Nutrition & Food Science

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Article information:

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Received 12 August 2017
Revised 13 October 2017
16 October 2017
16 October 2017
18 October 2017
Accepted 20 October 2017

Abstract

Purpose – Consumption of game meat is growing when compared to other meats. It is susceptible to adulteration because of its cost and availability. Spectroscopy may lead to rapid methodologies for detecting adulteration. The purpose of this study is to detect the adulteration of wild fallow deer (*Dama dama*) meat with domestic goat (G) (*Capra aegagrus hircus*) meat, for samples stored for different periods of time using Fourier transform infrared (FTIR) spectroscopy coupled with chemometric.

Design/methodology/approach – Meat was cut and mixed in different percentages, transformed into mini-burgers and stored at 3°C from 12 to 432 h and periodically examined for FTIR, pH and microbial analysis. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were applied to detect adulteration.

Findings – The PCA model, applied to the spectral region from 1,138 to 1,180, 1,314 to 1,477, 1,535 to 1,556 and from 1,728 to 1,759 cm^{-1}, describes the adulteration using four principal components which explained 95 per cent of variance. For the levels of Adulteration A1 (pure meat), A2 (25 and 50 %w/wG) and A3 (75 and 100 %w/wG) for an external set of samples, the correlation coefficients for prediction were 0.979, 0.941 and 0.971, and the room mean square error were 8.58, 12.46 and 9.47 per cent, respectively.

Originality/value – The PLS-DA model predicted the adulteration for an external set of samples with high accuracy. The proposed method has the advantage of allowing rapid results, despite the storage time of the adulterated meat. It was shown that FTIR combined with chemometrics can be used to establish a methodology for the identification of adulteration of game meat, not only for fresh meat but also for meat stored for different periods of time.

Keywords FTIR spectroscopy, Adulteration of game meat, Food authentication, Meat adulteration, PLS-DA

Paper type Research paper

1. Introduction

It is increasingly important to detect fraudulent food products, whether for economic, religious or public health reasons (Charlebois *et al.*, 2016; Manning and Soon, 2014). Food fraud is a common term used to include the intentional adulteration of foodstuff by substitution or addition of ingredients or misleading statements about a product, for economic gain (Spink and Moyer, 2011).

Nutrition & Food Science
Vol. 48 No. 2, 2018
pp. 245-258

0034-6659
DOI 10.1108/NFS-08-2017-0164

Game meat consumption has been increasing because of various motivations, such as the particular flavor, its healthier composition and lower fat and cholesterol contents. It is prone to adulteration because of its high price. Selective methods are necessary to verify its authenticity (Druml *et al.*, 2015).

Several analytical methods have been applied for the determination of meat adulteration, such as protein-based methods (Al Ebrahim *et al.*, 2013), the DNA-based methods (Lin *et al.*, 2014) and the real-time PCR (Druml *et al.*, 2015). These methods are laborious, expensive and destructive, and require sophisticated laboratory procedures.

Emerging technologies for authentication and traceability of fresh meat products include nuclear magnetic resonance imaging (Standal *et al.*, 2010), fluorescence (Karoui and Blecker, 2011), near and mid infrared absorption spectroscopy (NIR and MIR) (Alamprese *et al.*, 2013) and Raman spectroscopy (Zhao *et al.*, 2015) coupled with Fourier transform and multispectral and hyperspectral imaging (Pu *et al.*, 2015). These techniques are simple, non-destructive and allow real-time analysis.

Fourier transform infrared spectroscopy (FTIR) allows easy manipulation of the spectroscopic data (Bell, 2012). Transmission or attenuated total reflection (ATR) techniques are commonly used to obtain the absorption spectrum (Lohumi *et al.*, 2015).

There are several chemometrics methods applied in spectroscopy, namely principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), partial least squares regression and soft independent modeling of class analogy, among others (Gredilla *et al.*, 2016; Saraiva *et al.*, 2015). PLS-DA is a prevailing method for discriminant classification, particularly appropriate to deal with collinear data matrices, like in the case of spectroscopic data.

There are studies with FTIR for the detection of meat adulteration, for example, turkey meat in fresh, frozen-thawed and cooked minced beef (Alamprese *et al.*, 2016), beef meatball adulteration with pork (Rohman *et al.*, 2011), minced beef adulteration with turkey meat (Alamprese *et al.*, 2013) and rat meat in meatball formulation (Rahmania and Rohman, 2015).

The consumer purchases meat with a range of different periods of storage. Although there are studies on the use of spectroscopy and chemometrics to discriminate adulterated meat, to our knowledge, few studies extend their experiments to stored meat. The aim of this work was to explore the potential of FTIR in tandem with PCA and PLS-DA in detecting the presence/absence of adulteration of wild fallow deer (*Dama dama*) meat with domestic goat (*Capra aegagrus hircus*) meat, not only for fresh samples but also for samples stored for different periods.

