ULST Timisoara
Multidisciplinary Conference on Sustainable Development
30-31 May 2024

Preliminary results on meat authenticity testing by ATR-FTIR combined with chemometrics.

Sofia Kapageridou[1], Lamprini Dimitriou[2], Michalis Koureas[3], Athanasios Manouras[4], Eleni Malissiova[2]

[1] *Postgraduate Study Programme* Biotechnology-Quality Assessment in Nutrition and the Environment, *Department of Biochemistry And Biotechnology, University of Thessaly, Biopolis, 41500, Larisa, Greece*

[2] *Food of Animal Laboratory, Animal Science Department, University of Thessaly, Gaiopolis Campus, 41500 Larisa, Greece*

[3] *Laboratory of Hygiene and Epidemiology, Faculty of Medicine, University of Thessaly, 41222 Larissa, Greece*

[4] *Department of Nutrition and Dietetics, University of Thessaly, 42132 Trikala, Greece*

Sofia Kapageridou -sofikapa86@gmail.com Lamprini Dimitriou- ldimitriou@uth.gr Michalis Koureas - mkoureas@uth.gr Athanasios Manouras - amanouras@uth.gr *Eleni Malissiova - malissiova@uth.gr*

Abstract

Meat authenticity is a main concern for both consumers and authorities, leading to the need for quick, accurate and low cost techniques for fraud detection. The aim of this study was to investigate the utilization, of Attenuated Total Reflection-Fourier transform Infrared spectroscopy (ATR-FTIR) in combination with chemometrics in meat authenticity tests (species level), as well as to evaluate the type of samples (raw or freeze-dried) leading to higher discrimination rates. Ninety-two meat samples (sheep, goat and beef) were analyzed, in raw and freeze-dried form, by ATR-FTIR. Principal component analysis (PCA) and Partial least squares-discriminant analysis (PLS-DA) were applied. The results of this study indicated that PCA failed to distinguish the samples for any form and type of sample, whether PLS-DA revealed successful samples clustering. More specifically, the identification of pork was better for raw samples with sensitivity, specificity, and accuracy values of 100% and for beef for freeze-dried samples with a sensitivity rate of 0.90 and specificity of 0.64. These preliminary results indicate that ATR-FTIR combined with chemometrics may act as a valuable tool for quick, accurate and operational low cost analysis in detecting meat fraud in raw or freeze-dried samples.

Keywords: Authenticity, meat, adulteration, analytical techniques, spectroscopy techniques.

• Introduction

Meat is an essential food product, due to its high nutritional value and at the same time is very palatable [1]. Food and Agricultural Organization, the United Nations reported that meat consumption has significantly increased, globally over time and consequently the growth of the meat industry have exacerbated the frequency of adulteration in the meat chain ((FAO. FAOSTAT, 2021). So, meat authenticity is a global issue which concern consumers, food industries, researchers as well as regulators because is related to religious (e.g., halal), public health (e.g., allergies) and economic reasons and the detection of animal species in the meat supply chain is imperative [2]. This increased interest in meat authenticity may also be explained by the numerous food scandals over the last few years. Typical food scandals: in 2013 the case of adulteration of ground beef with horse meat and in 2008, Icelandic pork products detected with dioxin levels exceeding 200 times the recommended limit [3].

Meat authenticity can be assessed by several different analytical techniques such as molecular, isotopic chromatographic or a combination of all these. However, most of them are expensive, laborious, destructive and need complicated laboratory procedures [4,5]. Spectroscopic techniques have gained the interesting during the last few years because they are extremely fast methods, with high sensitivity, low operating cost and non-destructive for the product [6,7].

The aim of this study is to investigate the utilization of Attenuated Total Reflection-Fourier transform Infrared spectroscopy (ATR-FTIR) in combination with chemometrics in meat authenticity tests (species level), as well as to evaluate the type of samples (raw or freeze-dried) leading to higher discrimination rates.

