-Somovilla, España-España, De Pedro-Sanz, & Gaitán-Jurado, 2005).

Nevertheless, in spite of the broad body of literature available on applications of NIRS to meat composition and quality (Prieto et al., 2009; Weeranantanaphan, Downey, Allen, & Sun, 2011) no studies could be found concerning the identification and authentication of meats from less traditional and important sources of animal proteins in some regions of the world, such as llamas and horses, particularly when fresh meat or meat juices are employed. The use of meat juice appears as interesting, as some analysis could be performed on meat juice instead of muscle samples, providing a standardised extraction method and a suitable technique for spectra collection are employed. This could lead to a faster analysis, with easier homogenisation of a liquid sample and the requirement of a reduced sample size. Meat juice normally consists of a mixture of serum, lymph, and released intracellular liquid and its composition can be affected by different factors, including animal species (Piñeiro, Gymnich, Knura, Piñeiro, & Petersen, 2009).

The present study examined the accuracy of visible (VIS) and near-infrared (NIR) spectroscopy to indentify meat from beef, llamas and horses through the discriminant analysis of the spectra obtained from samples of meat and meat juices from these species.

2. Materials and methods

2.1. Samples

Meat samples (*Longissimus lumborum*) from beef, llamas and horses were purchased from different butcher shops and supermarkets in Chile. Beef and horse meat samples were purchased from the local markets in Valdivia and Temuco, llama meat samples were purchased from butcher shops in Arica and transported refrigerated to Valdivia via air-freight. To enhance diversity of samples, not more than one sample was purchased from one shop on the same week. Samples of beef and llama came from animals with 2 to 4 permanent teeth (1–2 and 2–3 years old, respectively), whereas age of horses could not be determined. All samples were identified, cut into 2.5 cm thick steaks and stored frozen in a commercial freezer at −20 °C until scanned. Sub-samples were set aside, cut into 1 cm^3 cubes and packaged in polyethylene bags, properly labelled, sealed and kept frozen for subsequently obtaining meat juice. All samples were frozen for approximately four weeks to standardise handling, since all meats were not purchased at the same time and the NIRS instrument was unavailable at the time of arrival of the first samples.

This study consisted of two trials. The first was a qualitative analysis of meat and the second of meat juices of the same species. In the first trial, 79 samples of *Longissimus lumborum*, 500 g each, were used: 31 from beef, 21 from llamas and 27 from horses. All samples were thawed at 4 °C for 24 h, then ground in a food processor (Moulinex®) and stored at 4 °C for 4 to 6 h before taking the spectra. For the second trial, some of the original samples were lost and only 58 samples of the same muscle (20 of beef, 19 from llama and 19 from horses) were used. To obtain the juice, each sample was thawed slowly at 4 °C for 24 h and the drained juice from each thawed sample was collected in 1.5 ml Falcon tubes and stored at 4 °C until spectra were collected.

2.2. Spectra collection and handling

Prior to spectroscopic analysis, meat was homogenised at room temperature (20 to 22 °C) and divided into three sub-samples. Spectra of meat samples were taken in reflectance, in ring cells (50 mm diameter, 10 mm depth) with quartz windows (part number NR-7072). Meat juice samples were divided in two sub-samples and agitated in a Vortex-Genie mixer, model k-550-GE (Scientific Industries, Bohemia, NY) immediately before each spectrum was taken. For the readings, 0.5 ml of each sub-sample was placed in a folded transmission (transflectance) cam-lock ring cell, 0.1 mm path length, with an aluminium plated reflector for liquid products (part number IH-0345-1). All readings were performed in a NIRSystems 6500® monochromator (NIRSystems, Silver Spring, MD, USA) with a spinning sample module. Every scan was composed of thirty-two readings per sample, plus a similar number of readings of a ceramic reference, taken through the visible (VIS) and near-infrared (NIR) range (from 400 to 2500 nm) every 2 nm. Spectral data collection and handling were performed using the software WinISI II version 1.02 (Infrasoft International, ISI, Port Matilda, PA, USA).The average spectra from the scanned sub-samples were stored in suitable files as [log (1/R)], where R is the reflectance energy recovered by two silicon (400–1100 nm) and four lead sulphide (1100–2500 nm) detectors positioned at 45° angle to the sample surface.

2.3. Spectral data analysis

In order to illustrate the effects of mathematical treatments on absorption signals, a second-order derivative transformation was applied to the spectral data to highlight useful information, such as to allow resolution of overlapping peaks (Shenk et al., 2008). A math treatment of 2–4–4 was applied to the spectra of meat and meat juice, that is, a second subtraction order across four data points (8 nm) with smoothing segments of four data points.

Principal components analysis of the spectra was performed to illustrate the distribution and potential clustering of samples according to the spectral features of each species. This method allows condensing of the information present in each full spectrum in a few synthetic independent variables (principal components) and to examine natural grouping and spectral patterns of samples according to the first PC's (Cozzolino & Murray, 2004; Robert, Devaux, & Bertrand, 1996).

Separate discriminant models were developed with the software WinISI II for meat and meat juice spectra by multivariate analysis regression technique (partial least squares, PLS). Three spectra files, one for each species, were entered to be discriminated within each sample type (meat or juice). In this procedure, a calibration matrix is built with all the samples through the creation of "dummy" variables with arbitrary values. In this way, a value of one is assigned if the spectrum belongs to the correct file, or zero if it does not belong to this group. The overall concept in this model is that a perfect match (a