2. Material and methods

2.1 Meat samples manufacture

Fallow deer (D) and goat (G) meat were excised from carcasses at 24 hours *post mortem*, cut in smaller pieces and minced. Four batches of fallow deer adulterated with goat were prepared and transformed in mini-burgers (samples). Five formulations with the values of 0, 25, 50, 75 and 100 per cent w/w of goat were considered.

The samples, weighing approximately 30 g were packed in air and overwrapped with polyethylene film. Following packaging, samples were stored at 3°C and examined after 12, 24, 36, 48, 72, 96, 120, 144, 168, 216, 264, 312, 360 and 432 h. At each sampling point (including t = 0), the samples were analyzed for three parameters, spectroscopic, pH and microbiological analysis. The microorganisms analyzed were total mesophilic (TVC) and psychrotrophs. Four batches were evaluated; each one constituted of 75 samples (15 sampling points and 5 formulations); therefore, 300 samples were analyzed.

2.2 Microbiological analysis

Aliquots (10 g) were homogenized with 90 ml tryptone salt (tryptone 0.1 per cent and NaCl 0.85 per cent) in a Stomacher for 90 s. Serial decimal dilutions were prepared in the same solution for microbiological determinations. TVC (ISO4833, 2003) and psychrotrophs (ISO6887, 2017) populations were obtained after incubation on plate count agar (Oxoid CM0325, England) at 30°C for 3 days and 7°C for 10 days, respectively. Results were expressed as logarithm of colony forming unit (cfu/g). In case the microorganism counts were below the detection limit, the result was considered to be zero for statistical purposes.

2.3 Physical-chemical measurements

2.3.1 pH. The pH was measured directly in the samples using a penetration electrode with a pH meter (Crison Instruments, Spain) and was evaluated in triplicate immediately after opening the packages (ISO2917, 2004).

2.3.2 FTIR measurement. Infrared spectra were collected in a FTIR spectrometer (Mattson, Unicam Research Series, USA) equipped with a single reflection ATR module (Golden Gate, UK), a DLaTGS detector and a KBr beamspliter. The equipment is controlled by the WinFirst software – v1.1. The samples were placed on top of the ATR crystal which was kept at 30°C, ensuring that the aerobic surface of the samples was in close contact with the crystal and then pressed. Supposing that samples are mainly composed of water, calculation using Equation (2).7 of the study conducted by Stuart (2000) showed that the evanescent field was probing a depth of approximately 1.0 μm. All infrared spectra were recorded from 500 to 4000 cm^{-1} by co-adding 128 interferograms at a resolution of 4 cm^{-1}. A new air background spectrum was taken after every scan. The ATR base was cleaned with ethanol and dried before measuring the next sample. The cleaning was verified by gathering a background spectrum and comparing to the previous one. For each sampling occasion, two replicates were analyzed by FTIR; for each replicate, two spectra were collected, and the average was calculated. These spectra were recorded as absorbance values.

2.4 Statistical analysis

Spectral data set was initially submitted to smoothing based on the Savitzky–Golay algorithm (Savitzky and Golay, 1964) and, afterwards, were mean-centered and standardized (SNV).

2.4.1 Principal component analysis. For a preliminary exploration, the spectral data set was handled by PCA, which allowed determining its main features and to highlight relations among the original variables (absorbances at different wavenumbers). The PCA projects the large number of potentially correlated original variables in a representation space of smaller dimensions and calculates new variables, called principal components (PC), that are linear combinations of the starting absorbances and, thus, reduces the size of the data set (Abdi and Williams, 2010).

2.4.2 Partial least squares discriminant analysis. For the classification of samples in three different levels of adulteration, A1 (pure meat, 0 per cent w/w G), A2 (adulterated, 25 and 50 per cent w/w G) and A3 (highly adulterated, 75 and 100 per cent w/w G), the PLS-DA algorithm was applied (Barker and Rayens, 2003). It is a supervised method applied for classification usually in multivariate regression (Wold *et al.*, 2001). The latent variables (LVs) that maximize discrimination between groups of samples are calculated from the spectral data (X matrix) maximizing the covariance, with classes defined in the Y matrix. When the number of classes is more than two, the Y matrix is composed of several variables and the PLS2 algorithm must be applied (Abdi *et al.*, 2016).

The data set was split into two subsets, for calibration (training set) and for prediction (external set). The number of LV of models was chosen by the criterion of lowest prediction error in leave-one-out cross-validation.

The quality of the models was scrutinized by the multiple coefficient of determination or regression coefficient (R^2, where R is the correlation factor) and the root mean square error of calibration (RMSEC) and of cross-validation (RMSECV) (Abdi *et al.*, 2016).

