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Differentiation of perirenal and omental fat quality of suckling lambs according to the rearing system from Fourier transforms mid-infrared spectra using partial least squares and artificial neural networks analysis

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ABSTRACT

Fourier transform mid-infrared (FT-IR) spectroscopy was evaluated as a tool to discriminate between carcasses of suckling lambs according to the rearing system. Fat samples (39 perirenal and 67 omental) were collected from carcasses of lambs from up to three sheep dairy farms, reared on either ewes milk (EM) or milk replacer (MR). Fatty acid composition of the samples from each fat deposit was first analyzed and, when discriminant-partial least squares regression (PLS) was applied, a perfect discrimination between rearing systems could be established. Additionally, FT-IR spectra of fat samples were obtained and discriminant-PLS and artificial neural network (ANN) based analysis were applied to data sets, the latter using principal component analysis (PCA) or support vector machines (SVM) as processing procedure. Perirenal fat samples were perfectly discriminated from their FT-IR spectra. However, analysis of omental fat showed misclassification rates of 9–13%, with the ANN approach showing a higher discrimination power.

1. Introduction

The production of suckling lambs to be slaughtered at about one month old, rendering carcasses of less than 7-kg-weight, is common in dairy sheep farms in Mediterranean countries because the meat is considered a valuable product due to its good eating quality (Vergara, Molina, & Gallego, 1999). In such farms, two different suckling lamb rearing systems can be followed. One consists of lambs being fed a milk replacer (MR) from the third day of life, while the other allows lambs to remain suckling with their dams (EM).

The effect of milk source on some suckling lamb meat quality traits has been investigated (Bas & Morand-Fehr, 2000; De la Fuente, Tejón, Rey, Thos, & López-Bote, 1998; Lanza et al., 2006; Napolitano, Cifuni, Pacelli, Riviezzi, & Girolami, 2002; Napolitano et al., 2006; Osorio, Zumalacárregui, Bermejo et al., 2007; Osorio, Zumalacárregui, Cabeza, Figueira, & Mateo, 2008; Osorio, Zumalacárregui, Figueira, & Mateo, 2007a; Osorio, Zumalacárregui, Figueira, & Mateo, 2007b; Santos-Silva, Bessa, & Santos-Silva, 2002; Vergara & Gallego, 1999; Vicenti, Colonna, Ragni, & Toteda, 2004). Apparently, milk source exerts a significant effect on fatty acid (FA) profile, volatile compounds, and colour and lipid oxidation stability. These studies agreed that meat from lambs fed with EM had higher saturated FA (SFA), lower monounsaturated FA (MUFA) contents and higher $n-3/n-6$ ratios than fat from lambs fed with MR. In addition, according to Osorio et al. (2008), meat from MR-fed suckling lambs, which had higher levels of vitamin E, was more stable to lipid oxidation compared to meat from EM-fed suckling lambs. Moreover, the type of rearing system had a significant effect on volatile compound profiles of the meat, leading to the suggestion that MR-meat might result in a different flavour from that expected from more traditional EM-fed suckling lamb meat. However, Napolitano et al. (2002) and Osorio et al. (2008) performed triangle tests and contradictory results were found. While in the former study the panel was able to distinguish between EM and MR meat samples, in the latter samples the rearing systems could not be discriminated.

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There seems to be a need to develop control methods for classifying suckling lamb carcasses according to the rearing system, since the regulation attached to several suckling lamb meat quality labels, such as "Lechazo de Castilla y León" Protected Geographical Indication (PGI) (Council Regulation 2081/92/EC), indicates that lambs must be reared exclusively on EM. Authentication of the type of feeding using analytical techniques will be a key issue in the certification of suckling lamb carcasses, with the rearing system being responsible for differences in price and quality.

Osorio et al. (2007a, 2007b) found that it is possible to classify suckling lamb carcasses according to the type of rearing system by means of FA analysis of different fat deposits. Furthermore, as a simpler, easier and more rapid alternative, infrared reflectance spectroscopy (NIRS) applied to perirenal fat samples has successfully been used for the discrimination of carcasses from suckling lambs reared with either EM or MR (Osorio, Zumalacárregui, Prieto et al., 2007).

FT-IR spectroscopy is a rapid and information rich technique for investigating the structure and composition of food components, allowing, in combination with chemometrics, the classification of foods without any chemical determination (Dupuy, Duponchel, Huvenne, Sombret, & Legrand, 1995). The introduction of the Attenuated Total Reflectance (ATR) technology extended the use of FT-IR in the food industry (Pedersen, Morel, Andersen, & Engelsen, 2003) where solid and semi-solid samples can be measured with high precision and reproducibility by use of the diamond ATR. The application of FT-IR to discriminate edible oils and fats has been proven to be successful in rapidly classifying these products (Dupuy et al., 1995; Vlachos et al., 2006; Yang, Irudayaraj, & Paradkar, 2005). According to Yang et al. (2005), FT-IR has more application for qualitative analysis than NIRS, because the 'fingerprints' of functional groups can be measured more narrowly and intensely in the MIR region (4000–400 cm^{-1}).

The aim of the present study was to investigate the potential of FT-IR spectroscopy for the discrimination of fat samples from carcasses of EM or MR reared suckling lambs. In addition, PLS multivariate regression and ANN models were applied to the spectral data and compared according to the rearing system.

