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Short communication

A novel method for discrimination of beef and horsemeat using Raman spectroscopy

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ABSTRACT

A new approach, based on the usage of Raman spectroscopy in combination with chemometrics, was developed for the rapid determination of beef adulteration with horsemeat. The data mining process of collected Raman spectra was performed with principal component analysis (PCA). Pure fat samples, extracted from forty-nine meat beef and horsemeat samples, were analysed using the Raman spectroscopy. All meat samples were classified successfully according to their origins. The presence of different concentrations (25%, 50%, 75%, w/w) of horsemeat in beef was also differentiated using the developed model system. This study offers a rapid assay for determination of meat adulteration by discriminating beef and horsemeat with high accuracy, a short analysis time (30 s) and no requirement for time-consuming sample preparation procedures.

1. Introduction

The ongoing meat adulteration scandal in Europe brings into question the usage of horsemeat as a new adulterant in the meat industry. The determination of horse DNA in frozen beef burgers on 15 January 2013 drew attention to the issue of meat adulteration (FSA, 2013). Horsemeat is alternatively used instead of beef due to lower the cost of raising. However in most cases, horses are being slaughtered at the end of their working lives when most of the essential compounds are depleted in their meats which have no desirable organoleptic or nutritional properties (Martuzzi, Catalano, & Sussi, 2001). While horsemeat is consumed as a healthy choice of red meat in many countries, there are many countries around the world in which horsemeat is considered a forbidden food due to ethical and religious reasons.

Proper implementation of food labelling has had ever-mounting importance in recent years. Identification of meat authenticity has a place in this context, as it constitutes a significant part of a healthy human diet. The potential for large financial profits and simplicity in substituting meats as a result of their close morphological and textural similarities has exposed the industry to a wide range of adulterations with poor quality and cheaper meat alternatives (Dean, Murphy, & Downey, 2006).

Protein-based (Alamprese, Casale, Sinelli, Lanteri, & Casiraghi, 2013; Mamani-Linares, Gallo, & Alomar, 2012), DNA-based (Ali et al., 2012; Haider, Nabulsi, & Al-Safadi, 2012; Mane, Mendiratta, & Tiwari, 2012; Sakaridis, Ganopoulos, Argiriou, & Tsaftaris, 2013; Soares, Amaral, Oliveira, & Mafra, 2013; Zhang, 2013) and fat-based methods (Abbas, Fernández Pierna, Codony, von Holst, & Baeten, 2009; Rohman, Sismindari Erwanto, & Che Man, 2011) have been intensively used for the detection of meat origin. DNA- and protein-based methods such as polymerase chain reaction (PCR) and enzyme linked immunosorbent assay (ELISA) are the most specific and sensitive techniques, although the requirement for expensive equipment and technical expertise restricts the usage of these techniques; in contrast, chromatographic methods generally suffer from a low rate of reproducibility. Fat-based methods, on the other hand, offer a simpler approach and mostly employ spectroscopic techniques. Fourier-transform infrared spectroscopy and Raman spectroscopy have been come into use for this purpose in recent decade (Che Man & Mirghani, 2001; Jaswir, Mirghani, Hassan, & Said, 2003; Rohman & Che Man, 2010). The main advantages of this technique are the ability to supply information about the chemical structure of molecules without causing any alterations (Marquardt & Wold, 2004), and requiring only a small amount of the sample (Wong, Choi, Phillips, & Ma, 2009).

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Meanwhile, Raman spectroscopy has high potency for the evaluation of food quality systems during handling, processing and storage (Herrero, 2008). The combination of Raman spectroscopy with chemometric data analysis methods enables researchers to determine food adulteration in a more rapid way (Boyaci, Genis, Guven, Tamer, & Alper, 2012; Özbalci, Boyaci, Topcu, Kadılar, & Tamer, 2013).

The above mentioned situation in the meat industry obliges scientists to find simple and rapid alternatives to presently available techniques for the discrimination of beef and horsemeat. Raman spectroscopy was introduced in the present study as a new approach since there is no study available, to our knowledge, focusing on the discrimination of horsemeat, which has been fraudulently substituted or combined with beef. To fulfill this purpose, the Raman spectra of various fat samples, which were extracted from different parts of horse and beef carcasses, were collected and the data were evaluated by PCA. Successful discrimination of beef and horsemeat was established and validation studies were carried out to support the reliability of the developed model. Success of the developed method was also investigated using beef samples adulterated with horsemeat in 0%, 25%, 50%, 75% and 100% by weight.

