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Identification of animal meat muscles by visible and near infrared reflectance spectroscopy

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Abstract

Visible (VIS) and near infrared reflectance spectroscopy (NIRS) was used to identify and authenticate different meat muscle species. Samples from beef (*n*: 100), lamb (*n*: 140), pork (*n*: 44) and chicken (*n*: 48) muscles were homogenised and scanned in the visible (VIS) and near infrared (NIR) region (400–2500 nm) in a monochromator instrument in reflectance. Both Principal Component Analysis (PCA) and dummy partial least-squares regression (PLS) models were developed to identify different meat species. The models correctly classified more than 80% of the meat sample muscles according with the muscle specie. The results showed the potential of VIS and NIR spectra as an objective and rapid method for authentication and identification of meat muscle species.

Keywords: Visible; Near infrared spectroscopy; Meat; Identification; Speciation; Principal component analysis; Partial least squares

1. Introduction

The determination of food authenticity and the detection of adulteration are major issues in the food industry, and are attracting and increasing amount of attention (Monin, 1998; Cordella, Moussa, Martel, Sbirrazzuoli, & Lizzani-Cuvelier, 2002). With meat and meat products major authenticity issues concern the substitution of high value raw materials with cheaper materials such as less costly cuts, mechanically recovered meat, offal, blood, water, eggs, gluten or other proteins of animal or vegetable origin (Hargin, 1996; Al-Jowder, Kemsley, & Wilson, 1997, 2002; Al-Jowder, Defernez, Kemsley, & Wilson, 1999; Cordella et al., 2002). In some countries the consumption of certain meats (e.g. pork) is proscribed for religious reasons. The intensification of agriculture and urbanisation over the last decades has created a major concern of many consumers about the authenticity and the safe of the meat (Al-Jowder et al., 1997, 1999, 2002; Murray, Aucott, & Pike, 2001). Therefore, analytical methods have focused on the identification of meat species in raw, cooked and processed products. Meat specication has been addressed by inmunological (Patterson & Jones, 1990; Smith, 1991) and enzymatic procedures (Sharma, Srivatava, Gill, & Joshi, 1994). These methods along with electrophoretic techniques have also been used to differentiate fresh from frozen meat (Sieberte, Beneke, & Bentler, 1994). These methods are cheap and have the ability to detect wide range and low levels of adulteration. However, spectroscopic methods are attractive options due to the speed of analysis and minimal sample preparation. Near Infrared Reflectance Spectroscopy (NIRS) was originally developed to provide a rapid measurement of the composition of grains and oilseeds (Deaville & Flinn, 2000). NIRS has emerged in the last 30 years as a rapid method for testing the quality of intact samples from the light they reflect (Osborne, Fearn, & Hindle, 1993; Deaville & Flinn, 2000) and it is likely to be the best means of achieving this quality control effectively and conveniently. Most of the established methods have involved the development of NIRS calibrations for the quantitative prediction of composition in meat (Ben-Gera & Norris, 1968; Lanza, 1983; Clark & Short, 1994). This