2. Materials and methods

2.1. Animals and sampling

Lambs originated from the flocks of three farms (A, B and C) affiliated to the 'Asociación Nacional de Criadores de Ganado Ovino de Raza Churra', which is a Churra breeders association from the region of 'Castilla-León' (Spain). Lambs were reared either exclusively on EM or MR (from up to three days after birth to slaughter). Both EM and MR lambs were from all three farms. Four different commercial MR were used, two in farm A (MR_{A1} and MR_{A2}) and one in each of the remaining farms (MR_B and MR_C). The four MR were those most frequently used by regional breeders. According to the MR labels, the proximate composition of the MR was: moisture, 4–5%, crude protein, 23–24%, crude fat, 23–25%, ash, 6.6–8.6%, starch, 0–3%, crude fiber, 0–0.5%; and their ingredients: powdered milk and milk solids, vegetable fats and oils, products and byproducts from cereals, mineral supplements, i.e. iron and copper and Vitamins, i.e. E and A.

Two different samplings were carried. The first lasted two months (April and May, 2006) during which, among all the 30-to-35-days-old and 11-to-14-kg-live-weight suckling lambs reared in farms A and B on EM or MR (MR_{A1} for farm A and MR_B for farm B), thirty nine were randomly selected and slaughtered in an industrial slaughterhouse. The second sampling was carried out in the following month and 28 suckling lambs of the same characteristics described above, reared on EM or MR but in farms A (using MR_{A2} as the MR), B (using MR_B) and C (using MR_C) were selected and slaughtered. Approximately four hours after slaughter a perirenal (approximately 20 g) and an omental fat sample (approximately 20 g) were obtained from each of the first thirty nine carcasses. For the remaining 28 carcasses, only the omental fat sample was obtained. Table 1 shows the distribution of samples according to sampling, fat deposit, type of rearing, farm, and milk replacer.

In addition, one sample of each of the MR used in the farms and one of the bulk EM tanks of those farms were collected. Fat, EM and MR samples were packed individually in Ziploc freezing plastic bags (SC Johnson, Racine, WI, USA) and frozen and stored at −40 °C for up to three months prior to analysis.

2.2. Fatty acid composition

For the analysis of the FA composition of the fat deposits, lyophilized EM and MR samples (0.15 g each) were analyzed by gas chromatography as described by Osorio et al. (2007a).

2.3. Spectroscopic analysis, Fourier transform mid-infrared (FT-IR) spectroscopy

After thawing (overnight at 4 °C), fat samples were homogenized using an IKALabortechnik A10 blender (IKA, Staufen, Germany) and analyzed using FT-IR. The MB100 FT-IR spectrometer (Arid-Zone™, Quebec, Canada) was used to record the IR spectra. All spectra were recorded from 4000 to 750 cm^{-1} with a resolution of 4 cm^{-1}. IR spectra of fat samples were performed using an Attenuated Total Reflectance (ATR) device with a Durascope diamond crystal (SensIR Technologies, Norwalk, CT, USA). The fat samples were squeezed against the ATR diamond crystal. A total of 32 scans were collected for each spectrum and the average calculated and subtracted from the background spectrum using an empty ATR diamond crystal. Duplicate spectra of each sample were collected. Data acquisition and processing software was Win-Bomem Easy (Galactic Industries Corp., Salem, NH, USA).

2.4. Data analysis

In order to recognize fat samples belonging to a particular rearing system, in the first place, FA contents of each fat deposit (per-

Table 1
Distribution of fat samples according to the sampling, fat deposit, rearing system, farm and milk replacer.

Sampling	1				2					
Fat deposit	Perirenal and omental				Omental					
Rearing system	EM		MR		EM			MR		
Farm code	A	B	A	B	A	B	C	A	B	C
MR code	EM_A	EM_B	MR_{A1}	MR_B	EM_A	EM_B	EM_C	MR_{A2}	MR_B	MR_C
Number of samples	11	8	11	9	11	2	4	4	3	4

EM: ewe milk.
MR: milk replacer.

irenal and omental) were analyzed using the discriminant-PLS (d-PLS) technique. In addition, spectroscopic data (FT-IR spectra; 4000–750 cm^{-1}; 1687 sampling points for each spectrum) were examined using: (a) d-PLS and (b) ANN.

In order to investigate the farm to farm effect or the MR to MR differences on discrimination ability, the study was conducted independently both with the data of samples obtained from farm A (using only MR_{A1}) and B (using MR_B), and from the whole data set: farms A (using MR_{A1} and MR_{A2}), B (MR_B) and C (MR_C). As can be seen from Table 1, the first data set had 39 instances for perirenal fat (19 EM and 20 MR), and 55 instances for omental fat (32 EM and 23 MR). The whole data set had 39 instances for perirenal fat (19 EM and 20 MR), and 67 instances for omental fat (36 EM and 31 MR).

PLS works on the basis of extracting a smaller number of orthogonal latent components that are linear combinations of the original ones. PLS was performed using the public domain iToolbox (Nørgaard et al., 2000). PLS analyses included up to 12 PLS components for the validation process. Data were pre-processed using multiplicative signal correction (MSC) followed by mean centering (Geladi, McDougall, & Martens, 1985; Martens, Nielsen, & Engelsen, 2003).

A calibration matrix was set up with all samples by creating "dummy variables", assigning a value of 0 if the spectrum belonged to EM-fed suckling lambs or 1 if it belonged to MR-fed suckling lambs. A sample was assigned to a specific rearing system if the predicted value was equal or ±0.5 of the dummy values. The model validation, considering up to 12 PLS components, was performed by 5-fold cross-validation with systematic exclusion (Venetian blinds).

