



Analytical Methods

Prediction of lamb meat fatty acid composition using near-infrared reflectance spectroscopy (NIRS)

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ABSTRACT

The aim of this study was to assess the feasibility of near-infrared reflectance spectroscopy (NIRS) for predicting lamb meat fatty acid composition. We compared ground vs. intact non-ground meat samples to determine whether grinding and homogenisation of meat samples improved the performance of the predictions. We used 76 male lambs, of which 32 were pasture-fed and 44 stall-fed with concentrate and hay. The reflectance spectrum of *Longissimus lumborum* muscle was measured at wavelengths between 400 and 2500 nm. Predictions were better with ground than with intact muscle samples. NIRS accurately predicts several individual fatty acids (FA) (16:0, 18:0, 16:1 $\Delta 9$ cis, 17:1 $\Delta 9$ cis, 18:1 $\Delta 9$ cis, 18:1 $\Delta 11$ cis and 16:1 $\Delta 9$ trans) and several FA groups (total linear saturated FA, total branched saturated FA, total saturated FA, total cis monounsaturated FA (MUFA), total trans MUFA, total MUFA and total polyunsaturated PUFA). These results show the potential of NIRS as a rapid, and convenient tool to predict the major FA in lamb meat.

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1. Introduction

Meat is an important source of fatty acids (FA) in the human diet, especially of saturated fatty acids (SFA). However, when consumed in excess, SFA are linked to certain health disorders associated with modern living, especially in developed countries. Some SFA (12:0, 14:0, 16:0) have been shown to be associated with a higher risk of cardiovascular accidents, although 18:0 has no adverse effect (Mensink, Zock, Kester, & Katan, 2003). Other work shows that SFA in excess of 14% of total energy supply increases insulin resistance, but has no detrimental effect below 10% (Vessby et al., 2001). The French National Nutrition Health Program 2006–2010 aims to reduce human consumption of SFA by 25%. Another feature of ruminant products is the presence of trans monounsaturated fatty acids (trans MUFA). The trans MUFA, abundant in human foods, are known to be generally more atherogenic than SFA (Sacks & Katan, 2002). However, the risk to human health varies widely according to the origin of trans FA. The “industrial” trans FA, rich in 18:1 $\Delta 9$ trans and $\Delta 10$ trans isomers, apparently increase cardiovascular accident risk, whereas the “natural” trans FA in ruminant products, rich in 18:1 $\Delta 11$ trans, do not seem to do so in humans (see review of Lock, Parodi, & Baumann, 2005). However, the amount and composition of “natural” trans FA in ruminant products are strongly modulated by animal feeding con-

ditions, such as composition of basal diet or lipid supplements (Bispo-Villar et al., 2009). By contrast, *n*-3 polyunsaturated fatty acids (*n*-3 PUFA), which are abundant in meat products, appear to be beneficial to consumer health, decreasing blood pressure, triglyceridemia, morbidity and mortality following cardio-vascular accidents and tumour growth (Bougnoux, 1999). The AFSSA (www.afssa.fr) (Agence Française de Sécurité Sanitaire des Aliments) report on *n*-3 PUFA recommends a ratio of *n*-6 PUFA to *n*-3 PUFA of about 5 for a balanced diet. Given current human diets in developed countries, this means reducing *n*-6 PUFA while sustaining or increasing *n*-3 PUFA. Nutritionally, another FA family is of interest; the conjugated linoleic acids (CLA). Some of these FA reduce fat deposition in rodents and pigs (Pariza, 1999) and inhibit degenerative cellular proliferation (Khanal & Olson, 2004).

There has accordingly been a growing interest in the FA profile of meat in recent years and in ways to control it by acting on various factors, especially diet composition (Berthelot, Bas, & Schmidely, 2010; Scollan et al., 2005). Concurrently, there has been an increasing demand from human nutritionists and dieticians, and also from consumers, for more accurate and systematic information on the nutritional quality of foods.

In addition to its utility for diet authentication purposes (Dian, Andueza, Jestin, Prado, & Prache, 2008), NIRS has potential for rapid routine measurements of meat FA composition. NIRS has been used to determine the total fat and FA composition of rabbit meat (Pla, Hernández, Ariño, Ramírez, & Díaz, 2007), chicken (Berzaghi, Della Zotte, Jansson, & Andrighetto, 2005) and pork (Pérez-Marín, De Pedro, Guerrero-Ginel, & Garrido-Varo, 2009) or pork products

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(Fernandez-Cabanas, Polvillo, Rodriguez-Acuña, Botella, & Horcada, 2011). Information on the FA analysis of ruminant products by NIRS is relatively scant and mainly concerns FA prediction for milk (Coppa et al., 2010) and cheese (Lucas, Andueza, Ferlay, & Martin, 2008) and for bovine meat (Realini, Duckett, & Windham, 2004; Sierra et al., 2008; Windham & Morrison, 1998).

The current reference method for analysis of meat FA composition by gas-liquid chromatography (GLC) requires work-up, such as solvent extraction and derivatization. The method performs well, but is polluting, costly and time-consuming, whereas NIRS analysis is simple, fast and chemical-free.

The aim of the present study was to determine whether NIRS was feasible for accurate predictions of lamb meat FA. We compared two preparations of meat for NIRS analysis (ground vs. intact, non-ground meat samples).

2. Materials and methods

The lambs were raised at the experimental farm of the Monts-d'Auvergne Experimental Unit (UE1296), Site d'Orcival, of the INRA Centre of Clermont-Ferrand Theix, France. The animals were handled by specialised staff to ensure their welfare in accordance with European Union Directive No. 609/1986.

