Fast detection and quantification of pork meat in other meats by reflectance FT-NIR spectroscopy and multivariate analysis

Fazal Mabood[a,*] mehboob@unizwa.edu.om, Ricard Boqué[b,*] ricard.boque@urv.cat, Abdulaziz Y. Alkindi[c], Ahmed Al-Harrasi[d,*] aharrasi@unizwa.edu.om, Issa S. Al Amri[a], Salah Boukra[a], Farah Jabeen[e], Javid Hussain[a], Ghulam Abbas[a], Zakira Naureen[a], Quazi M.I. Haq[a], Hakikulla H. Shah[a], Ajmal Khan[d], Samera K. Khalaf[c], Isam Kadim[a,*] isam@unizwa.edu.om

[a]Department of Biological Sciences & Chemistry, College of Arts and Sciences, University of Nizwa, Nizwa 616, Oman

[b]Universitat Rovira i Virgili, Department of Analytical Chemistry and Organic Chemistry, Tarragona, Spain

[c]DARIS Centre for Research and Technology Department, University of Nizwa, Oman

[d]Natural and Medical Sciences Research Center, University of Nizwa, PC 616, Nizwa, Oman

[e]Department of chemistry, University of Malakand, lower Dir KP, Pakistan

*Corresponding authors.

A~~BSTRACT~~bstract

This study aimed to develop a fast analytical method, combining near infrared reflectance spectroscopy and multivariate analysis, for detection and quantification of pork meat in other meat samples. A total of 5952 mixture samples from 39 types of meat were prepared in triplicate, with the inclusion of pork at 0%, 1%, 5%, 10%, 30%, 50%, 70%, 90% and 100%. Each sample was scanned using an FT-NIR spectrophotometer in the reflection mode. Spectra were collected in the wavenumber range from 10,000 to 4000 cm^{-1}, at a resolution of 2 cm^{-1} and a total path length of 0.5 mm. Principal Component Analysis (PCA) revealed the similarities and differences among the various types of meat samples and Partial Least-Squares Discriminant Analysis (PLS-DA) showed a good discrimination between pure and pork-spiked meat samples. A Partial Least-Squares Regression (PLSR) model was built to predict the pork meat contents in other meats, which

provided the R^2 value of 0.9774 and RMSECV value of 1.08%. Additionally, an external validation was carried out using a test set, providing a rather good prediction error, with an RMSEP value of 1.84%.

Keywords: Near infrared reflectance spectroscopy; ~~p~~Pork meat; PCA; PLSR; PLS-DA

~~1.~~1 I~~NTRODUCTION~~ntroduction

Meat is one of the most commonly consumed foods around the world. It is an essential healthy nutrient and an excellent source of complete proteins. However, in some cases, the presence of meat of one animal species in another one is considered a food fraud and it is a crucial issue in the meat industry Masiri et al. (2016). The two main origins of the presence of pork meat in other meats are: 1) economically motivated adulteration, where significant amounts of low cost product (e.g. pork) is added for economical gain, and 2) contamination, when several types of meat are processed and meat from other species gets contaminated with pork meat. This leads to issues around labelling.

Adulteration and/or contamination has been a widespread problem and the analysis of pork meat in other meat products has become an emerging challenge because the consumption of pork meat is forbidden for the people practicing the Islamic and the Jewish faiths (Ortea et al., 2012). Meats with Halal or Kosher certification are readily accepted by Muslim and Jewish consumers to whom consumption of pork and its derivatives in any product is prohibited. Hsieh and his co-workers reported that multispecies contamination was found in some meat products (Hsieh, Chen, & Sheu, 1997). Moreover, products labeled exclusively as beef are often intentionally adulterated with pork meat (Singh & Neelam, 2011; Soares, Amaral, Oliveira, & Mafra, 2013).

Labeling regulations are required for providing consumers with accurate information about the food product, and for fair competition among producers (Hsieh, 2000). Labeling regulations require that the origin of meat type must be mentioned on the label because of the consumers' personal food preferences, religious food ethics, and medical issues (Ballin, 2010). However, many processed meat products are still lacking the proper labeling for meat species, especially pork (Tanabe, Miyauchi, Muneshige, & Kazuhiro, 2007). The specification and labeling of meat products are very crucial for maintaining the national standards as well as for protecting the consumers' interests (Cai et al., 2017).

For instance, beef mixed with pork or horse meat, and pork adulterated with poultry have been detected in many meat brands in retail markets in the last few years (Ali, Hashim, Mustafa, Chen Man, & Dhabi, 2012; Djurdjevic & Hsieh, 2005; Mandli, Fatimi, Seddaoui, & Amine, 2018; Soares, Amaral, Mafra, & Oliveira, 2010; Tanabe et al., 2007). Pork meat tissues are genetically different from all other meat tissues (Trivedi et al., 2016).