Classification evaluation was assessed in terms of the overall misclassification error rate and the class conditional error rates: EM and MR error rates. Moreover, the variability between the predicted value and the reference is also analyzed with the root mean square error of cross validation (RMSECV), the standard error of prediction (SEP) and the square of the correlation coefficient (R^2). Results were estimated by 10-fold cross-validation and the results are given as an average over 10 runs. Note that in each run, the data set is divided in 10-folds, where 1-fold is employed for evaluation purposes and the other 9-folds to calibrate or train the model. As mentioned above, 5-fold cross-validation was performed with the training data (belonging to the 9-folds mentioned before) in order to select the model complexity.

The RMSECV was computed by cross-validation as $\mathrm{RMSECV} = \sqrt{\frac{\sum d_i^2}{N}}$, where N stands for the total number of samples, and d_i is the difference between the reference value and that predicted by the classification model.

The SEP corrects the bias and was calculated by cross-validation as $\mathrm{SEP} = \sqrt{\frac{\sum (d_i - \mathrm{bias})^2}{N-1}}$, where the bias is computed as the average difference between the reference and the prediction ($\mathrm{bias} = \frac{\sum d_i}{N}$).

ANNs are known to efficiently model highly nonlinear classification boundaries. An ANN based on multi-layer perceptron (MLP) architecture (with three layers) is capable of modeling an arbitrarily complex mapping between the input (feature) and output (class) spaces (Samarasinghe, 2006); consequently, they are called universal approximators. The cost function for the training stage was the mean square error (MSE), optimized by the back-propagation algorithm with adaptive learning rate and momentum.

To overcome the problem of high dimensionality, the original feature set can be reduced by selecting the most relevant features and by extracting new ones (hence called components, as in the case of PLS). Once the dimensionality reduction is performed, the ANN operates with the new data set.

Two different approaches were assessed in the context of ANN classifiers: (a) an ANN with different number features (up to 1000) selected by SVM; and (b) an ANN with components extracted by PCA (up to 24). A technique based on SVM (Guyon, Weston, Barnhill, & Vapnik, 2002) was used to rank the original features according to its discriminatory power which allows selecting subsets of the most relevant ones. Furthermore, the PCA technique was used to project the data to a space with lower

Table 2
Fatty acid composition of the ewe milk and the milk replacer collected in different farms (expressed as percentage of total fatty acids).

Rearing system	EM						MR			Sign.
Farm code	A	B	C	Mean	A	A	B	C	Mean	
MR code	EM_A ($n = 1$)	EM_B ($n = 1$)	EM_C ($n = 1$)		EM_{A1} ($n = 1$)	EM_{A2} ($n = 1$)	EM_B ($n = 1$)	EM_C ($n = 1$)		
C10:0	8.9	10.8	8.8	9.5 ± 1.1	5.8	1.2	2.6	2.6	3.1 ± 1.9	***
C12:0	5.0	6.5	4.8	5.4 ± 0.9	18.4	8.4	16.4	17.7	15.2 ± 4.6	**
C14:0	12.6	14.0	9.2	11.9 ± 2.5	7.6	4.3	7.2	7.6	6.7 ± 1.6	**
C15:0	1.7	1.1	1.1	1.3 ± 0.3	tr	tr	tr	tr	tr	–
C16:0	17.4	15.9	26.2	19.8 ± 5.6	16.4	28.8	19.4	18.9	20.8 ± 5.4	NS
C16:1	1.7	1.6	2.0	1.8 ± 0.2	2.1	4.1	3.1	2.5	3.0 ± 0.8	NS
C17:0 br	0.9	1.0	1.3	1.1 ± 0.2	tr	tr	tr	tr	tr	–
C17:0	1.4	1.2	1.0	1.2 ± 0.1	tr	tr	tr	tr	tr	–
C17:1 $n-7$	0.5	0.6	tr	0.5 ± 0.1	tr	tr	tr	tr	tr	–
C18:0	11.7	10.6	12.9	11.7 ± 1.2	10.1	11.5	12.9	10.8	11.3 ± 1.2	NS
C18:1	27.5	26.8	22.8	25.7 ± 2.6	29.8	34.0	31.7	30.6	31.5 ± 1.9	*
C18:2	3.7	3.6	2.4	3.2 ± 0.7	6.9	6.5	4.3	6.9	6.2 ± 1.2	*
C18:3	1.0	0.6	1.8	1.1 ± 0.6	0.8	tr	tr	0.3	0.4 ± 0.3	NS
CLA	0.8	0.7	1.0	0.8 ± 0.1	tr	tr	tr	tr	tr	–
SFA	63.3	64.6	68.1	65.4 ± 2.5	59.1	55.2	59.7	58.6	58.2 ± 2.0	***
MUFA	30.4	29.8	25.9	28.7 ± 2.4	32.7	38.8	35.2	33.9	35.2 ± 2.6	*
PUFA	6.3	5.6	6.0	5.9 ± 0.3	8.1	6.6	5.1	7.5	7.0 ± 1.4	NS

EM: ewe milk; MR: milk replacer.
br: branched.
Sign.: Significance of the effect of type of milk (EM or MR); *,**,*** indicate P levels lower than 0.05, 0.01, and 0.001, respectively.
NS: no significance ($P > 0.05$).
CLA: conjugated linoleic acid.
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
tr: traces; ⩽0.2.

dimensions, retaining the maximum variance. Feature extraction was performed by creating a number of new uncorrelated components formed by linear combination of the original correlated features. Feature ranking based on SVM was carried out with WEKA (a suite of machine learning software; Witten & Frank, 2005) and feature extraction based on PCA with MATLAB 6.5 (the language of technical computing; The MathWorks, Inc., Natick, Massachusetts, USA). The ANN training is also carried out with MATLAB and the Neural Network Toolbox (Demuth & Beale, 2002). The number of nodes in the hidden layer, the number of training cycles as well as the number of features in the low dimensional space was determined by cross-validation with 10-folds. In addition, classifier evaluation was carried out as described previously for the PLS model. The ANN model output (sigmoid) gave a

Table 3
Fatty acid composition of perirenal fat deposit (expressed as percentage of total fatty acids).