• Material and method

- **Sample collection.**
  Ninety two raw meat samples (approximately 100g) ware collected from different slaughter houses and market in Thessaly, Greece (33 sheep, 39 beef, 20 pork). All samples ware stored at -18^{0}C, until analysis was performed.
- **Sample preparation**.
  Visible skin, fat and connective tissue were excised as they could interfere in the analysis. Then samples were divided into two equal parts in smaller tubes. The first group of samples used as raw meat for analysis and the second group was freeze- dried (Christ Alpha 1-2 1Dplus) for 24 hours.
- **FTIR spectra measurement.**
  Fourier Transform Infrared Spectroscopy (FTIR Nicolet 6700) using detector DTGS (deuterated triglycine salfate) was connected to software OMNIC. The meat samples were directly placed into multibounce attenuated total reflectance (ART) crystal and scanned using resolution of 4 cm^{-1} and number of scanning 32 at room temperature. The surface of the ATR interface was cleaned with ethanol and dried before the next sample. All spectra were measured at mid infrared region (4000-650 cm $^{-1}$) using air as background.
- **Data analysis.**
  FTIR data were visually inspected and further examined using principal component analysis (PCA) and partial least squares (PLS) analysis. To evaluate the power of discrimination classification models for each species, classification figures including sensitivity (Sen), specificity (Spe) and accuracy (Acc) were used and were derived from a confusion matrix.

• Conclusions

Regarding the increasing meat fraud during these years and the demand for screening methods for meat authenticity, in this study, the application of ATR-FTIR spectroscopy combined with PLS-DA chemometric method was assessed for discrimination of pork, beef and sheep meat. ART-FTIR discriminant modeling gave the highest classification rates for the pork raw meat with values 100%. Crucial for the performance of this screening method is the choice of sample form (raw or freeze-dried). Raw meat samples showed better performance rather than freeze-dried following by PLS-D analysis. Further research can be done by expanding sample, as to assess the clustering of meat originating from different geographical areas, or from different farming methods (organic-conventional).

• References

1. Pereira CC, Vicente AF (2013) Meat nutritional composition and nutritive role in the human diet. Meat Science 93: 586-592.
2. M.Á. Sentandreu, E. Sentandreu (2014) : Authenticity of meat products: Tools against fraud.Food Res. Int., 60 (2014), pp. 19-29
3. Smith R, Manning L, McElwee G (2022) The anatomy of 'So-called Food-Fraud Scandals' in the UK 1970-2018: Developing a contextualised understanding. Crime Law Soc Change 78: 535-558.
4. Li T, Wang J, Qiao L, et al. (2021) Quantitative determination of mutton adulteration with single-copy nuclear genes by real-time PCR, Food Chem 344: 128622.
5. Vlachos, A., Arvanitoyannis, I. S. & Tserkezou, P. (2016). An Updated Review of Meat Authenticity Methods and Applications. Critical Reviews in Food Science and Nutri-tion,56(7),1061-1096.
6. Candoğan, K., Altuntas, E.G. & Iğci, N. Authentication and Quality Assessment of Meat Products by Fourier-Transform Infrared (FTIR) Spectroscopy. Food Eng Rev 13, 66–91 (2021)
7. Hassoun, A.; Heia, K.; Lindberg, S.-K.; Nilsen, H. Spectroscopic Techniques for Moni-toring Thermal Treatments in Fish and Other Seafood: A Review of Recent Develop-ments and Applications. Foods 2020, 9, 767.

• Results and discussion

FTIR spectra plot of three raw meat species are shown in Fig 1, A. The mean spectra of the classes show visual differences in 2800-3000 cm^{-1} which is related to the C-H stretching and is associated with lipids. In the bands 3000-4000 cm^{-1} which is related to the stretching vibration of O-H and N-H bonds associated with protein amino acids were not observed visible differences due to the presence of excess water. However, upon removal of water on the freeze-dried samples the N-H stretching vibration is visible (Fig 1, B).