The predictive ability of the model is evaluated from root mean square error of prediction (RMSEP), which is a measure of how well the model will make predictions, and is calculated exactly as RMSEC and RMSECV, except that the estimates of mean errors are now values obtained from the external set.

In addition, the evaluation of the PLS-DA models was performed using the goodness-of-fit parameter R^2Y, which represents the variation in class membership explained by the model, and the predictive ability parameter Q^2, known as the goodness of prediction (Sanz-Cortés *et al.*, 2013). Fisher's probability was used as a quality probe of the classification (< 0.01 usually considered a good prediction) (Whitlock, 2005).

Statistical calculations were performed using the Excel-based XLSTAT V2006.06 package (Addinsoft, Inc, NY, USA).

3. Results and discussion

3.1 Microbial and pH data

The evolution of TVC and psychrotrophs with storage time is shown in Table I for the five formulations. The TVC at the onset of storage (fresh samples) of fallow deer and of goat samples were (mean/standard deviation) 3.10 ± 0.30 and 4.85 ± 0.40 log cfu g^{-1}, respectively, and for samples stored for 432 h (completely spoiled), the values were 9.69 ± 0.45 and 10.50 ± 0.36 log cfu g^{-1}. Regarding psychrotrophs, for fresh samples of fallow deer and of goat the values were 2.50 ± 0.25 and 4.14 ± 0.36 log cfu g^{-1}, respectively, and for samples stored for 432 h (completely spoiled), the values were 8.43 ± 0.39 and 9.35 ± 0.38 log cfu g^{-1}. The TVC and psychrotrophs counts increase with storage time, as expected, following and exponential growth. The pure fallow deer has fewer initial TVC and psychrotrophs counts than goat, and therefore, the mixtures with increasing goat concentration have higher counts.

The pH value of meat is a parameter that indicates the quality of the meat (Watanabe *et al.*, 1996). The evolution of the pH with the storage time for the five formulations and four sets of samples is shown in Table I. While for fresh samples, the pH was in a small range from 5.73 to 5.85, at the end of the storage period, the pH values spanned from 6.29 to 7.54 for the pure fallow deer and goat, respectively. These results confirm that the samples are highly deteriorated before the end of the storage time and are within the published values for game meat, for similar storage conditions (Spaziani *et al.*, 2011). As can be seen in Table I, the time evolution of the pH for samples with 25, 50 and 75 per cent w/w of goat follows, within the experimental error, the corresponding values for pure fallow deer and pure goat. The high pH values observed in the goat samples at end of storage may be because of the production of basic compounds by some deteriorative microorganisms that degrade the nitrogen fraction of meat (Ammor *et al.*, 2009).

3.2 FTIR measured spectra

The infrared spectra of meat can provide information on biochemical changes occurring during storage (Ellis *et al.*, 2004). The peptide group, the structural repeat unit of proteins, gives up to 9 bands called amide A, B, I, II, . . ., VII. Absorption bands because of Amide A, I and II are two major contributors of the protein infrared spectrum (Krimm and Bandekar, 1986). Absorption

Table I.
Evolution of TVC, psychrotrophs and pH with storage time for the five formulations of fallow deer adulterated with goat