2. Materials and methods

2.1. Chemicals

Analytical grade *n*-hexane was purchased from Riedel–de Haen (Seelze, Germany) and glass beads (1 mm in diameter) were provided by Marienfeld-Superior Co. (Lauda-Königshofen, Germany). Nitrogen gas was supplied by a local company.

2.2. Sample preparation

Meat samples were drawn out from beef and horse carcasses. Beef samples were provided from local supermarkets in Turkey while horsemeat samples were imported from local markets in Kazakhstan. To obviate the differences originating from the meat

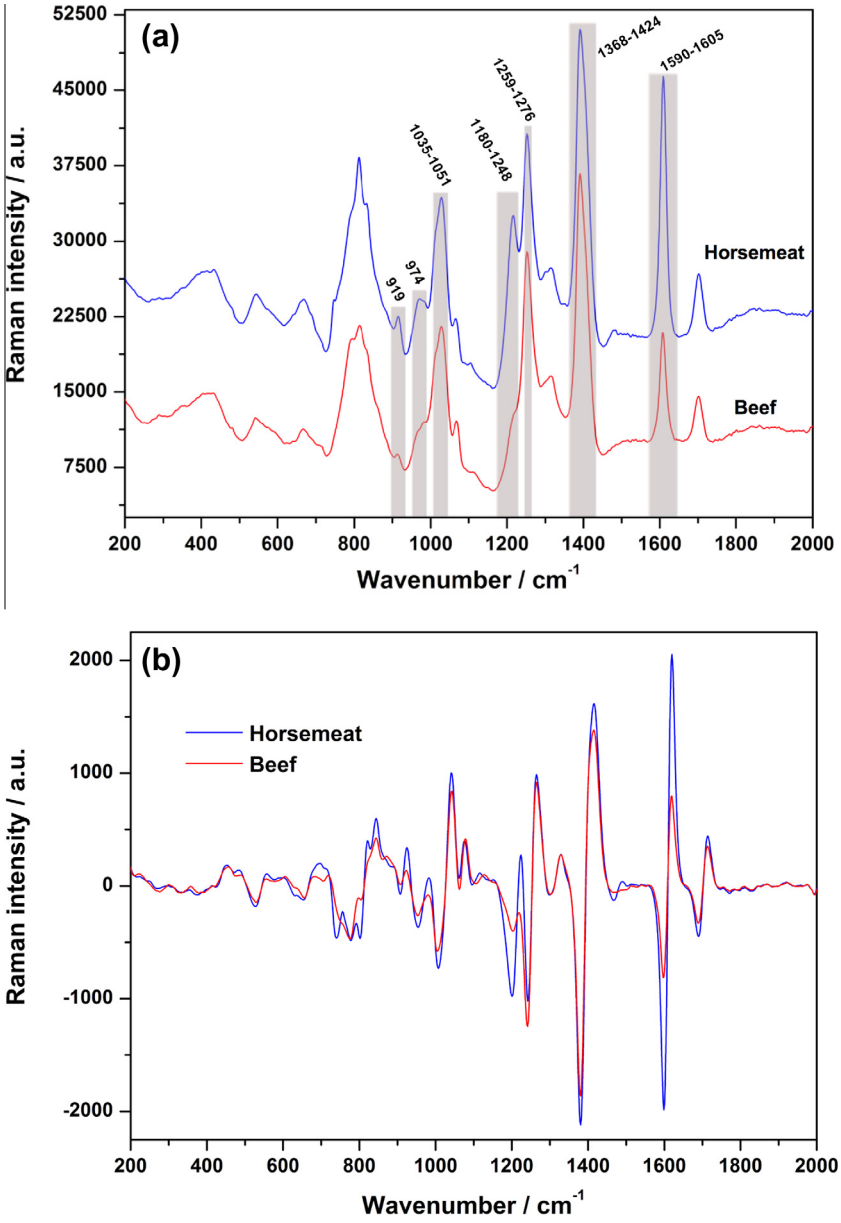

Fig. 1. Original (a) and first derivative (b) Raman spectra of horsemeat and beef samples.