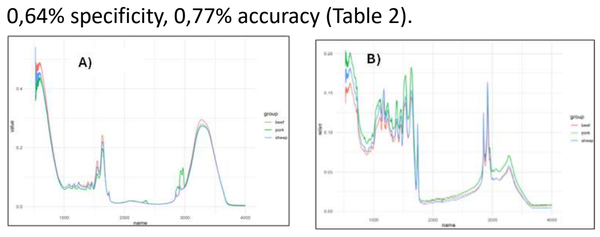
PCA analysis was used to explore and visualize the variability of the spectra and to assess if the three different meat samples are classified into clusters. In Figure 2.A, the scores plot for three raw meat samples are presented. There are not clear clusters classified between pork, beef, and sheep meat on the scores of the two main components PC 1 and PC 2 which explain 79,3% of the volatility. Improved clustering was observed in the PCA plot when using part of the spectrum (600-1800 cm^{-1}) without observing clear classification between three meat samples (Fig 2.B). Similar results were obtained from the freeze-dried samples analysis (Figure 2.C-2. D). According to the PCA analysis the dominant variations are not related with the meat species. However, supervised discriminant analysis methods like PLS-DA may be able to classify the three different meat samples to categories. The FTIR spectral data subjected to supervised discriminant analysis PLS to create a discrimination and classification model for the three meat samples.

Results of raw meat samples.

The train data included sixty-five samples (28 beef, 14 pork and 23 sheep). The predictive classification models were validated using thirteen latent variables. The performance of the PLS-DA model evaluated in the control set where actual values compared with predicted. The test data contained twenty-six samples, (6 samples were pork, 11 beef and 9 sheep). For the total samples, the accuracy of the model was 0,92. 95% CI, (0,7487, 0,9905). The results in spectra showed that the pork can be easily distinguished from the beef and sheep by the greatest total of PLS-DA accuracy values 100% (Table 1).

Results of freeze-dried meat samples.

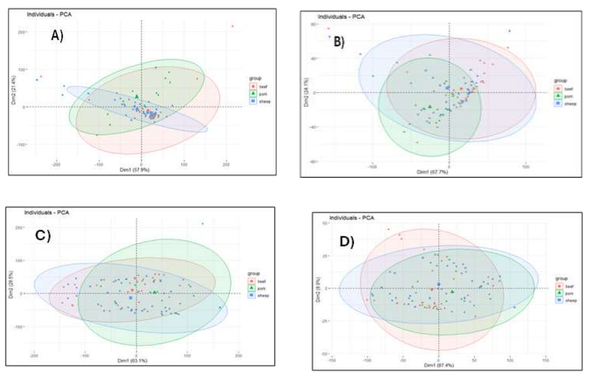
The same analysis was implemented for the freeze-dried meat samples. The predictive classification models were validated using fourteen latent variables. The performance of the PLS-DA model evaluated in the control set where actual values compared with the predicted. The test data contained twenty-five samples, (5 samples were pork, 11 beef and 9 sheep). For the total samples, the accuracy of the model was 0.72, 95% CI: (0.5061, 0.8793). The results in spectra showed that the beef can be easily distinguished from the pork and sheep by the greatest total of PLS-DA values 0,90% sensitivity, 0,64% specificity, 0,77% accuracy (Table 2).



Finger 1: A) Spectra plot of raw meat samples, B) Spectra plot of freeze-dry meat samples.

Table.1: Resolution assessment of the PLS-DA model of raw samples

Class	Sensitivity	Specificity	Accuracy
Beef	0,91	0,93	0,92
Pork	1	1	1
Sheep	0,89	0,94	0,91



Finger 2: A) PCA plot of raw meat samples, B) PCA plot of raw meat samples (spectrum 600-1800 cm 1), C) PCA plot of freeze-dry meat samples, D) PCA plot of freeze-dry samples (spectrum 600-1800 cm 1).

Table.2: Resolution assessment of the PLS-DA model of freeze-dry samples

Class	Sensitivity	Specificity	Accurency
Beef	0,9	0,64	0,77
Pork	0,2	1	0,6
Sheep	0,77	0,87	0,82

Acknowledgement: we extend thanks to the staff of the Food of Animal Origin and the Lab of Food Chemistry, Biochemistry and Technology at University of Thessaly, for their kind support in the experimental procedure.