(% w/w goat)[b] Time (h)	TVC[a] (log cfu g^{-1})					Psychrotrophs (log cfu g^{-1})					pH				
	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100
0	3.1 (0.3)	3.3 (0.1)	3.6 (0.2)	4.0 (0.2)	4.8 (0.4)	2.5 (0.2)	2.8 (0.2)	3.2 (0.3)	3.9 (0.2)	4.1 (0.3)	5.72 (0.03)	5.84 (0.06)	5.82 (0.01)	5.85 (0.04)	5.78 (0.02)
12	3.2 (0.1)	3.4 (0.2)	4.1 (0.3)	4.3 (0.3)	4.9 (0.0)	2.6 (0.2)	3.0 (0.3)	3.3 (0.2)	4.1 (0.3)	4.4 (0.2)	5.79 (0.03)	5.91 (0.05)	5.86 (0.04)	5.88 (0.04)	5.83 (0.01)
24	3.2 (0.4)	3.6 (0.3)	4.3 (0.1)	4.7 (0.2)	4.9 (0.3)	2.8 (0.3)	3.0 (0.2)	3.4 (0.3)	4.0 (0.3)	4.4 (0.2)	5.74 (0.02)	5.82 (0.05)	5.86 (0.02)	5.90 (0.04)	6.01 (0.02)
36	3.2 (0.3)	3.9 (0.2)	4.4 (0.2)	4.8 (0.2)	5.1 (0.2)	2.7 (0.2)	2.9 (0.3)	3.6 (0.2)	4.0 (0.3)	4.3 (0.2)	5.92 (0.02)	5.93 (0.02)	5.97 (0.05)	6.03 (0.04)	6.09 (0.00)
48	3.6 (0.2)	4.0 (0.2)	4.7 (0.3)	5.3 (0.2)	5.5 (0.3)	2.8 (0.2)	2.8 (0.3)	3.5 (0.2)	4.0 (0.2)	4.4 (0.2)	5.94 (0.05)	5.99 (0.04)	6.02 (0.04)	6.09 (0.02)	6.16 (0.02)
72	3.7 (0.2)	4.4 (0.2)	4.9 (0.2)	5.3 (0.2)	6.1 (0.3)	2.8 (0.3)	2.9 (0.3)	3.5 (0.4)	4.3 (0.2)	4.4 (0.2)	5.90 (0.02)	5.97 (0.02)	6.00 (0.02)	6.10 (0.06)	6.12 (0.04)
96	3.8 (0.2)	4.8 (0.2)	5.3 (0.3)	5.7 (0.2)	6.5 (0.5)	2.8 (0.3)	3.1 (0.3)	3.6 (0.2)	4.5 (0.3)	4.9 (0.2)	5.92 (0.04)	5.98 (0.04)	6.03 (0.04)	6.08 (0.03)	6.18 (0.04)
120	3.9 (0.2)	5.1 (0.2)	5.9 (0.3)	6.5 (0.2)	7.2 (0.2)	3.2 (0.3)	3.3 (0.3)	3.8 (0.3)	5.4 (0.3)	6.1 (0.2)	5.95 (0.01)	6.01 (0.04)	6.06 (0.04)	6.12 (0.04)	6.16 (0.03)
144	4.0 (0.3)	5.2 (0.2)	6.0 (0.3)	6.8 (0.2)	7.6 (0.2)	3.3 (0.3)	3.7 (0.3)	4.8 (0.2)	6.1 (0.2)	7.1 (0.3)	5.92 (0.06)	5.99 (0.02)	5.99 (0.03)	6.07 (0.05)	6.11 (0.03)
168	4.0 (0.3)	5.8 (0.2)	6.4 (0.2)	7.1 (0.3)	7.8 (0.2)	3.1 (0.3)	4.0 (0.2)	5.5 (0.2)	6.6 (0.3)	7.7 (0.3)	6.02 (0.05)	6.09 (0.02)	6.09 (0.04)	6.17 (0.05)	6.21 (0.01)
216	4.7 (0.2)	6.0 (0.1)	7.1 (0.3)	7.8 (0.3)	8.8 (0.3)	3.3 (0.3)	4.7 (0.3)	6.5 (0.3)	7.4 (0.2)	7.9 (0.3)	6.04 (0.05)	6.07 (0.03)	6.15 (0.04)	6.22 (0.05)	6.33 (0.05)
264	6.2 (0.2)	7.7 (0.3)	8.4 (0.3)	9.2 (0.13)	9.7 (0.3)	4.2 (0.3)	5.8 (0.3)	7.1 (0.2)	8.2 (0.2)	8.8 (0.2)	6.02 (0.03)	6.03 (0.03)	6.18 (0.04)	6.45 (0.01)	6.51 (0.03)
312	8.4 (0.2)	8.8 (0.2)	9.3 (0.3)	9.7 (0.3)	10.3 (0.2)	5.0 (0.2)	6.6 (0.3)	7.8 (0.3)	8.9 (0.3)	8.8 (0.2)	6.10 (0.03)	6.24 (0.04)	6.50 (0.03)	6.60 (0.01)	6.67 (0.01)
360	9.1 (0.2)	9.2 (0.3)	9.5 (0.3)	10.1 (0.2)	10.4 (0.3)	6.3 (0.2)	8.0 (0.2)	8.6 (0.3)	9.1 (0.2)	9.2 (0.2)	6.15 (0.05)	6.32 (0.01)	6.74 (0.03)	7.24 (0.04)	7.39 (0.05)
432	9.6 (0.4)	9.8 (0.2)	9.9 (0.2)	10.2 (0.3)	10.5 (0.3)	8.4 (0.3)	8.5 (0.2)	8.7 (0.2)	9.2 (0.2)	9.3 (0.3)	6.29 (0.06)	6.62 (0.03)	6.96 (0.06)	7.28 (0.07)	7.54 (0.02)

Notes: Numerical values in parentheses are standard deviation of measurements; [a] TVC – total viable counts; and [b] five formulations with %w/w of goat from 0 to 100. The TVC and psychrotrophs counts increase with storage time, following an exponential growth. The pure fallow deer has fewer initial TVC and psychrotrophs counts than goat, and therefore, the mixtures with increasing goat concentration have higher counts. The pH is a parameter that indicates the quality of the meat. While for fresh samples the pH of both meats is similar, at the end of the storage period, the pH values spanned a broader range. These vales confirm high deterioration, particularly evident in mixtures with 75 and 100 % of goat meat, at the end of the storage time

bands related with water, fat, carbohydrates and proteins appear in the region from, approximately, 1750 to 900 cm^{-1}, loosely called "fingerprint region" (Ammor *et al.*, 2009).