Numerous analytical methods have been used for the identification and quantification of the meat species. Most of these methods are based on either protein testing or DNA analysis (Mane, Mendiratta, & Tiwari, 2012). A real-time PCR analysis was recently used, which confirmed the fallow deer meat adulteration in commercial food (Maria, Rupert, & Margit, 2018). Similarly, pork meat adulteration was detected in beef meat

using a label free electrochemical immuno-sensor (Syazana & Minhaz, 2016). A specific primer was developed to detect pork meat adulteration in different meat products, even after heating treatment (Ha et al., 2017). Electrophoretic and chromatographic methods for separation and detection of meat adulteration have also been reported. However, the requirement of expensive instruments and lengthy preparation procedures limits the application of these methods for routine analysis (Ballin, Vogensen, & Karlsson, 2009). Moreover, proteins are known to be very sensitive to heat and the thermally treated meat has a spectrum that is different from that of raw meat. Therefore, many scientists are nowadays relying on DNA analysis, rather than protein analysis for authentication of food (Bhat, Salahuddin, Mantoo, Jalal, & Pal, 2016). These methods have the advantage that can be applied to thermally treated meats in addition to raw meats. However, the drawback of these methods is that they cannot determine the percentage of adulteration, and they are very prone to contamination. With regard to spectroscopic techniques, animal proteins were reported as potential adulterants in minced beef and pork in a study using Vis-NIR spectroscopy (Ahmed & Akinbode, 2018). An FT-IR spectroscopic method was applied by Yang et al. (2018), the main drawback being the tedious sample preparation step, as they prepared meat sample powders that were then mixed with potassium bromide (KBr) to perform the measurement. Therefore, a reliable and cost-effective technique for detecting and quantifying the presence of pork meat in other meats is needed. Herein, we present a study aimed at developing a quick, low cost, method using near infrared reflectance spectroscopy and multivariate analysis for detecting and quantifying the amount of pork meat in other meats. The advantage of this method is that the sample preparation process is short and simple. All the meat samples in the mince mixture (in the solid form) were scanned with the NIR spectrophotometer and then multivariate chemometric models was built to detect and quantify the pork contents in other meats samples.

~~2.~~2 M~~ATERIALS~~aterials ~~AND~~and ~~METHODS~~methods

~~2.1.~~2.1 Meat samples spiked with pork meat preparation

In the current study, 39 different types of meat samples including beef, mutton, lamb, pork, camel, chicken and veal were collected from different local markets in Oman. As pork is not popular in Oman, some pork samples were purchased in local markets as well as in superstores in Oman and Malaysia (see Table 1.) The samples were transported in coolers containing ice packs and temperature loggers. Upon receipt, the packages were immediately transferred to a 4 °C refrigerator and stored for a maximum of 24 h until sample processing could be done, or frozen at−20 °C if processing could not be done within 24 h of receipt.

alt-text: Table 1

~~Table 1.~~Table 1

The presentation of Tables and the formatting of text in the online proof do not match the final output, though the data is the same. To preview the actual presentation, view the Proof.

Meat sample names and their corresponding codes.

Sample name	Code
Australian lamb	CAULB
Australian lamb	LAULB
Australian lamb	SAULB
Brazil beef	CBRBF
Denmark Pork (Dry)	FDP
Dhofar Chicken	CDHCH
Indian mutton	IFM
Indian lamb	CINLB
Indian mutton	LINMT
Indian veal	CINVE
Indian veal	LINVE
Italian Pork (Dry & Flavored)	ITP
Kenyan Pork (Fresh)	KEP
Kenyan lamb	LKNLB
New Zealand beef	CNZBF
New Zealand beef	LNZBF
New Zealand lamb	CNZLB
New Zealand lamb	LNZLB
Old Sample of Pork (Fresh)	PORK1
Omani beef	LOMBF
Omani beef	LSLBF
Omani camel	LSLCL
Omani camel	SOMCL
Omani mutton	NOM
Omani mutton	RUOM
Pakistani beef	LPKBF
Pakistani beef	SPB
Pakistani camel	SPCL
Pakistani beef	SPC

Pakistani mutton	CPKGO
Pakistani mutton	LPKMT
Pork	PORK
Somali mutton	LSMMT
Somalian beef	SSMBF
Somalian mutton	SSMG
Somalian lamb	SSMLB
Spain Pork (Fresh)	SPP
UK Pork (Fresh)	UKP
USA Smoked Pork	USSMP

All the 39 meat samples were crushed and ground to prepare mince. The minced meat samples from different species were mixed with each of the 8 pork varieties at 8 levels of concentration (i.e. 0%, 1%, 5%, 10%, 30%, 50%, 70%, and 90% pork). All prepared samples were tested in triplicate. Subsequently, a total of 31~~x~~ × 8~~x~~ × 8~~x~~ × 3 = 5952 meat samples were analyzed along with 8 pork samples.