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was a rational strategy to pursue during the initial stages of its application, given the type of equipment available, the state of development of the emerging discipline of chemometrics and the overwhelming commercial interest in solving such problems (Downey, 1994, 1996, 1998). One of the advantages of NIRS technology is not only to assess chemical structures through the analysis of the molecular bonds in the near infrared spectrum, but also to build a characteristic spectrum that represents the "finger print" of the sample. This opens the possibility of using spectra to determine complex attributes of organic structures, which are related to molecular chromophores, organoleptic scores and sensory characteristics (Hildrum, Nilsen, Mielnik, & Naes, 1994; Hildrum et al., 1995; Park, Chen, Hruschka, Shackelford, & Koohmaraie, 1998). In addition, the application of statistical packages such as principal component or discriminant analysis provides the possibility to understand the optical properties of the sample and classify them without the need for chemical information. NIRS has been examined to assess its suitability for this application by different authors in different types of products such as fat substitutes in sausages (Ellekjaer, Naes, Isaksson, & Solheim, 1992), fat in milk (Sato, Kawano, & Iwamoto, 1990), several foods (Downey, 1996), fresh and frozen beef (Downey & Beauchene, 1997; Thyoldt & Isaksson, 1997). Routine assessment of meat composition using optical devices is now widely in use in the oilseed, milk and cereal industry for quality purposes (Osborne et al., 1993). At present there is little information on how NIRS can determine the quality of the muscle "as meat" and few reports were found in the literature in relation to the use of NIRS for species identification in meat. These reports account in the use of NIRS to discriminate between kangaroo and beef meat (Ding & Xu, 1999), chicken meat cuts (Ding, Xu, & Chan, 1999; Fumiere, Sinnaeve, & Dardenne, 2000), lamb and beef mixtures (McElhinney, Downey, & O'Donnel, 1999) and between beef, pork, chicken and pork (Downey, 2000; Arnalds, Fearn, & Downey, 2002). The aim of this work was to study the reliability and accuracy of the visible and NIRS for identification and authentication of raw meat species such as pork, chicken, lamb and beef without depend on chemical information.

2. Materials and methods

2.1. Samples and sample preparation

One hundred (*n*: 100) beef muscle samples (*longissimus dorsi*); one hundred and forty (*n*: 140) lamb muscle samples (*longissimus dorsi*, *infraspinatus*, *supraspinatus*, *rectus femoris*, *semitendinosus* and *semimembranosus*), forty-eight (*n*: 48) chicken muscle samples (breast and thigh) and forty-four pork samples (*n*: 44) (*longissimus dorsi*) were analysed. Samples were kept frozen in a commercial freezer (−4°C) until NIRS analysis were done. About 100–200 g of muscle was thawed at room temperature (20–22°C) and homogenised for one to two minutes with a Philips multiprocessor blender (RI-3142, Brazil). The blender cup was washed first with hot water, followed by cold water and towel dried between samples. Minced thawed samples were flattened, then sub-samples were taken randomly for further chemical analysis. Intact samples were prepared by cutting slices parallel to the longitudinal orientation of the muscle fibres (60–100 mm × 20–50 mm × approximately 20 mm thick) from the thawed muscle sample. Information about sample characteristics, process, slaughter conditions are detailed in previous reports (Cozzolino, Murray, Paterson, & Scaife, 1996; Cozzolino, Murray, Scaife, & Paterson, 2000; Cozzolino, De Mattos, & Vaz Martins, 2002).

2.2. Near infrared analysis

Samples were scanned minced in reflectance mode (400–2500 nm) in a scanning monochromator NIRS 6500 (NIRSystems, Silver Spring, MD, USA). Samples were scanned in a circular cup (50 mm diameter, 10 mm depth) (Part number IH-0307, NIRSystems, USA) sealed with disposal paper back. Samples were not rotated when spectra collection was made. Spectral data collected were recorded in the form of the logarithm of reciprocal of reflectance [$\log(1/R)$] with 2 nm interval (1050 data points). Two pairs of lead sulphide detectors collected the reflectance spectra and the readings were referenced using a ceramic disk. The spectrum of each muscle sample was the average of 32 successive scans (16-32-16 sequence). Spectral data collection and manipulation were performed using NIRS 2 (1995) software, version 3.01, from Infrasoft International (ISI, Port Matilda, PA, USA).

2.3. Chemometric analysis

Spectra were exported from the NIRS 2 software as NSAS file for chemometric analysis. Both Principal Component Analysis (PCA) and dummy partial least-squares regression (PLS) analysis were performed using The Unscrambler version 7.8 (CAMO ASA, Oslo, Norway). PCA, is a mathematical procedure for resolving sets of data into orthogonal components whose linear combinations approximate the original data to any desired degree of accuracy (Martens & Naes, 1996; Martens & Martens, 2000). PCA was used to derive the first 20 principal components from the condensed spectral data. These were used in further analysis to examine the natural groupings of the samples. In order to visualise the relative position of