Rearing system	EM			MR			Sign.
Farm code	A	B	Mean	A	B	Mean	
MR code	EM_A ($n = 11$)	EM_B ($n = 8$)		EM_{A1} ($n = 11$)	EM_B ($n = 9$)		
C10:0	0.97	0.80	0.89 ± 0.33	0.23	0.23	0.23 ± 0.11	***
C12:0	1.86	1.46	1.67 ± 0.72	4.10	3.34	3.76 ± 1.49	***
C14:0	9.07	8.38	8.74 ± 1.77	8.61	8.36	8.50 ± 1.16	NS
C15:0	0.84	0.80	0.82 ± 0.26	0.33a	0.10^b	0.22 ± 0.19	***
C16:0	19.78	20.06	19.91 ± 1.94	20.22	18.67	19.52 ± 2.66	NS
C16:1	2.56	2.64	2.60 ± 0.25	2.11	1.91	2.02 ± 0.53	***
C17:0 br	1.85	1.56	1.71 ± 0.38	0.58	0.22	0.42 ± 0.51	***
C17:0	1.89	1.73	1.81 ± 0.32	0.74^a	0.27^b	0.53 ± 0.46	***
C17:1 *n*−7	0.54	0.80	0.66 ± 0.44	0.33	0.20	0.27 ± 0.29	**
C18:0	18.90	19.85	19.35 ± 2.70	13.05	15.71	14.25 ± 3.67	***
C18:1	32.40	34.27	33.29 ± 3.53	37.48^b	42.46^a	39.72 ± 3.90	***
C18:2	3.79	3.50	3.65 ± 0.64	7.77^a	6.03^b	6.99 ± 1.35	***
C18:3	0.82	0.51	0.67 ± 0.39	1.14^a	0.27^b	0.75 ± 0.59	NS
CLA	0.73^a	0.51^b	0.62 ± 0.21	0.38	0.39	0.39 ± 0.26	**
SFA	57.42	56.45	56.96 ± 2.95	49.10	47.53	48.39 ± 3.10	***
MUFA	36.83	38.72	37.72 ± 3.39	41.23^b	45.46^a	43.13 ± 3.37	***
PUFA	5.75	4.84	5.32 ± 1.04	9.67^a	7.01^b	8.47 ± 1.83	***

EM: ewe milk; MR: milk replacer.
br: branched.
Sign.: significance of the effect of rearing system; *, **, *** indicate *P* levels lower than 0.05, 0.01, and 0.001, respectively.
NS: no significance ($P > 0.05$).
CLA: conjugated linoleic acid.
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
For each milk source, mean values in the same row with different letter (a, b) presented significant differences ($P < 0.05$).

Table 4
Fatty acid composition of omental fat deposit (expressed as percentage of total fatty acids).

Rearing system	EM				MR					Sign.
Farm code	A	B	C	Mean	A	A	B	C	Mean	
MR code	EM_A ($n = 22$)	EM_B ($n = 10$)	EM_C ($n = 4$)		EM_{A1} ($n = 11$)	EM_{A2} ($n = 4$)	EM_B ($n = 12$)	EM_C ($n = 4$)		
C10:0	1.16	1.03	0.76	1.08 ± 0.43	0.22^c	0.17^d	0.24^a	0.23^b	0.22 ± 0.04	***
C12:0	2.43^a	1.60^b	0.75^c	2.01 ± 1.13	4.75^a	2.60^c	3.29^b	2.53^d	3.57 ± 1.51	***
C14:0	11.82^a	10.88^b	7.54^c	11.08 ± 2.45	9.87^a	6.16^d	9.59^b	8.58^c	9.07 ± 1.60	***
C15:0	0.96	0.91	0.73	0.92 ± 0.26	0.21^b	0.25^a	0.12^d	0.13^c	0.17 ± 0.07	***
C16:0	25.23^b	24.87^a	21.17^c	24.68 ± 2.24	23.21^b	24.72^a	21.92^c	20.91^d	22.48 ± 2.07	***
C16:1	2.59	2.47	2.39	2.53 ± 0.66	1.95^c	2.35^b	1.84^d	2.39^a	2.02 ± 0.34	***
C17:0 br	0.91^c	1.31^b	1.58^a	1.09 ± 0.40	0.18	0.25	0.15	0.14	0.17 ± 0.07	***
C17:0	1.21^c	1.63^b	1.88^a	1.40 ± 0.43	0.39^b	0.42^a	0.31^c	0.29^d	0.35 ± 0.09	***
C17:1 *n*−7	0.38^c	0.62^b	0.68^a	0.48 ± 0.27	0.15	0.15	0.14	0.16	0.15 ± 0.04	***
C18:0	12.43^c	13.46^b	17.07^a	13.23 ± 2.54	9.30	11.09	10.25	12.19	10.27 ± 2.59	***
C18:1	33.17	33.80	37.62	33.84 ± 4.08	39.20^d	40.34^c	44.47^a	40.46^b	41.55 ± 4.51	***
C18:2	3.43	3.23	3.81	3.41 ± 0.62	7.54^c	9.35^a	5.46^d	9.19^b	7.18 ± 1.64	***
C18:3	1.03	0.88	1.03	0.97 ± 0.20	1.04^a	0.47^c	0.32^d	0.54^b	0.90 ± 0.85	NS
CLA	0.52^b	0.42^c	0.53^a	0.49 ± 0.10	0.26^b	0.08^d	0.43^a	0.10^c	0.28 ± 0.16	***
SFA	57.81	57.47	53.04	57.19 ± 3.85	48.74	46.19	46.42	45.73	46.87 ± 4.08	***
MUFA	36.74	37.52	41.19	37.45 ± 3.90	42.02^b	43.35^b	47.02^a	43.78^b	44.35 ± 4.47	***
PUFA	5.45	5.00	5.77	5.36 ± 0.82	9.24^b	10.46^a	6.55^b	10.50^a	8.78 ± 2.23	***