~~2.2.~~2.2 NIR Sspectroscopic analysis

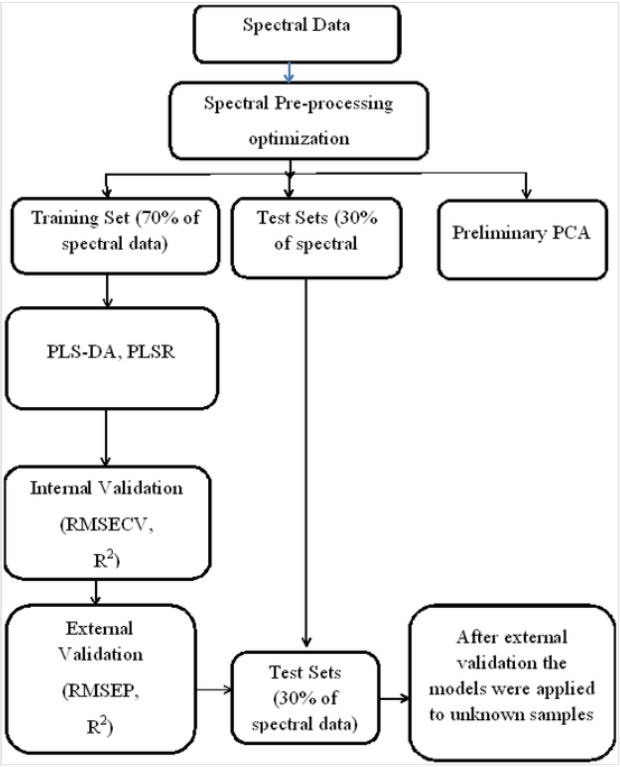
Each meat sample (in the solid form) was scanned by using a Frontier NIR spectrophotometer (BSEN60825-= 1:2007 by Perkin Elmer) in reflection mode. Spectra were collected in the range 10,000 to 4000 cm^{-1}, at 2 cm^{-1} resolutions and a total path length of 0.5 mm, using a calcium fluoride sealed cell with a path length of 0.2 mm.

~~2.3.~~2.3 Multivariate analysis

The Unscrambler (version 10.4) and Microsoft Excel 2016 softwares were used for performing the multivariate analysis of the NIR spectral data. PCA, PLS-DA and PLSR models were built, as it will be explained in the Results and discussion section. Fig. 1 summarizes the flowchart of the multivariate analysis performed.

alt-text: Fig. 1

~~Figure 1.~~Fig. 1



Flowchart of the multivariate analysis performed.

The NIR spectra of the meat samples were preprocessed prior to building the multivariate models. Standard normal variate (SNV) and unit vector normalization were applied to minimize the scattering effects and to remove the noise from the spectral data. Scattering is a common physical phenomenon in near-infrared (NIR) analysis. It is produced by particles, which randomly deviate the light from its original trajectory, causing undesired nonlinearities in the spectral data. The effect can be corrected by applying suitable spectral preprocessing techniques before chemometric modeling. The algorithm investigated in this study was Standard Normal Variate (SNV). SNV is a transformation usually applied to spectroscopic data, to remove scatter effects by centering and scaling each individual spectrum (i.e. a sample-oriented standardization).

The selection of the optimal combination of spectral pre-processing and spectral region was made based on a compromise between the lowest RMSECV, the highest possible R2 value and the least number of factors, for the calibration set. The transformed NIR spectra were split into two sets, i.e. a training test was including 70% of all the spectral data to build the PLSR model and a test set was including 30% of spectral data to externally validate PLSR model. The test set samples from the training set were randomly selected. The PLSR model was internally also validated, using a full cross validation procedure.

PLS-DA models were also built using Kernel-NIPALS algorithm with iterations value of 100 under the same optimized spectral transformation as used for PLSR model. Unlike the PLSR models, for PLS-DA, as the number of samples was not so high (around 45), we decided to internally validate the models using a cross validation strategy, leaving out several blocks of samples containing each around 10% of the samples of the training set, that is 4–5 samples in each split.

The level of adulteration providing complete discrimination between pure meats and meats adulterated with pork was then optimized. For this purpose, three levels of pork meat adulteration were tried i.e. 1%, 5% and 10% as shown in Table 3. Table 3 shows the classification rates of the PLS-DA models, expressed as Sensitivity, Selectivity and Accuracy. Sensitivity measures the number of adulterated samples correctly predicted as adulterated, Selectivity measures the number of non-adulterated samples correctly predicted as non-adulterated and, finally, accuracy measure the total number of samples correctly classified (both adulterated and non-adulterated). The three measure were calculated using a threshold value of 0.5.

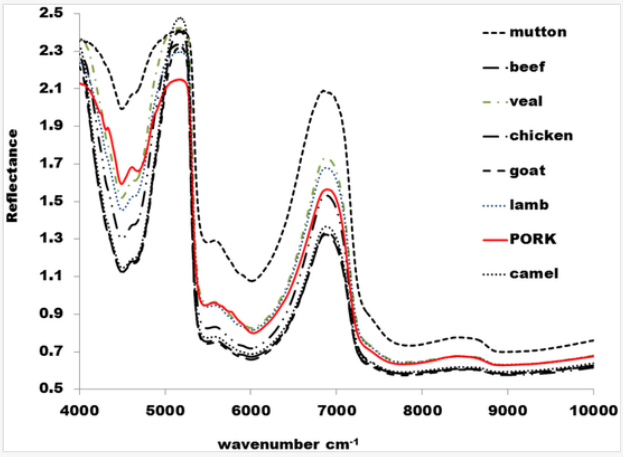
~~3.~~3 R~~ESULTS~~esults ~~AND~~and ~~DISCUSSION~~discussion

~~3.1.~~3.1 NIR spectra

The representative NIR spectra (not pre-processed) for all the meat samples are shown in Fig. 2.

alt-text: Fig. 2

~~Figure 2.~~Fig. 2



Representative NIR spectra (not pre-processed) of various types of meat.