the different muscle species samples were graphically displayed by means of the first three PCs. Discriminant analysis was performed using the dummy regression technique as described elsewhere by other authors (Osborne et al., 1993; Ding et al., 1999). Briefly, 50 beef samples, 24 chicken samples, 22 pork samples and 70 lamb samples were randomly selected to form the calibration set while the remaining samples were used as validation set ($n = 166$). Samples of beef muscle were assigned a dummy value 1, pork muscle the value 2, chicken muscle the value 3 and lamb muscle the value 4. Both principal component regression (PCR) and partial least-squares regression (PLS) were used to develop calibration equations to predict dummy values of samples in the validation set from their spectral data. Three wavelength regions: visible 400–700 nm, near infrared 1100–2500 nm and visible and near infrared region 400–2500 nm were used. Neither scatter correction nor mathematical treatments were used to perform the calibration models. A sample was classified as muscle belongs to a specific category (species) if the predicted value was equal or ± 0.5 the dummy values. Cross validation was used in all cases as internal validation. Cross validation estimates the prediction error by splitting the calibration samples into groups (four in this study). One group was reserved for validation and the remaining groups were used for calibration. The process was repeated until all groups have been used for validation once (Martens & Naes, 1996; Martens & Martens, 2000).

3. Results and discussion

3.1. Spectra characterisation

The typical spectra of beef, sheep, pork and chicken muscle samples in the VIS and NIR regions are shown in Fig. 1. Visual differences were observed between sample species in the visible region (respiratory pigments) and in the near infrared region (fat content). Absorption band at 430 nm is related with Soret absorption bond (Stryer, 1995), attributed to traces of erythrocytes of haemoglobin and absorption band at 574 nm related with oxyhaemoglobin absorption (Mitsumoto, Maeda, Mitsuhashi, & Ozawa, 1991; Swatland, 1995; Cozzolino et al., 2000; Cozzolino & Murray, 2002). In the near infrared region absorption bands were observed at 980, at 1460 and at 1960 nm related with OH third, second and first OH stretch overtones, mainly related with water content of the samples. Water is the main component of fresh samples ranging for 70–85%. Around 1200 nm, absorption bands are related with CH second overtone. At 1738 nm with CH_2 stretch first overtone related with both fat and fatty acids and at 2310 nm with CH combinations associated with fat

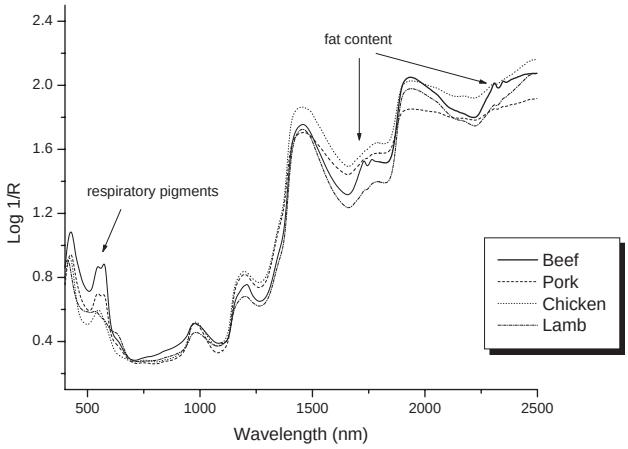

Fig. 1. Visible and near infrared mean spectrum of pork, beef, lamb and chicken samples.

content and with saturated and unsaturated fatty acids, respectively (Murray, 1986). Lamb muscles had absorption bands at 424 and at 550 nm in the visible region related to the Soret band and oxymyoglobin absorption band, respectively (Swatland, 1995; Cozzolino et al., 2000). Both absorption bands at 540 and at 580 nm are related with both myoglobin and oxymyoglobin (Lawrie, 1985; Mitsumoto et al., 1991; Swatland, 1995) while myoglobin has an absorption band at 555 nm (Lawrie, 1985; Mitsumoto et al., 1991; Swatland, 1995; Delpy & Cope, 1997). In the near infrared region the mean spectrum shows absorption bands at 762 nm related to the OH third overtone (Murray, 1986) or an absorption band produced by the oxidation of the myoglobin (deoxymyoglobin) (Swatland, 1995; Delpy & Cope, 1997; Cozzolino & Murray, 2002). Most of the spectral information used for the discrimination analysis is contained in the VIS region (400–700 nm) and in the NIR region around (1400–2300 nm) due to both different pigments present in each specie and to matrix characteristics (intra-muscular fat, fatty acids, moisture) (Fig. 1). These results are consistent with those reported by other authors (Downey, McElhinney, & Fearn, 2000; Alomar, Gallo, Castaneda, & Fuchslocher, 2003).