In the region from 4000 to 500 cm^{-1}, there is a very broad band at around 3360 cm^{-1} related to, symmetric and asymmetric, N-H and O-H stretching of amide A and water, respectively. Around 1640 cm^{-1}, there is a large absorption band related to the O-H bending (present in water) and to the C=O stretching of amide I (from protein). At 1,550 cm^{-1}, there is a band because of the N-H bending of Amide II mixed with C-N stretching. Absorption bands at 1,458 and 1,401 cm^{-1} are because of C-H stretching of methylene (CH_2) and methyl (CH_3) groups (present in fat). Peaks at 1,398, 1,314 and 1,238 cm^{-1} are related to C-N stretch, N-H bend, C-O stretching and O = C-N bending of Amide III. The region from 1,200 to 950 cm^{-1} is associated with C-O and C-C stretching vibrations (because of carbohydrates). Moreover, the peaks at 1,460, 1,240 and 1,175 cm^{-1} can be attributed to C-O and at 1,740 cm^{-1} to C=O (fat). Finally, the peaks arising from 1,025 to 1,140 cm^{-1} could be absorbance because of amines (C-N stretch) (Sinelli *et al.*, 2010).

3.3 Preliminary analysis of the spectral data set

The FTIR spectra were exported from the equipment in native format and imported directly into the statistical software for all multivariate procedures. Data in the range 500 to 4,000 cm^{-1}, composed of 875 variables (wavenumbers), were used in further chemometric analysis.

First, the spectra set expressing various combinations of the five formulations and storage periods were smoothed, mean-centered and standardized (SNV), and then, PCA was used to determine the major sources of variance and to detect any unusual or outlying samples. The FTIR spectrum of the samples are very similar, meaning that gathering information requires the use of multivariate analysis.

The appropriateness to perform PCA was confirmed by Bartlett's sphericity test ($p < 0.0001$). The number of components retained in the final solution was based on the Kaiser–Meyer–Olkin (KMO) criterion for the analysis of eigenvalues (>1) and the proportion of variance retained (> 70 per cent), usually seen as the minimum needed to make the model suitable for explaining the original data. It was concluded that 88 PCs describe the variance of the data set represented by the original 875 variables. However, approximately 95 per cent of the total variance was explained by the first nine PCs, among which PCs 1, 2 and 3 accounted for 51.9, 21.3 and 5.4 per cent of the variability, respectively.

The variance that a given wavenumber is accounted for by all the PCs is given by the sum of the squared PCs loadings for all PCs for that given wavenumber, which is frequently called communality, and its initial value in PCA is 1. Therefore, it determines the variance in a given wavenumber explained by all the PCs (Abdi and Williams, 2010). Communality values smaller than 0.6 indicate that the wavenumber do not fit well in the PCA solution and should be release (Field, 2005; Stevens, 2002). Wavenumbers for which the communality value are higher than 0.6 are important to explain the variance and are potential wavenumbers to differentiate the five formulations (Nychas and Tassou, 1997).

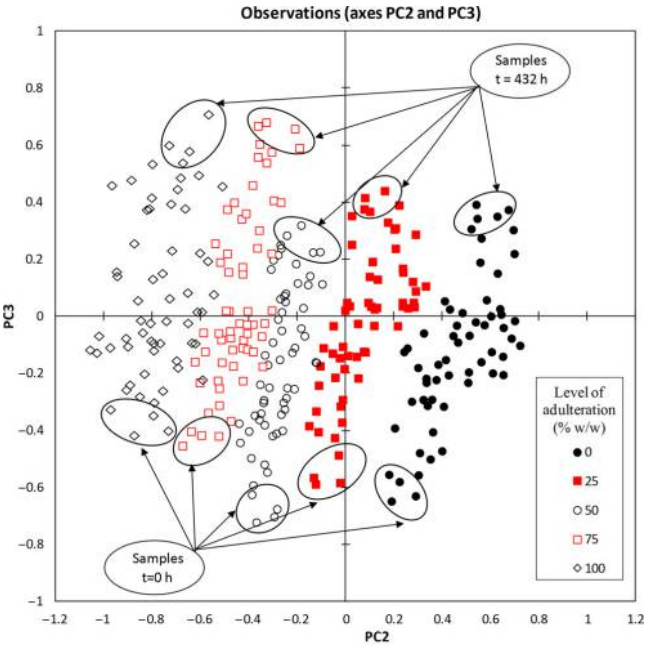
From the PCA, the average communality of the wavenumbers from 2,000 to 900 cm^{-1} (275 variables) was higher than 0.45, with most of the wavenumbers having communality higher than 0.55. This range of wavenumbers were then selected for additional analyses.