The near infrared spectra of all meat samples indicate that pork meat samples (Fig. 2) have a different reflectance pattern as compared to other meat samples, which could be due to differences in meat muscle type and composition, fibre type, molecular weight of various muscle proteins, and fatty acid profile of the intramuscular fats (Listrat et al., 2016). For instance beef, lamb, mutton and camel are ruminants, which means that their meat contains more saturated fatty acids than unsaturated, while the monounsaturated fatty acids are the dominant ones in pork and chicken meat samples (William, 2007). Moreover, the anatomical structure of pork muscles is different from those of other meats due to the presence of a high glycogen content (Milan, Jeon, & Looft, 2000).

As shown in Fig. 2, there is an offset in the spectral baseline due to scattering effects. The scattering effect of the spectra is due to the solid nature of meat samples. Various types of spectral pretreatments, such as baseline correction, standard normal variate (SNV) or first derivative, were applied to minimize the scattering effect

and to remove the noise from the spectral data (Table 2). Apart from the spectral transformations, different spectral regions were selected and studied, to check whether the models improved or not. The selection of the optimal combination of spectral pre-processing and spectral region was made based on a compromise between the lowest RMSECV, the highest possible R^2 value and the least number of factors, for the calibration set.

alt-text: Table 2

~~Table 2.~~Table 2

The presentation of Tables and the formatting of text in the online proof do not match the final output, though the data is the same. To preview the actual presentation, view the Proof.

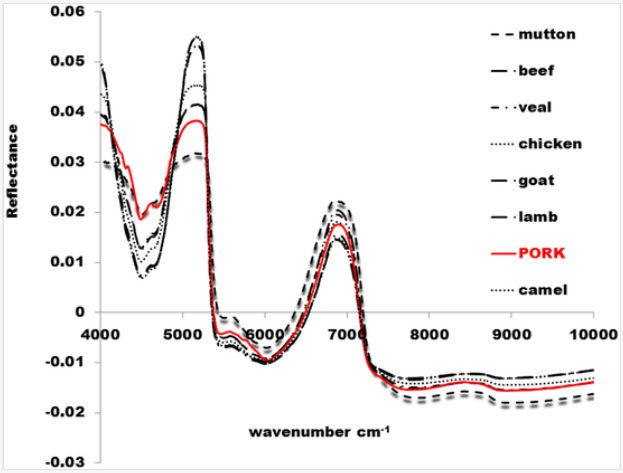
Results of the PLSR models for different spectral pre-processing and spectral region combinations.

Type of spectra	Transformation	Calibration		Validation		Factors
		RMSECV (%)	R^2	RMSEP (%)	R^2	
Full Spectra (4000 to 10,000 cm^{-1})	none	2.35	0.825	2.68	0.780	6
Spectra (4000 to 7300 cm^{-1})	none	2.18	0.822	2.89	0.791	6
Full Spectra (4000 to 10,000 cm^{-1})	Baseline	1.63	0.825	2.26	0.767	6
Spectra (4000 to 7300 cm^{-1})	Baseline	1.75	0.74	2.53	0.723	6
Full Spectra (4000 to 10,000 cm^{-1})	SNV+ Unit Vector Normalization	0.077	0.977	1.183	0.992	3
Spectra (4000 to 7350 cm^{-1})	SNV+ Unit Vector Normalization	0.185	0.961	2.123	0.965	4
Full Spectra (4000 to 10,000 cm^{-1})	1st derv. ~~w~~With 11 smoothing points	3.52	0.92	4.96	0.982	8
Spectra (4000 to 6400 cm^{-1})	1st derv. ~~w~~With 11 smoothing points	3.74	0.966	3.84	0.983	8

Table-2 shows that the application of standard normal variate (SNV) and unit vector normalization improved the values for all parameters, including RMSECV, R^2 and RMSEP in the case of full spectra. The multivariate model using this transformation was the simplest one (only needed 3 factors) and showed the minimum error and the highest correlation values. SNV and unit vector normalization lead to the minimization of the level of noise and scattering effect of the spectral data as shown in Fig. 3.

alt-text: Fig. 3

~~Figure 3.~~Fig. 3



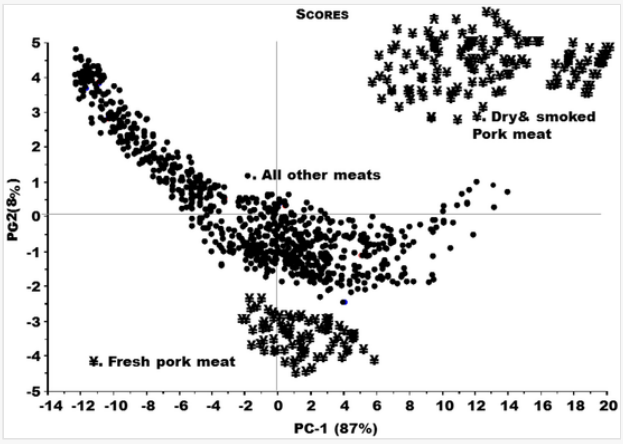
Representative NIR spectra (pre-processed) of various types of meat.