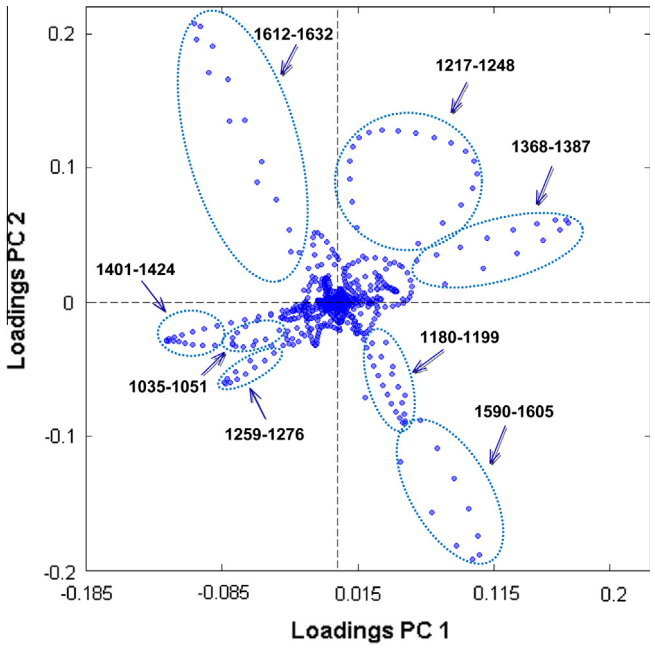

Fig. 2. PCA loadings plot of Raman spectra of horsemeat and beef samples.

samples, they were provided from different body parts of each animal such as the back, abdomen, leg, kidney and other organ-surrounding fats. Performance of the developed model was tested using beef samples adulterated with horsemeat in different ratios. Horsemeat is mostly used in high ratios to provide intended profit growth. Reported meat adulteration cases are also supporting this consideration as 40–100% horsemeat was used in most of these cases (FSA, 2013). In these premises, beef samples containing horsemeat 0%, 25%, 50%, 75% and 100% by weight were investigated in the scope of our study.

2.3. Fat extraction

Sufficient amounts of each meat sample were put into blender in order to homogenise the sample. In samples with a hard texture, a sufficient amount of hexane (2–3 ml) was added for a more effective blending. Subsequently, the blender was carefully washed with boiling water and detergent. In order to avoid errors originating from fat contamination, the blender was rewashed with distilled water and completely dried prior to the next blending. Fifty grams of each homogenised sample were placed in a mortar and 50 ml of hexane were added. Glass beads were added to the mortar for proper processing and extraction. The fat-hexane mixtures were centrifuged at 7500 rpm (4779 g) for 5 min to remove impurities originating from the meat sample. The centrifuged mixture was passed through filter paper for the complete separation of impurities. Afterwards, the sample was placed in a water bath at a temperature of 70 °C and purged with N_2 gas to facilitate the evaporation of hexane from the filtrate. The pure fat samples were stored in glass vials at 4 °C in the refrigerator.

2.4. Instruments and data collection

Raman spectra were obtained using a DeltaNu Examiner Raman Microscopy system (DeltaNu Inc., Laramie, WY, USA) with a 785 nm laser source and a cooled charge-coupled device (CCD at 0 °C) detector. The sample was put into a water bath for 1 h and heated to 70 °C, then the sample was immediately placed in the Raman spectroscopy system. Spectra were obtained in the range of 200–2000 cm^{-1} at a resolution of 2 cm^{-1}. Constant measurement parameters were as follows: integration time of 30 s and 100 mW laser power. Measurements were conducted in duplicate for each sample. Stand-alone Chemometrics Software (Version Solo 6.5 for Windows 7, Eigenvector Research Inc., Wenatchee, WA, USA) was used for the PCA analysis.

2.5. Data processing and chemometric analysis

In order to facilitate the analysis of the large number of spectra obtained from the samples, a new application combining chemometric methods with Raman spectroscopy was developed. In this study, a PCA model was developed using the collected Raman data.