2.1. Animals, diets and slaughter procedures

A total of 76 Limousine male lambs were used, 32 pasture-fed (Group P) and 44 stall-fed (Group S); 28 were slaughtered in 2005 (8 P and 20 S) and 48 in 2006 (24 P and 24 S).

We used both pasture-fed and stall-fed lambs to ensure a wide variability of meat FA composition. It is now well known that the intramuscular lipids from pasture-fed lambs contain more *n*-3 PUFA and CLA and less *n*-6 PUFA and palmitic acid than the intramuscular lipids from stall-fed lambs (Aurousseau, Bauchart, Calichon, Micol, & Priolo, 2004).

The experiment lasted from April to October in both years. The S lambs were fed *ad libitum* with a commercial concentrate and hay. The P lambs were offered permanent pasture *ad libitum* and they received no supplementation at pasture. The same pasture was used in both years and it was maintained in a leafy, green, vegetative state. Animals were weaned at 75 and 90 d on average for S and P lambs, respectively.

Lambs were slaughtered when they had reached a target fat class of 2–3 (on a scale of 1–5), manually assessed by skilled technicians. The animals were transported by truck to the INRA slaughterhouse located within 25 km of the pasture and stalls. Immediately after their arrival the animals were electrically stunned and slaughtered by a throat cut. Carcasses were placed in a chiller at 4 °C until 24 h *post mortem*.

2.2. Measurements

2.2.1. Carcass measurements and sampling

Lambs were weighed just before slaughter. Carcass weight was measured 24 h *post mortem*. The perirenal fat and the kidneys were then removed from the carcass. The fat was separated from the kidneys using a knife and then weighed. Two samples of *Longissimus lumborum* were taken from each lamb at 24 h *post mortem*. The first one (10–20 g) was packed in aluminum foil, placed in a plastic bag under vacuum, and stored in a conventional freezer at –20 °C before NIRS scanning as an intact muscle sample. The second one (60–80 g) was cut into small pieces and frozen in liquid nitrogen. Muscle tissue samples were ground in liquid nitrogen in a mill (model M20, IKA-Werke, Staufen, Germany) to produce a fine homogeneous powder. Ground muscle was divided into

two parts and stored at –80 °C for lipid and fatty acid analysis and NIRS scanning respectively.

2.2.2. Fatty acid analysis using gas-liquid chromatography (GLC): the reference method

Lamb *L. lumborum* muscle dry matter was assayed gravimetrically after drying at 80 °C for 48 h. Total lipids of *L. lumborum* muscle were extracted by mixing 6 g of muscle powder with chloroform-methanol according to the method of Folch, Lees, and Stanley (1957) and assayed gravimetrically. Total FA were extracted and transmethylated from total lipids at 20 °C, first with Na methanolate for 20 min, and second with BF₃-methanol 14% for an additional 20 min (Glass, 1971). Concentration and detailed FA composition of *L. lumborum* muscle samples were determined by gas-liquid chromatography (GLC) using the Peri 2100 chromatograph model (Perichrom, Saulz-les-Chartreux, France) equipped with a CP-Sil 88 glass capillary column (Varian, USA) (length 100 m, ID 0.25 mm, H₂ as carrier gas). Temperatures of the split/splitless injector and of the flame ionisation detector were 200 and 230 °C respectively, and the oven temperature was programmed as follows: 70 °C for 4 min, 70–175 °C at 20 °C/min, 175 °C for 25 min, 175–215 °C at 10 °C/min and a final plateau at 215 °C for 42 min. FA were quantified using 19:0 as the internal standard. Identification of muscle FA was based on comparison of the retention time of individual FA with commercial standard of individual FA or FA mixtures (FAME Mix C4–C24) (Supelco, USA). The response coefficient of each individual FA was calculated using the quantitative mix C4–C24 FAME (Supelco, USA).

Standard error of the reference method (SERM) was assessed by analysing, in triplicate, FA from total lipids of three lamb *L. lumborum* muscles in the same single day. *L. lumborum* muscles were here collected from three lambs, one of them stall-fed indoors with concentrate and hay and the other two pasture-fed.

2.2.3. Reflectance spectrum of *L. lumborum* muscle in the visible and near infrared range (400–2500 nm)

The reflectance spectrum of *L. lumborum* muscle in the visible and near infrared range was measured on ground samples and on intact non-ground samples. The non-ground muscle sample was a slice of intact muscle tissue. Ground and non-ground tissue samples were thawed at room temperature for approximately 2 h. Portions weighing about 3 g were then scanned in the reflectance mode (400–2500 nm) in a NIRS 6500 scanning monochromator (NIRSystems, Silver Spring, MD, USA) using ISI software, version 3.01, from Infracsoft International (Infracsoft International, South Atherton St. State College, PA 16801, USA) equipped with a spinning module. Muscle samples were scanned in a circular cup (diameter 50 mm, depth 10 mm) (Part number IH – 0307, NIRSystems, Infracsoft International, South Atherton St. State College, PA 16801, USA), compressed and sealed with a disposable paper-backed wrap. Reflectance data were recorded at 2 nm intervals and stored as log (1/reflectance).

2.3. Calibrations and statistics

Calibrations were performed using WinISI II version 1.60 (Infracsoft International, South Atherton St. State College, PA 16801, USA). NIR calibration equations were obtained by modified partial least squares (MPLS) regression using the range 400–2500 nm or 700–2500 nm. To optimise the accuracy of the calibration, the data underwent (i) different combinations of scattering corrections (no correction, detrending, standard normal variate, standard normal variate + detrending, multiplicative scatter correction) and (ii) mathematical pre-treatments (no derivative, first and second derivatives applied on different gaps; 0,0,1,1; 1,4,4,1; 1,8,8,1; 2,5,5,1 and 2,10,10,1; where the first digit is the number of the

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