EM: ewe milk; MR: milk replacer.
br: branched.
Sign.: significance of the effect of rearing system; *, **, *** indicate *P* levels lower than 0.05, 0.01, and 0.001, respectively.
NS: no significance ($P > 0.05$).
CLA: conjugated linoleic acid.
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
For each milk source, mean values in the same row with different letter presented significant differences ($P < 0.05$).

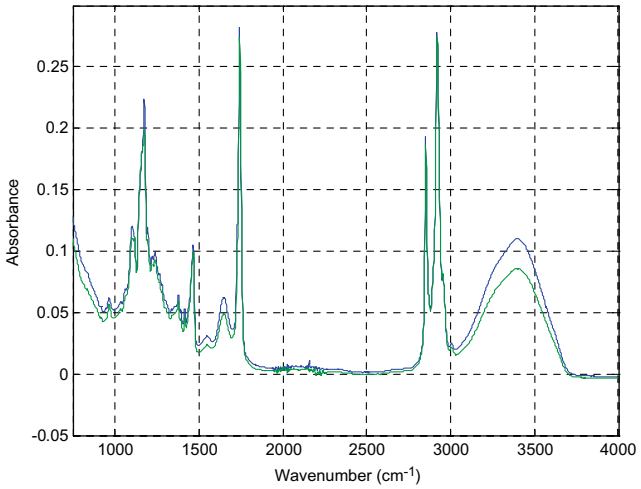
value between 0 and 1, so that any output value higher than 0.5 was assigned to class MR, and lower than 0.5 to class EM. Before the ANN based analysis, for both approaches, the data corresponding to the regions from 1800 to 2700 cm^{-1} and 3600 to 4000 cm^{-1} were omitted to avoid nonsense overfit information selected from the noisy background. From the remaining regions, 1014 sampling points were available for each spectrum.

3. Results and discussion

3.1. Fatty acid composition

The FA composition of EM and the four different commercial MR samples collected from the different farms are shown in Table 2. The contents of several FA differed significantly between EM and MR samples. As found in other studies (Lanza et al., 2006; Napolitano et al., 2002; Osorio et al., 2007b), EM fat had higher amounts of C15:0, C17:0, branched-chain C17:0, C10:0, C14:0 and the sum of SFA than MR fat, and lower amounts of C12:0 and the sum of MUFA. These differences have been explained by the fact that vegetable fats were included in MR formulations.

FA compositions of perirenal and omental fat samples and the effect of the milk source and farm on their compositions are pre-



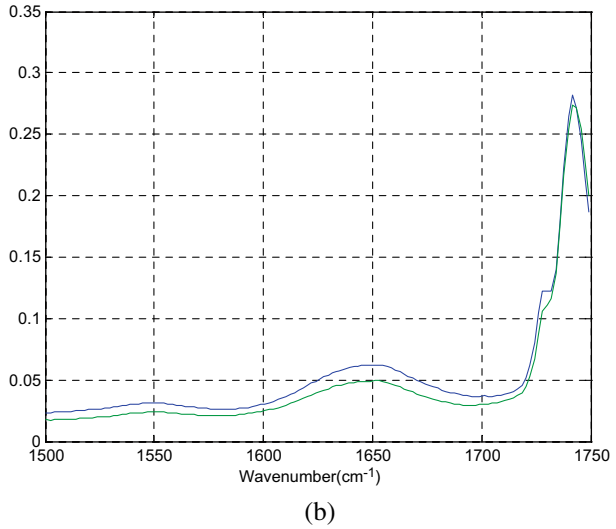
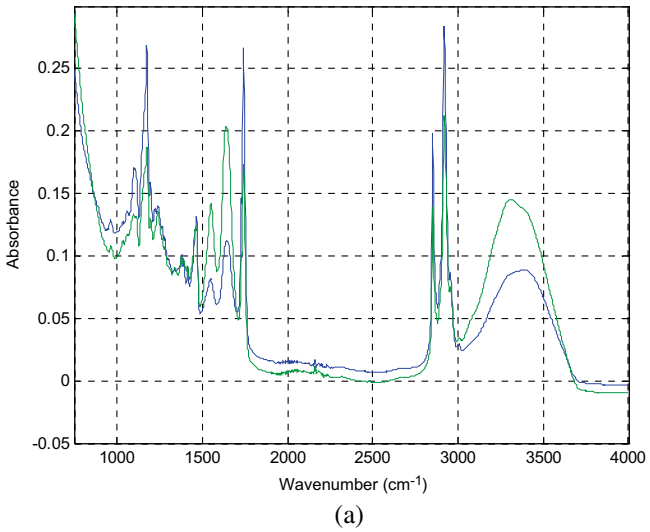


Fig. 1. FT-IR spectra of perirenal fat samples (—: EM; —: MR): (a) from 750 to 4000 cm^{-1}. (b) Zoom on the region 1500–1750 cm^{-1}.