3.2. Principal component and discriminant analysis

Fig. 2 showed the discriminant plots for the raw meat species analysed using the first three PC's. The score-plots showed clusters of samples related with the different meat species. This confirms the assumption that different spectral attributes between samples were associated with characteristics of the muscles depending on the species. Moisture, pigments and fat could explain the discrimination between the different muscle species. The PCs loading showed a shape similar to the mean spectrum in PC1 (Fig. 3). PC1 explains 68% of the total

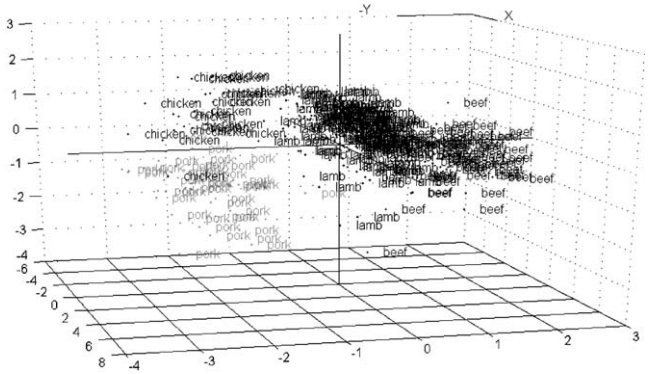

Fig. 2. PCA score for the three PCs used for discrimination between animal species.

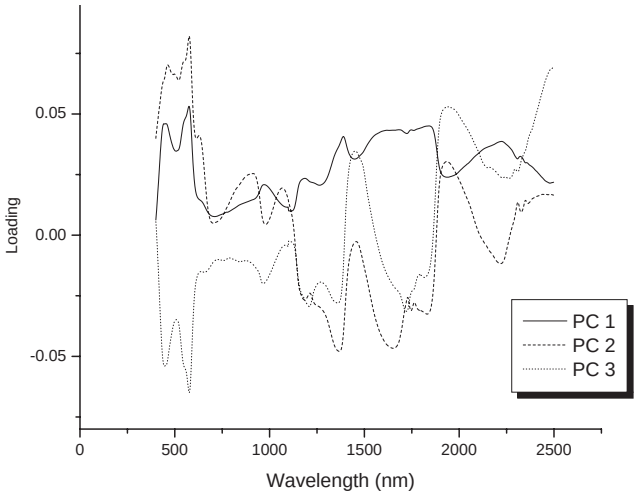

Fig. 3. PC loadings used for the meat sample discrimination.

variance in the samples. The highest loading on PC1 were found around at 410 and at 598 nm related with meat pigments (Lawrie, 1985), at 980 and 1980 nm related with water content. Around 1500–1800, and at 2304 nm with fat (intra-muscular fat and fatty acids). PC2 explains 19% while PC3 explains 8% of the total variance, respectively. The highest loading on PC2 were found around 510–630 nm (meat pigments), 1496 nm (water), 1742 and 1766 nm (fatty acids and fat), 1930 nm (water) and around 2300 nm (saturated and unsaturated fatty acids). Some authors reported that absorbance shoulders between 450 and 510 nm correspond to light absorption by muscle pigments. Absorption bands between 1750 and 1760 nm were related to the type of fat present in the sample (Murray, 1986; Osborne et al., 1993). Spectral bands between 2200 to 2300 nm were related to unsaturated =C–H and C=C groups which suggests that differences in polyunsaturated fatty acids may also contribute for further muscle species classification (Murray, 1986). The inclusion of information originating in the visible region was essential for the development of the most accurate discriminant model. These results agreed with those reported elsewhere by others (Ding & Xu, 1999; Downey et al., 2000). The loading of the PC3 shows predominantly absorption bands related with water.