A new PCA was then calculated (using the aforementioned wavenumber range), and it was determined that the variance could be explained by 29 PCs, among which the first four explain approximately 95 per cent of the total variance. The KMO measure of sampling adequacy is 0.854. The communality value of the first four principal components was higher

than 0.6 for wavenumbers from 1,138 to 1,180, 1,304 to 1,477, 1,535 to 1,556 and from 1,728 to 1,759 cm^{-1}.

Among these wavenumbers, those in the range 1,304 to 1,322 and from 1,372 to 1,403 cm^{-1} are related to biomolecules such as amides, amines and the biochemical changes during storage of the meat, as mentioned earlier by other authors (Ammor *et al.*, 2009; Ellis *et al.*, 2004; Ellis *et al.*, 2002). The wavenumbers from 1,728 to 1,759 cm^{-1} are related to the lipid constitution of each type of meat and, in our opinion, explain the separation achieved between the five different mixtures of fallow deer and goat. This is, probably, an effect of different feeding regimes (Kim *et al.*, 2017; Paengkoum *et al.*, 2013).

From the observation diagram PCs 2 and 3, represented in Figure 1, is evident the separation of the samples according to the formulation and, within each formulation, it is clearly seen the effect of the storage time. From this diagram, it may be concluded that it is possible to distinguish the level of adulteration, disregarding the storage time of the meat. It must be observed that while the samples corresponding to the five formulations are distributed along the PC 2 axis, the PC 3 coordinate is closely correlated to the storage time for each formulation.



Notes: Cluster of samples corresponding to the five formulations are easily distinguishable. Moreover, fresh (t = 0) and spoiled (t = 432h) samples are located in opposite sides of the diagram. This preliminary result indicates that infrared spectroscopy can identify different level of fallow deer adulteration, even in the case of samples stored for long periods. PCs 2 and 3 – principal components of the principal components analysis – PCA

Figure 1. Observations diagram from the PCA of the spectral data for the five formulations of fallow deer adulterated with goat: 0, 25, 50, 75 and 100 %w/w of goat

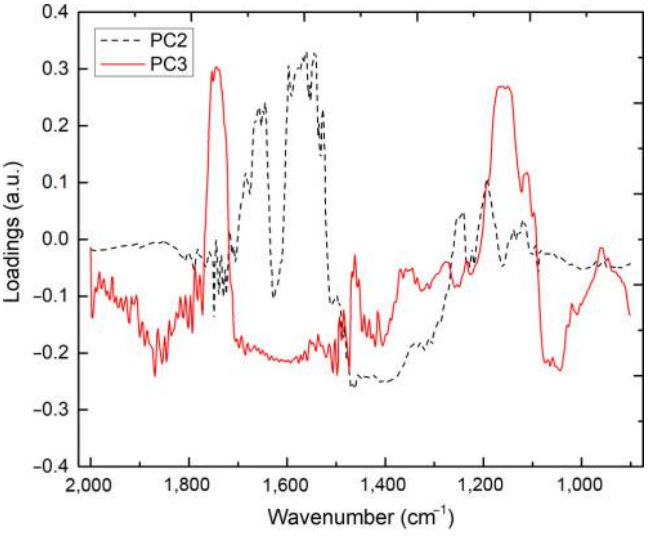
The loading plots of the PCs 2 and 3 are represented in Figure 2 whose interpretation is not straightforward, but a few correspondences to important functional groups can be pointed out.

Analysis of the PC 2 loading plot reveals that the major features are mostly related to the lipid content of the samples. Namely, besides the bands at 1,312 and 1,400 cm^{-1}, related to Amide II and, from 1,030 to 1,180 cm^{-1}, related to amines, the bands at 1,174, 1,236 and 1,476 cm^{-1} and, most important, the band at 1,745 cm^{-1} are because of lipid content.

The loading plot for PC 3 are related not only to the content and structure of protein, but also to carbohydrates and lipids. It may be seen a band at 1,460 cm^{-1} related to lipids, a band at 1,194 cm^{-1} because of carbohydrates, but the most important bands are at 1,640 cm^{-1}, because of Amide I and, at 1,546 cm^{-1}, related to Amide II.

As indicated above, component PCs 2, mostly related to the lipid content, accounts for the separation obtained between the two types of meat. It is well-known that animal feed plays a role in the characteristics of the meat. For instance, dietary plant extract affects lipid oxidation levels of pork meat (Rossi *et al.*, 2013). As stated above, it is reasonable to assume that the fallow deer, feeding from the wild, may possess fat tissues with characteristics that act as a fingerprint.