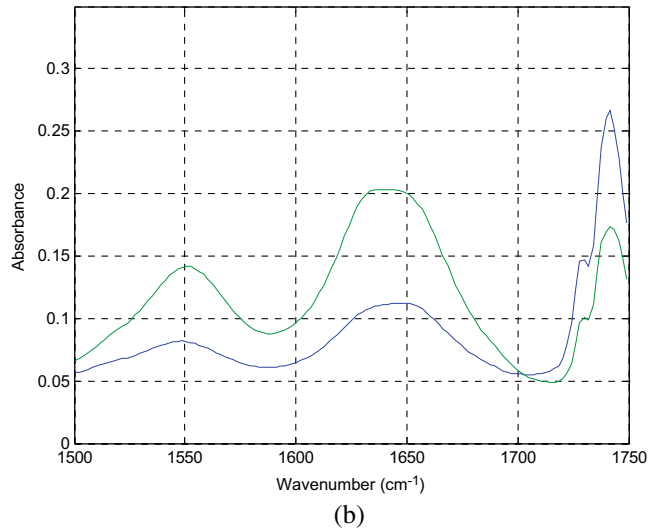


Fig. 2. FT-IR spectra of omental fat samples (—: EM; —: MR): (a) from 750 to 4000 cm^{-1}. (b) Zoom on the region 1500–1750 cm^{-1}.

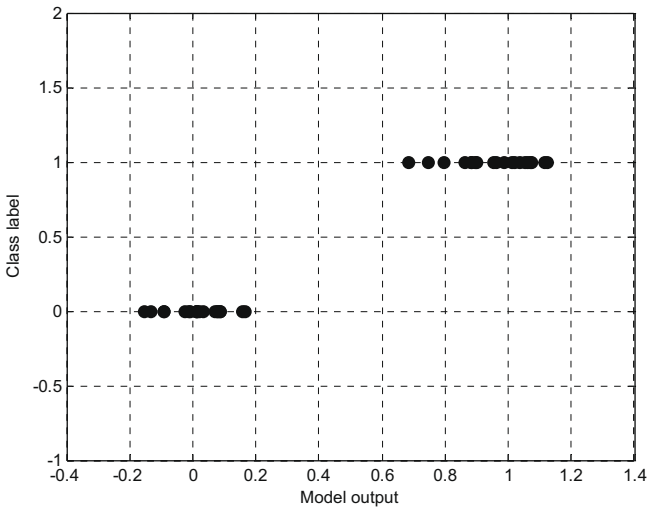


Fig. 3. PLS discriminant analysis using 12 PLS components for the perirenal fat samples. The class label is plotted against the PLS model output.

sented in Tables 3 and 4. Both fat deposits showed similar FA profiles. However, rearing system had a significant effect on FA composition. Fat of EM reared suckling lambs showed a higher content of SFA ($P < 0.001$) and lower content ($P < 0.05$) of MUFA and polyunsaturated FA (PUFA) than fat of MR reared animals. Differences between types of rearing were significant in most FA's. Levels of C10:0, all Odd chain fatty acids, C16:1, C18:0 and conjugated linoleic acid (CLA) were found to be higher in the fat of EM reared lambs. On the contrary, higher contents of C12:0, C18:1 and C18:2 were found in fat of MR reared lambs, which is in agreement with Osorio et al. (2007a).

Within each rearing system, significant differences ($P < 0.05$) were found for several individual FA's between farms. For MR samples, significant differences in total MUFA or PUFA between farms were observed. However, as expected, dispersion in the FA contents between farms or MR, within each rearing system, was much lower than the variation in the FA contents between rearing systems.

3.2. FT-IR spectra interpretation

The average spectra of perirenal and omental fat samples from lambs reared on EM or MR are shown in Figs. 1 and 2, respectively. The strongest peaks for both fat deposits were observed at the following wavenumbers: 3300–3400 cm^{-1}, assigned to –OH stretching and –NH stretching vibrations; 2925 cm^{-1} and 2850 cm^{-1}, which are specific for the asymmetric and symmetric C–H stretching vibrations of the aliphatic $–CH_2–$ groups of fatty acids; 1746 cm^{-1} which is due to the –C=O ester carbonyl stretching vibration of triglycerides; and 1163 cm^{-1}, which is associated with the stretching vibration of the –C–O bond of the glycerol skeleton of triglycerides. Other medium intensity peaks in the spectra for both groups were observed at: 1654 cm^{-1} and 1550 cm^{-1}, which could be primarily assigned to amide I and amide II bands of the proteins (Pedersen et al., 2003), as well as to some hidden information about the –C=C– (*cis*-) stretching vibrations; 1465 cm^{-1} and 1377 cm^{-1}, which can be explained by the bending vibrations of the aliphatic groups ($–CH_2–$ and $–CH_3$); peaks at 1238 cm^{-1} and 1097 cm^{-1} are primarily ascribed to the –C–O stretching. Finally, weak peaks can also be seen at: 965 cm^{-1}, which can be attributed to –HC=CH– bending out-of-plane vibrations of fatty acid *trans*-double bonds; 1725 cm^{-1}, which might be attributed to free fatty acids or monoacylglycerols; and 3005 cm^{-1}, which is due to olefinic =C–H strectching vibrations. All the above peaks are characteristic for animal fat and connective tissues.