3. Results and discussion

Meat samples from various parts of the body were obtained from horse and beef carcasses. An extraction procedure was applied to each sample to obtain pure animal fat and the Raman

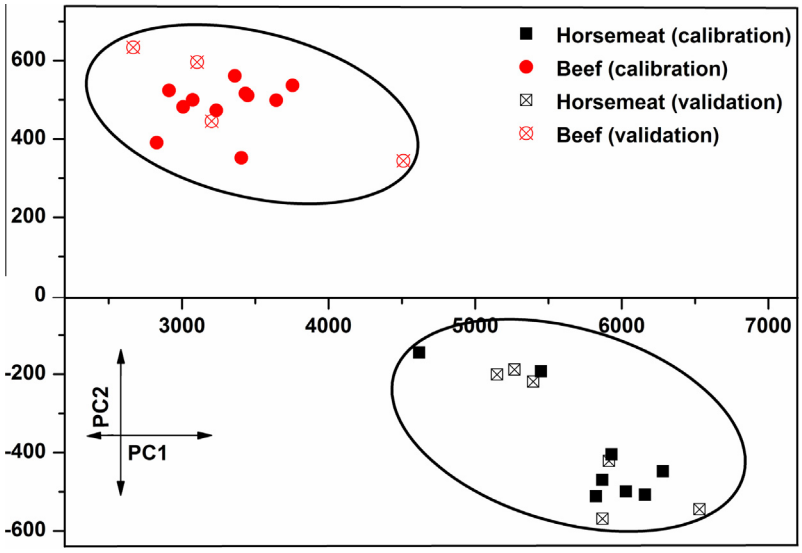

Fig. 3. PCA score plot for Raman spectra of horsemeat and beef samples.

spectra of the samples were collected. The obtained data were analysed with the developed PCA method. In order to separate animal samples more clearly, the score results of PCA analysis were used.

3.1. Raman spectra of the samples

The Raman spectra of meat samples were collected between 200 and 2000 cm^{-1}, as shown in Fig. 1a. The spectra of the horse and beef samples seemed to be similar; however, there were spectral differences due to intensity differences in the same band intensity and some unique bands belonging to horse fat. As shown in Fig. 1a, the following Raman bands were observed in both horse and beef samples: 555, 678, 815, 1032, 1265, 1392, 1611 and 1706 cm^{-1}. The spectral difference between samples of horse and beef arose from the unique bands of horse fat that were positioned at 919, 974 and 1215 cm^{-1}. The strong band positioned at 1265 cm^{-1} belongs to δ(C–H) bending at the cis double bond in R–HC=CH–R (El-Abassy, Eravuchira, Donfack, von der Kammer, & Materny, 2011). The bands (815 and 1032 cm^{-1}) in the range of 800–900 and 1000–1100 cm^{-1} are due to the vibration of skeletal C–C bonds in $-(CH_2)_n-$ molecules (Baeten, Hourant, Morales, & Aparicio, 1998). One of the bands distinguishing horse and beef at 974 cm^{-1} belongs to =C–H out-of-plane deformation (Afseth, Wold, & Segtnan, 2006). In addition, the distinctive band between horse and beef samples at 1213 cm^{-1} is due to anti-symmetric phosphoryl stretching corresponding to the vibrations of phospholipids and fatty acid chains (Gallier, Gordon, Jiménez-Flores, & Everett, 2011).