The difference in the features of the NIR spectral pattern of pork meat are related to differences in muscle composition (contents of fatty acids, proteins, water, etc.), which in turn may be due to different factors such as muscle type, production system or processing, among others. However, it is difficult to link a specific spectral band to a given constituent without having measured the exact composition.

In order to visualize and explore the similarities and differences among the various types of meat samples the exploratory tool principal component analysis (PCA) was applied to the NIR spectra, as shown in Fig. 4. PCA is used to reduce the dimensionality of a multidimensional data set to extract the most important information from the data, to detect outlier samples and to visualize groups or trends in the data based on similarities and dis-similarities of the samples.

alt-text: Fig. 4

~~Figure 4.~~Fig. 4



PCA score plot of all meat samples.

The PCA score plot in Fig. 4 shows the complete segregation of pork meat samples from the other meats. All the pork meat samples were clustered together in two different regions. The two clusters of pork meat correspond to fresh pork meat and to dry and smoked pork meat samples, respectively, and are very well differentiated along PC-2.

In order to see that what part of the spectra contributed to the PCA model, the loading plots for PC-1 and PC-2 are shown in Figs. 4S-1 and 4S-2 (supplementary material). The X-loading plot of PC-1 explains 87% of the total spectral variability, while PC-2 explains 8%. However, the separation between pork meats and other meats is clearly seen along PC-2. The key bands important for the separation are in the NIR region from 4000 t0 5318 cm^{-1} of PC2.

PlS-DA models were also built to optimize at which level of adulteration the disrcimination is complete in between pure meats and meats adulterated with pork. For this purpose three levels of pork meat adulteration were tried i.e. 1%, 5% and 10% as shown in Table 3.

alt-text: Table 3

~~Table 3.~~Table 3

The presentation of Tables and the formatting of text in the online proof do not match the final output, though the data is the same. To preview the actual presentation, view the Proof.

Results of PLS-DA models for discrimination of adulterated and un-adulterated meat samples based on the presence of pork meat.

		Measured				
		Non-adult	**Adult**	**Sensitivity**	**Selectivity**	**Accuracy**
Predicted 0–1% adult	Non-adult	22	3	0.85	0.88	0.87
	Adult	3	17			
Predicted 0–5% adult	Non-adult	23	2	0.93	0.92	0.92
	Adult	2	26			
Predicted 0–10% adult	Non-adult	22	0	1.00	1.00	1.00
	Adult	0	27			

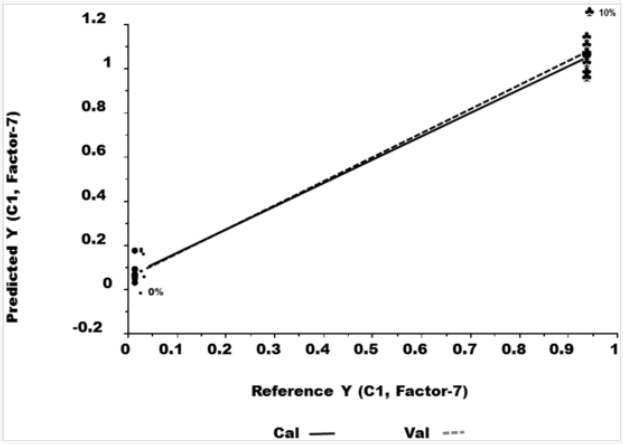
Table 3. Optimization of the levels of pork meat adulteration for PLS-DA model.

Table 3 summarise the values of RMSECV, and R^2 to find the optimum level of pork meat adulteration for complete discrimination of PLS-DA model. It can be seen from Table 3 that the lowest level of contamination that can be detected is 10% of pork meat adulteration, because it has the minimum value of error i.e. 0.07637RMSECV and having good value of correlationship i.e. R^2 = 0.9805.