3.3. Classification

Table 1 showed the calibration statistics for the dummy classification models using both PCR and PLS regression methods. All the models developed had a coefficient of determination in calibration (R^2_{cal}) higher than 0.90, determining that more than 90% of the variation is explained by these regression models. The prediction statistics and the performances of the classification models are summarised in Tables 2 and 3, respectively. Most of the samples are classify correctly by the dummy models (>90%). The PLS models gave the best classification results in the three wavelength segments used. The best classification using both PCR and PLS methods were achieved using either the NIR or the VIS+NIR region. The visible region alone did not gave good classification results. Similar discrimination results using PLS and the raw spectra were reported previously by other authors (McElhinney & Downey, 1999). Spectroscopic techniques as generally applied to authenticity issues are nonselective. Rather spectra contain information about the complete composition and physical state of the material under analysis, yield structural information that constitutes the fingerprint of a sample (Downey, 1998; Cordella et al., 2002). In general, supervised classification (discriminant analysis) is used to test similar known authentic samples. Central to the philosophy of discriminant techniques is the belief that the spectra of samples of a given material are similar and different from spectra of other materials when the raw spectra is compared (Downey, 1998). The ability of a NIRS model to discriminate or identify species is based on the vibrational responses of chemical bonds to NIR radiation and it is probable that the

Table 1
Visible and near infrared calibration statistics using PLS and PCR for meat speciation

	SEC	R^2_{CAL}	SECV
PCR (nm)			
400–700	0.35	0.95	0.40
1100–2500	0.31	0.93	0.33
400–2500	0.30	0.94	0.31
PLS (nm)			
400–700	0.30	0.94	0.35
1100–2500	0.33	0.94	0.35
400–2500	0.28	0.94	0.31

PCR: principal component regression; PLS; partial least square regression; SEC: standard error in calibration; SECV: standard error in cross validation.

Table 2
Visible and near infrared prediction statistics using PLS and PCR for meat speciation

	r	SEP	Slope	Bias
PCR (nm)				
400–700	0.94	0.39	0.89	0.04
1100–2500	0.96	0.34	0.93	0.01
400–2500	0.97	0.38	0.94	0.04
PLS (nm)				
400–700	0.95	0.42	0.90	0.01
1100–2500	0.96	0.32	0.93	0.002
400–2500	0.97	0.28	0.94	0.02

PCR: principal component regression; PLS; partial least square regression; SEP: standard error of prediction, *r*: coefficient of correlation.

Table 3
Classification for meat species using PLS and PCR regression models

Spectra segment (nm)	Classification model of dummy regression			
	PCR		PLS	
	CC	IC	CC	IC
400–750	90 (81%)	21 (19%)	94 (85%)	17 (15%)
1100–2500	104 (94%)	7 (6%)	104 (94%)	7 (6%)
400–2500	101 (91%)	10 (9%)	107 (96%)	4 (3%)

CC: correct classify; IC: nonclassify.

higher the variability in these chemical entities, which respond to this range of electromagnetic spectrum, the better the accuracy of the model can be (Alomar et al., 2003). This indicated that the not only the pigments but also the composition of the muscle gave information to be used for identification purposes. Intra-muscular fat, fatty acids and other characteristics (e.g. muscle structure, type of muscle fibres) could add information to the model and allowed the discrimination between them.

4. Conclusions

Optical properties of the samples gave an excellent differentiation of muscle specie. The results reported here lead to conclude that NIRS can be used as a helpful tool for identifying muscle species on an objective basis. The visible and NIRS models classify correctly more than 85% of samples from different muscle species. Multivariate classification methods like discriminant analysis or dummy regression using PLS can be used to classify different muscle species. Two new experiments should be carried out in order to develop models capable to discriminate meat species on a large data set and to validate the pre-existing models.

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