3.2. Data analysis

PCA was employed as the data processing method in the present study. To distinguish pure horse and beef samples from each other, PCA was performed on the entire spectrum between 200 and 2000 cm^{-1}. Creating calibration and validation steps required two-step analysis. There were a total of forty-nine meat samples including eighteen horse and thirty-one beef. The calibration data set, which was used to create a PCA model, was formed using thirty-nine samples, composed of fourteen horse and twenty-five beef samples. Before the model was created, different preprocessing techniques (different derivative orders, baseline correction, mean center, normalising, smoothing and auto-scaling) were employed to enhance the performance and the decomposition ability of the model. Among these techniques, the best performance was obtained by the application of the first derivative. The first-derivative Raman spectra of the samples are shown in Fig. 1b. As a result of taking the first derivative transformation of the spectra, the baseline shift was removed, the overlapped bands were resolved and the spectral differences were enhanced; taking the first derivative of the spectrum was a prerequisite to obtain a model that could be transported to the next stage of the analysis. In addition, PCA loadings plot is shown in Fig. 2. The loadings indicated that a cluster of eight band ranges explained the most of the variance. As can be seen in Fig. 1a, these band ranges coincide with 1035–1051, 1180–1199, 1217–1248, 1259–1276, 1368–1387, 1401–1424, 1590–1605, and 1612–1632 cm^{-1} in Raman spectra. Then, the model was created and scores of the principal components were used to differentiate the horse and beef samples positioned in the different regions of the score plot. The first two principal components, which had the highest cumulative variance, were chosen to draw a score plot. Principal component 1 explained 96.32% of the total variance and principal component 2 explained 3.20% of the total variance. The PCA score plot of the calibration and validation data is shown in Fig. 3. The horse and beef samples were distinguished so clearly that the samples of horsemeat are positioned on the upper side and the beef samples appear on the lower side of the graph (Fig. 3). Clusters of each meat species were obtained by means of this calibration score graph. After the development of the calibration model, a validation step was performed to evaluate the performance and success of the model using a validation data set composed of four horsemeat and six beef samples. The scores of the validation data were obtained and added to the score plot. As shown in Fig. 3, the validation scores (horsemeat and beef) were positioned in their own clusters in the plot.

Performance of the developed model was tested using beef samples adulterated with horsemeat in 0%, 25%, 50%, 75% and 100% by weight. PCA was used to draw a score plot showing the clustering of the samples. Mean centering and taking the first derivative of the spectra, was applied simultaneously, as preprocessing techniques. Pure beef and horsemeat samples were positioned in the two edges of the plot while the samples prepared as binary mixtures (50% horsemeat + 50% beef) were in the middle of these to edges. On the other hand, samples containing 75% horsemeat were closer to the cluster of pure horsemeat samples and samples containing 75% beef were closer to the cluster of pure beef samples (Fig. 4). Based on these results, it is possible to say

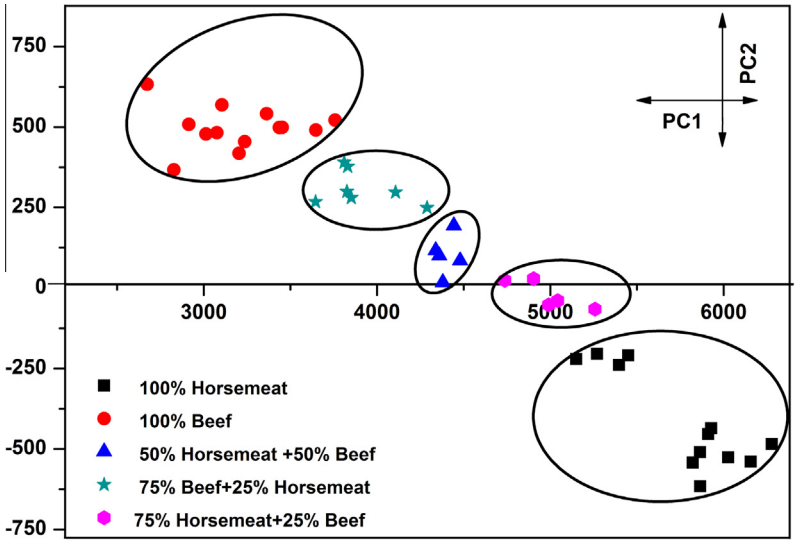

Fig. 4. PCA score plot for Raman spectra of beef samples adulterated with horsemeat.

that the developed Raman method is capable to determine 25% of horsemeat in beef, successfully.

4. Conclusions

Discrimination of beef and horsemeat samples was accomplished in the present study. A novel fat-based method was developed which uses Raman spectroscopy in combination with principal component analysis. Performance of the developed model system was good enough to differentiate beef samples adulterated with horsemeat in 0%, 25%, 50%, 75% and 100% by weight. Advantages such as the short analysis time and straightforward sample preparation make this system a promising technology for the determination of meat authenticity.

The present study has the features of a pioneer work for the determination the origin of unprocessed horsemeat and beef samples by Raman spectroscopy. Further studies are being carried out for development of the system to increase the sensitivity and usability for processed food products.

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