The PLS-DA model for 10% pork meat adulterant, is shown in Fig. 5 under the same optimized spectral transformation as used for the PLSR model.

alt-text: Fig. 5

~~Figure 5.~~Fig. 5



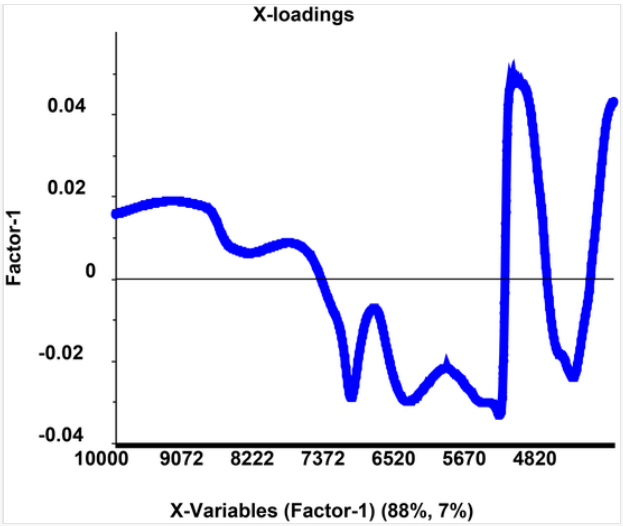
PLS-DA model between non-adulterated meats samples and meat samples containing 10% of pork meat adulteration.

The results of the PLS-DA model in Fig. 5 reveal that the discrimination is complete and the lowest level at that discrimination is complete is 10-% of pork meat adulteration. However, results form 5% adulteartion are fairly good too. Between 5% and 10% the samples detected as adulterated should be taken with care and probably ~~analysed~~analyzed with a confirmatory method. The PLS-DA model was found to be an excellent tool to detect the presence of pork meat adulteration in all other different meat samples.

The loading plots of factor-1 of PLS-DA model is also shown in Fig. 6.

alt-text: Fig. 6

~~Figure 6.~~Fig. 6



loading plots of factor-2 of PLS-DA model.

Near-infrared spectra could record the multifrequency and co-frequency information of organic molecules, which involves the response of molecular bonds of C—H, N—H, C—O, and O—H. The chemical composition of meat is moisture, fat and protein, and some peaks and valleys representing the characteristics of meat including hidden information of different elements were obviously shown in the spectra. The NIR spectra present broader peaks, but it is still much easier to assign specific lipid, protein, or water peaks. Strong absorption bands can be observed at a number of wavelengths. The original spectrum reveals clear wide peaks only at about 8264.46 cm-1, 6896.55 cm−1, and 5263.16 cm−1, which were appeared in the main absorbing wavelengths. The case of C—H bonds corresponds to the 6172.84–5617.98 cm−1and 4545.45–4000.00 cm−1 regions of the spectrum (first C—H overtone and C—H bond vibration, respectively), and that of N—H and O—H bonds corresponds to the 7042.25–6172.84 cm−1 and 5555–4545.45 cm−1 regions (first N—H and O—H overtones, and N—H and O—H vibrations, respectively).[32–34] The main meat component was moisture and has the highest absorption at wavelengths in the NIR region. The absorbance spectra for meat samples are dominated by moisture absorption bands at 10000 and 6896.55 cm−1. The typical peaks of fat are located at about 8264.46 cm−1 (C—H second overtone), and 5263.16 cm−1, which agrees with other studies[Rødbotten, Nilsen, & Hildrum, 2000; Cozzolino, Barlocco, Vadell, Ballesteros, & Gallieta, 2003; Cozzolino & Murray, 2004; Leroy et al., 2004].

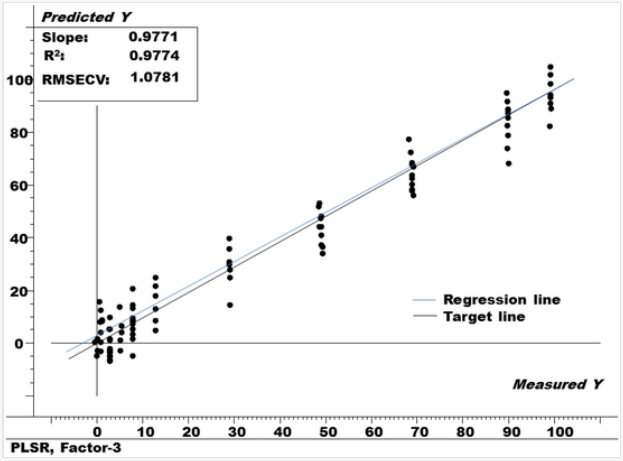
The loading plots of factor-2 of PLS-DA model as shown in Fig. 6b illustrates that the NIR peaks in the spectral range from 7000 to 4730 cm^{-3} are contributing more to differentiate among the meat samples. This variation in their spectral data is due to higher percentages of polyunsaturated fatty acids in both muscle and adipose tissues of pork meat as compared to sheep and cattle meat. The percent composition of polyunsaturated fatty acids such as linoleic acid in adipose tissue (g/100 g fatty acids) in pork, sheep and cattle was found to be 14.3%, 1.3% and 1.1%, respectively, while arachidonic acid remained 0.2% in pork meat while it was absent in sheep and cattle meat. Similarly, polyunsaturated fatty acid composition in muscle tissue was also different as linoleic acid in pork was 14.2%, sheep 2.7% and cattle 2.4% while, arachidonic acid was found as 2.21% in pork, 0.64% in sheep and 0.63% in cattle (Wood et al., 2008).