On the other hand, component PCs 3, correlated with the storage time, possesses loadings features related to biochemical changes in the meat because of a combination of autolytic and microbiological proteolysis of meat muscle proteins. In fact, an increase in absorption at certain wavenumbers was reported, suggesting the production of free amino acids and



Notes: Major features of PC 2 are related to the lipid content of the samples and biogenic amines and PC 3 is mostly related to the protein content, carbohydrates and lipids. The lipid content, accounts for the differences between the five formulations. It is reasonable to assume that fallow deer, feeding from the wild, has fat tissues that are a fingerprint. PCs 2 and 3 – principal components of the principal components analysis – PCA

Figure 2.
Loading plots calculated from the PCA of the spectral data for the five formulations of fallow deer adulterated with goat: 0, 25, 50, 75 and 100 % w/w of goat

peptides (Alexandrakis *et al.*, 2012). Hydrolysis of proteins points to the production of metabolites related to spoilage such as ammonia and volatile amines (Ammor *et al.*, 2009).

3.4 Partial least square discriminant analysis

After selecting the wavenumbers that provide more information for the discrimination, the next step was to build a classification model using PLS-DA to evaluate the possibility of predicting the level of adulteration. The wavenumbers between 2,000 and 900 cm^{-1} were used as X variables, while the Y variables were associated with three different levels of Adulteration A1, A2 and A3. Each Y variable corresponds to a different level of adulteration, with the value 1 or 0 depending on whether or not it belongs to a certain level of adulteration.

A subset of 225 samples, the training set, was used to calculate the RMSEC and the RMSECV parameters of the PLS-DA model and are shown in Table II. High correlation factors and low RMSE values were obtained: RMSEC between 5.1 and 9.2 and RMSCV between 4.2 and 10.3. Figure 3 represents the scores plot of the PLS-DA, where the three levels of Adulteration A1, A2 and A3 are clearly discriminated.

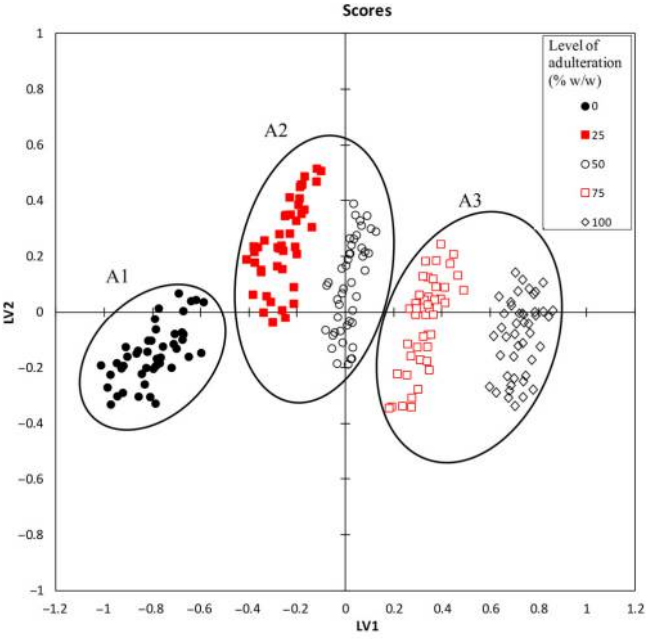
For a second subset of 75 samples, the external set, (of which 15 belong to A1, 30 belong to A2 and 30 belong to A3), reasonable values of R^2 (between 0.94 and 0.98) and RMSEP between 8.6 and 12.5, were obtained. Results of the application of the model to the prediction of the level of adulteration for those samples is presented in Table III. Predicted level of adulteration variable (Apred) near 1 means belonging to the corresponding class, and on the other hand, a value near 0 means not belonging to the that class. Therefore, very accurate classification was achieved because the predicted values were always very close to 1 for classification within the class, and values were near to 0 for classification outside the class. Therefore, it was confirmed the quality of the predictive ability of the PLS-DA classification model, presenting a goodness-of-fit value of $R^2Y = 0.62$ and a goodness-of-prediction value of $Q^2 = 0.51$ [$Q^2 > 0.5$ is generally considered good, (Sanz-Cortés *et al.*, 2013)] and p-value = 0.0027. We may be say that the present predictive model is capable of classifying real samples with 100 per cent accuracy.