A visual comparison of average spectra from perirenal and omental fat deposits (Figs. 1 and 2) showed that the peaks assigned to amide bands were far more prominent in the latter. This is in agreement with a previous study (Osorio et al., 2007a) where higher content of collagen was found in omental than in perirenal fat deposits. Moreover, differences in the FT-IR spectra between types of rearing can be observed, with differences being the result of specific characteristics of the fat samples. Among both fat deposits, the average spectra of omental fat showed more variation. More specifically, peaks showing the highest differences in intensity between the two types of rearing system were at 3400, 1654, 1550 and 1163 cm^{-1} – the intensity of the first three peaks was higher in the spectrum of omental MR samples, while the opposite was true for the last one. Differences in the amide peaks (at 1654 and 1550 cm^{-1}) due to rearing system can be related to differences in collagen content, which according to Osorio et al. (2007a) is significantly higher in omental fat from MR reared suckling lambs than in that from lambs reared on EM.

3.3. Partial least squares

When applying the d-PLS analysis to the FA data from farms A (using MR_{A1} as the MR and EM_A as the EM) and B (using MR_B as the MR and EM_B as the EM), and to the whole data set, a perfect discrimination (100%) of both perirenal and omental samples, according to the rearing system, was achieved (data not shown).

D-PLS analysis of FT-IR spectra also showed a perfect classification (0% misclassifications) when considering perirenal fat samples. Fig. 3 shows the results for the d-PLS classification of the MR and EM samples – it depicts the true sample label (dummy value) against the PLS model output. Note that for MR samples (class label 1) the PLS model output was always higher than 0.5 and for the EM samples (class label 0) less than 0.5.

Table 5
Classification error rate for the omental fat samples when considering 2 farms (A and B) and one milk replacer for each farm (MR_{A1} and MR_B, respectively).

Approach	Number of descriptors	Error rate (%)	EM error rate (%)	MR error rate (%)	R^2	Bias	SEP	RMSEP
PLS	4 Components	10.0	12.3	6.8	0.64	−0.042	0.316	0.316
Neural network (PCA)	20 Components	9.0	9.2	9.1	0.65	−0.021	0.307	0.306
Neural network (SVM)	60 Features	9.9	9.4	10.2	0.64	−0.002	0.316	0.316

PCA: principal component analysis.
SVM: support vector machines.
R^2: correlation coefficient.
EM: ewe milk; MR: milk replacer.
SEP: standard error of prediction.
RMSEP: root mean squared error of prediction.

Table 6
Classification error rate for all omental fat samples, when considering the three farms (A, B, C), and the four milk replacers (MR_{A1}, MR_{A2}, MR_B and MR_C).

Approach	Number of descriptors	Error rate (%)	EM error rate (%)	MR error rate (%)	R^2	Bias	SEP	RMSEP
PLS	4 Components	12.9	15.2	9.6	0.56	−0.039	0.359	0.358
Neural network (PCA)	20 Components	8.9	9.9	7.6	0.65	−0.021	0.310	0.310
Neural network (SVM)	60 Features	9.0	8.6	9.5	0.65	−0.005	0.310	0.310

PCA: principal component analysis.
SVM: support vector machines.
R^2: correlation coefficient.
EM: ewe milk; MR: milk replacer.
SEP: standard error of prediction.
RMSEP: root mean squared error of prediction.

A subsequent analysis focused on the omental fat samples obtained from the same lambs as used in the analysis above. The study (Table 5) showed a misclassification rate of 10.0%, a RMSECV of 0.316 and a correlation coefficient, R^2, of 0.64. These results were inferior to those achieved with the discrimination based on the perirenal fat samples. In addition, considering all omental fat samples, the PLS model with 4 components (determined by cross-validation) showed a misclassification error rate equal to 12.9% (Table 6). Out of the total 36 EM samples, an average of 5.5 samples was classified incorrectly (15.2%). Moreover, out of the 31 MR samples, an average of 3 samples (9.6%) was assigned to the EM class.

The lower discrimination ability between rearing systems obtained when using FT-IR spectra of omental fat with respect to that obtained from FT-IR spectra of perirenal fat could be attributed to subtle differences in the composition between fat deposits. Thus, in omental fat the discrimination ability might have been interfered with by the presence of bands different from those assigned to fatty acids and glycerol. It must be noted that the number of farms, or MR used in those farms, hardly influenced the percentage of misclassifications.

3.4. Artificial neural network

Having explored by cross-validation several architectures (up to 30 nodes in the hidden layer) and different numbers of training cycles (up to 10000), the optimal configuration found for the data from perirenal fat was a ANN with 15 nodes in the hidden layer and 7000 training cycles. Likewise, the optimal ANN for the omental fat deposit had 10 nodes in the hidden layer and was trained during 700 cycles.

The ANN that classified the perirenal fat samples achieved perfect classification whether it used 20 PCA components or SVM-selected features (5 out of 1014). These relevant wavenumbers for the perirenal fat are shown in Fig. 4, and belong to the following set (expressed in cm^{-1}): 1215, 1217, 1213 and 2952. None of these wavenumbers corresponded with the strong or medium intensity bands described above (see Section 3.2). The latter is the frequency from –C–H (CH_3) asymmetric stretching (Guillén & Cabo, 1997).