~~3.2.~~3.2 PLS regression

A PLSR model (Fig. 7) was built using the training set of the NIR spectral data spiked at the levels of: 1-%, 5 %, 10-%, 30-%, 50-%, 70-%, and 90-% of pork meat.

alt-text: Fig. 7

~~Figure 7.~~Fig. 7



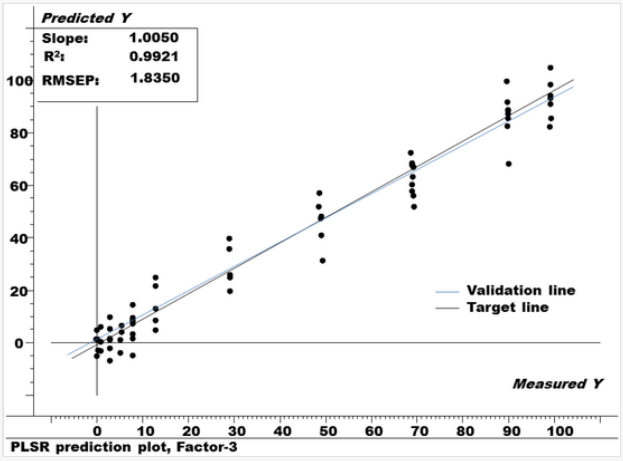
PLSR validation plot using training set.

The optimal PLSR model (Fig. 7) was built with 3 factors and shows a significant value of coefficient of determination, R^2 = 0.9774 and a rather good cross-validation error, RMSECV = 1.08%.

The performance of the PLSR model was also externally validated by using the test set of samples, as shown in Fig. 8.

alt-text: Fig. 8

~~Figure 8.~~Fig. 8



PLS prediction plot using the test set.

The PLS prediction plot illustrates that PLSR model displayed a very good prediction ability (RMSEP value = 1.84%, with a high determination coefficient (R^2 = 0.9921).

For PLS regression and prediction models the standard normal variate (SNV) and unit vector normalization transformation were applied on the full spectra. After the application of these transformations the scattering effects by centering and scaling of each individual spectrum was removed and the errors of the model were reduced to minimum values while the correlation values were improved to high.)

~~4.~~4 Conclusion

Reflectance NIR spectroscopy coupled with multivariate data analysis can be deployed as a quick, and low cost, analytical method for detecting the presence of pork meat in other meats. The analytical method presented in this study avoids lengthy sample preparation and provides real time analysis of meat adulteration. The current study revealed that PLS-DA model can be a fast discrimination tool, the only limitation being the lowest level of adulteration that can be 100% correctly detected is 10%. The PLSR model can be used as a rapid quantification tool to know the content of pork meat in any other meats.

Funding

The authors declare that this study was not financialy funded by any organization.

Informed consent

Not applicable.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Uncited references

Al-Jowder et al., 1999

Arvanitoyannis and Van, 2003

Celio, 2003

Fazal et al., 2017

Thienes et al., 2017

Walker et al., 2013

Warris and Brown, 1998

Declaration of Competing Interest

Authors declare that they have no conflict of interest.

~~Appendix A.~~Appendix A Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2020.108084.

References

The corrections made in this section will be reviewed and approved by journal production editor.

Ahmed, R., & Akinbode, A. (2018). Assessing different processed meats for adulterants using visible-near-infrared spectroscopy. *Meat Science*, 136, 59–67.

Ali, M. E., Hashim, U., Mustafa, S., Chen Man, Y. B., & Dhabi, T. S. (2012). Analysis of pork adulteration in commercial meatballs targeting porcine-specific mitochondrial cytochrome b gene by TaqMan probe real-time polymerase chain reaction. *Meat Science*, 91, 454–459.

Al-Jowder, O., Defernez, M., Kemsley, E. K., & Wilson, R. H. (1999). Mid-infrared spectroscopy and chemometrics for the authentication of meat products. *Journal of Agriculture and Food Chemistry.*, 47, 3210–3218.

Arvanitoyannis, S., & Van, H. K. M. (2003). Implementation of chemometrics for quality control and authentication of meat and meat products. *Critical Reviews in Food Science and Nutrition*, 43, 173–218.

Ballin, N. Z. (2010). Authentication of meat and meat products. *Meat Science*, 86, 577–587.

Ballin, N. Z., Vogensen, F. K., & Karlsson, A. H. (2009). Species determination – Can we detect and quantify meat adulteration?. *Meat Science*, 83, 165–174.

Bhat, M. M., Salahuddin, M., Mantoo, S. A., Jalal, H., & Pal, A. (2016). Species-species identification of adulteration in cooked mutton Rista (a Kashmiri Wazwan cuisine product) with beef and buffalo meat through multiplex polymerase chain reaction. *Veterinary World. EISSN*, 2231–0916, 226–230.

Cai, Y., He, Y., Lv, R., Chen, H., Wang, Q., & Pan, L. (2017). Detection and quantification of beef and pork materials in meat products by duplex droplet digital PCR. *PLoS One*, 12(8). doi:10.1371/journal.pone.0181949. (e0181949).