In principle, the difference of lipids and proteins in the two types of meat was the key to the discriminate fallow deer meat from goat meat by spectroscopy and chemometrics. In general, the greater the difference in meat composition, the more accurate can be achieved by infrared spectroscopy. When using infrared spectroscopy to detect different kinds of meat, such as cattle, llama and horse (Mamani-Linares *et al.*, 2012), pork and beef (Kuswandi *et al.*, 2015), pork and lamb (Kamruzzaman *et al.*, 2013), the accuracy of meat classification

	R^{2b}			Error (%w/w of goat)		
Classes[a]	Calibration	Validation	Prediction	Calibration	Validation	Prediction
A1	0.99	0.99	0.98	5.1	7.2	8.6
A2	0.97	0.95	0.94	9.2	10.3	12.5
A3	0.99	0.98	0.97	7.4	9.4	9.5

Notes: Parameters of the partial least squares discriminant analysis (PLS-DA); [a]Classes of adulteration: A1 (pure meat), A2(adulterated) and A3(highly adulterated); and [b]regression coefficients, all with $p < 0.05$. Two set of samples were used: a "traning set" and an "external set" (samples not used to build the predictive model). The expected error when measuring real adulterated samples using the spectroscopic method is below approximately 12 %w/w of goat. Previous development of models ("Calibration") and internal verification ("Validation") use the training set. High regression coefficients (>0.9), with low p-value, and low error means high accuracy and predictive quality of the models developed

Table II. Quality of the predictive model. The effective (in real world) predictive ability ("prediction") of the model was tested using the external set



Notes: Groups of samples corresponding to three levels of Adulteration, A1 (pure meat), A2 (adulterated) and A3 (highly adulterated) are positively noticeable. Furthermore, pure fallow deer samples are detached from any adulterated sample, even in the case of samples stored for long periods. This plot indicates that infrared spectroscopy can identify pure fallow deer from adulterated meat. LVs 1 and 2 – latent variables of the partial least squares discriminant analysis, PLS-DA. Goodness-of-fit $R^2Y = 0.62$; goodness-of-prediction $Q^2 = 0.51$; p-value = 0.0027

Figure 3.
Scores plot of PLS-DA predictive model using the spectroscopic data

was very high. However, for similar breeds of meat, such as white pigs and Iberian pigs (Guillén *et al.*, 2010), adult steers and young cattle (Prieto *et al.*, 2008), Duroc and Iberian pork (Del Moral *et al.*, 2009), the accuracy declined and the meat samples were easily confused. It is conceivable that when the variety approaches, the chemical composition of the meat itself is more similar, reflecting the smaller difference in the spectrum.

The wild fallow deer is a ruminant mammal belonging to the family Cervidae. The domestic goat is a subspecies of goat domesticated from the wild goat of southwest Asia and Eastern Europe and is a member of the family Bovidae. They belong to different families; thus, it is expected that the discrimination would be very complex.

The results presented in this paper showed that wild fallow deer meat can be reliably identified using the mid infrared spectroscopy combined with the PLS-DA method. However, the model is only applicable to the distinction of these two types of meat. To identify more types of meat, we need to increase the number of species and of samples. The model will be more adaptable and robust if the calibration set is extended with more samples, of different breeds and areas (Mamani-Linares *et al.*, 2012).

Classes[a]	A1		A2		A3	
	Aref[b]	Apred[c] (std)[d]	Aref[b]	Apred[c] (std)[d]	Aref[b]	Apred[c] (std)[d]
A1	1	1.010 (0.009)	0	−0.077 (0.016)	0	0.007 (0.004)
A2	0	0.008 (0.001)	1	0.953 (0.102)	0	0.220 (0.060)
A3	0	−0.008 (0.001)	0	0.197 (0.049)	1	1.096 (0.125)

Notes: Partial least squares discriminant analysis (PLS-DA); [a]Classes of adulteration: A1 (pure meat – 15 samples), A2 (adulterated – 30 samples) and A3 (highly adulterated – 30 samples); [b]Aref – actual classification (0/1);[c] Apred – average predicted classification for each class; and [d]std – standard deviation of predicted classification for each class. Goodness-of-fit R^2Y = 0.62; goodness-of-prediction Q^2 = 0.51; p-value = 0.0027. A very accurate predictive model classify a sample blonging to a certain with a value Apred = 1 (means belong to the class) or Apred = 0 (means not belong to the class). Application of the predictive model to the external data set (samples not used in development of predictive models) led to average Aprev values near 1 for classification within the classe, and average values near 0 for classification outside the classes; in both cases, the standard deviation of predicted classification is very small

Table III. Results of the application of the model. The present predictive model is capable of classifying real samples with high accuracy

4. Conclusions

A reliable and low-cost method based on the combination of mid infrared spectroscopy with the PLS-DA technique has demonstrated great potential in the discrimination and in the classification of fallow deer meat adulterated with goat. Using this methodology, the two types of meat can be easily distinguished with high accuracy.

The PCA analysis led to the conclusion that the most important absorption bands for discriminating the adulteration level of the fallow deer are contained in the wavelength range from 2,000 to 900 cm^{-1}, encompassing specific bands normally associated to lipid and protein compounds.

The process of classification using the PLS-DA allowed discrimination of samples in three different levels of Adulteration A1 (pure meat), A2 (adulterated) and A3 (highly adulterated) was successfully carried out using fresh samples and stored for different periods of time and at diverse stages of the deterioration process.

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