Regarding the omental fat samples, the ANN(PCA) based on 20 PCA components yielded an error rate equal to 9.0% when including EM_A, EM_B, MR_{A1} and MR_B in the analysis, and 8.9% when the entire data set was included. In comparison, the misclassification error rates were 9.9% and 9.0%, respectively, for an ANN (SVM) with 60 features selected by SVM (Tables 5 and 6). Fig. 5 shows the averaged spectra obtained with all omental fat samples and the set of 60 wavenumbers selected by SVM for which the classifier model achieved the lowest classification error. Only some of the relevant wavelengths selected by SVM were related to the major bands attributed to functional groups of FA and glycerol, i.e. 1163, 1747, 2800 and 2925.

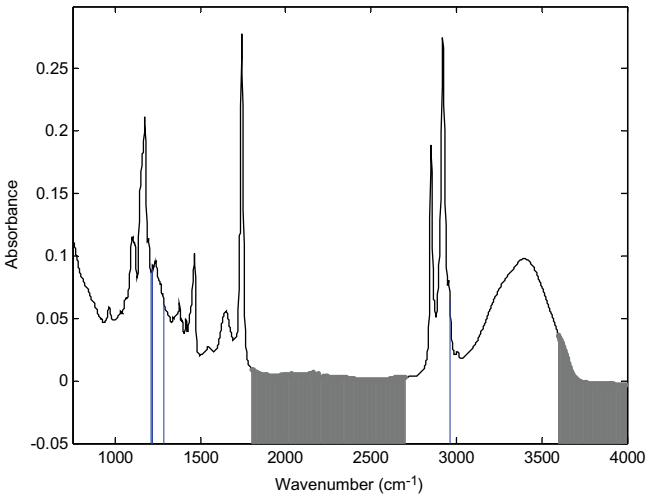


Fig. 4. Average FT-IR spectrum of perirenal fat samples (––: 5 features selected by SVM: 1215.1; 1217.0; 1213.1; 2962.4).

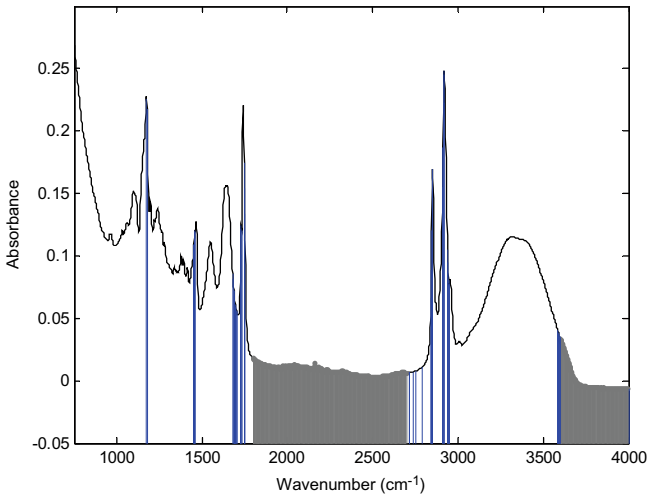


Fig. 5. FT-IR spectrum of omental fat samples (––: 60 features selected by SVM: 1726.2; 4000.1; 1724.2; 1728.1; 1458.1; 2740.6; 1460; 2848.7; 1456.1; 1730; 2941.2; 2715.6; 2846.7; 2943.2; 2914.2; 2916.2; 1454.2; 3598.9; 1452.3; 2717.5; 2850.6; 3597; 1703; 3593.1; 1701.1; 2912.3; 3595; 1697.2; 2939.3; 2844.8; 1699.2; 3591.2; 1695.3; 2945.1; 2792.7; 1176.5; 2947; 2918.1; 1693.4; 3589.3; 2719.4; 1691.4; 1751.2; 1731.9; 1679.9; 1749.3; 2910.4; 3587.3; 2948.9; 2713.6; 1689.5; 1681.8; 1753.2; 1178.4; 3585.4; 1687.6; 1747.4; 2752.2; 2842.9; 2950.9).

4. Conclusions

FT-IR spectroscopy applied to fat samples has shown good potential to discriminate suckling lamb carcasses according to their rearing system. By direct analysis of fat samples, obtained from carcasses in a practically non-destructive way, a perfect classification model (0% error rate) was achieved when considering perirenal fat. Moreover, a reasonably good classification model (9% error rate) was obtained for omental fat.

For perirenal fat analysis, in terms of classification accuracy, the three data treatment approaches (d-PLS, ANN(PCA) and ANN(SVM)) yielded a perfect model (0% error rate). For omental fat analysis, the d-PLS classification error rate was outperformed by the ANN. The ANN (SVM) showed similar results to ANN(PCA), but has the advantage of selecting the regions of the spectrum with higher discriminatory power, which may help to better understand the specific functional groups associated with the classification.

Subtle differences between the composition of perirenal and omental fat deposits could be the cause of the difference in the classification accuracy between the fat deposits. It must be emphasized that the number of farms sampled (2 or 3) or MR used in those farms (two or four) hardly influenced the percentage of misclassifications for omental fat samples.

For omental fat samples, the fact that the analysis of FT-IR spectra showed misclassifications between rearing systems, in spite of the perfect discrimination obtained when using the fatty acid composition, lead to the following suggestion: discrimination ability between rearing systems from FT-IR spectra could be interfered with by the presence of bands different from those assigned to fatty acids and glycerol. This is reinforced by the number of wavenumbers selected by SVM, in the ANN analysis, which did not correspond to the characteristic bands of fatty acids or glycerol.

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