Celio, P. (2003). ~~Review, Near Infrared Spectroscopy: fundamentals, practical aspects and analytical applications~~Review, near infrared spectroscopy: fundamentals, practical aspects and analytical applications. *Journal of Brazillian Chem. Soc.*, 14, 198–219.

Cozzolino, D., Barlocco, N., Vadell, A., Ballesteros, F., & Gallieta, G. (2003). ~~The Use of Visible and Near-Infrared Reflectance Spectroscopy to Predict Colour on Both Intact and Homogenised Pork Muscle~~The use of visible and near-infrared reflectance spectroscopy to predict colour on both intact and homogenised pork muscle. *LWT Food Science and Technology*, 36, 195–202. doi:10.1016/S0023-6438(02)00199-8.

Cozzolino, D., & Murray, I. (2004). ~~Identification of Animal Meat Muscles by Visible and near Infrared Reflectance Spectroscopy~~Identification of animal meat muscles by visible and near infrared reflectance spectroscopy. *LWT Food Science and Technology*, 37, 447–452. doi:10.1016/j.lwt.2003.10.013.

Djurdjevic, N., & Hsieh, Y. P. (2005). Quantitative detection of poultry adulterants in cooked meat products. ~~*Food Science.*~~ *Food Science*, 70, C586–C593.

Fazal, M., Farah, J., Javid, H., Ahmed, A., Ahmad, H., Saaida, A. A. A., … Alamgir, K. (2017). FTNIRS coupled with chemometric methods as a rapid alternative tool for the detection and quantification of cow milk adulteration in camel milk samples. *Vibrational Spectroscopy*. doi:10.1016/j.vibspec.2017.07.004.

Ha, J., Kim, S., Lee, J., Lee, S., Lee, H., Chol, Y., … Yoon, Y. (2017). Identification of pork adulteration in process meat products using the development mitochondrial DNA-based primers. *Korean Journal of Food Science Animal Research.*, 37, 464–468.

Hsieh, Y. H. P. (2000). Current development for the detection of meat species adulteration. *53rd. Annual Recip*. *Meat Conference Proceeding* (pp. 72–73).

Hsieh, Y. H. P., Chen, F. C., & Sheu, S. C. (1997). AAES research developing simple, inexpensive tests for meat products. *Highlights Agriculture Research.*, 44, 19–20.

Leroy, B., Lambotte, S., Dotreppe, O., Lecocq, H., Istasse, L., & Clinquart, A. (2004). ~~Prediction of Technological and Organoleptic Properties of Beef Longissimus Thoracis from Mear-Infrared Reflectance and Transmission Spectra~~Prediction of technological and organoleptic properties of beef Longissimus Thoracis from Mear-infrared reflectance and transmission spectra. *Meat Science*, 66, 45–54. doi:10.1016/S0309-1740(03)00002-0.

Listrat, A., Lebret, B., Louveau, I., Astruc, T., Bonnet, M., Lefaucheur, L., … Bugeon, J. (2016). ~~How Muscle Structure and Composition Influence Meat and Flesh Quality~~How muscle structure and composition influence meat and flesh quality. *The Scientific World Journal.*, 14.

Mandli, J., Fatimi, I. E., Seddaoui, N., & Amine, A. (2018). Enzyme immunoassay (ELISA/immunosensor) for a sensitive detection of pork adulteration in meat. ~~*Food chemistry*~~ *Food Chemistry*, 255, 380–389.

Mane, B. G., Mendiratta, S. K., & Tiwari, A. K. (2012). Beef specific polymerase chain reaction assay for authentication of meat and meat products. ~~*Food Control.*~~ *Food Control*, 28, 246–249.

Maria, K., Rupert, H., & Margit, C. (2018). Development and validation of a fallow deer (*Dama dama*)-specific TaqMan real-time PCR assay for the detection of food adulteration. *Food Chemistry*, 243, 82–90.

Masiri, J., Benoit, L., Barrios-Lopez, B., Thienes, C., Meshgi, M., Agapov, A., … Samadpour, M. (2016). Development and validation of a rapid test system for detection of pork meat and collagen residues. ~~*Meat science*~~ *Meat Science*, 121, 397–402.

Milan, D., Jeon, J. T., & Looft, C. (2000). A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. *Science.*, 288, 1248–1251.

Ortea, I., Pascoal, A., Canas, B., Gallardo, J. M., Barros-Velazquez, J., & Calo-Mata, P. (2012). Food authentication of commercially-relevant shrimp and prawn species: From classical methods to foodomics. *Electroph.*, 33, 2201–2211.

Rødbotten, R., Nilsen, B.-N., & Hildrum, K.-I. (2000). ~~Prediction of Beef Quality Attributes from Early Post-Mortem near-Infrared Reflectance Spectra~~Prediction of beef quality attributes from early post mortem near infrared reflectance spectra. *Food Chemistry*, 69, 427–436. doi:10.1016/j.meatsci.2008.03.009.

Singh, V. P., & Neelam, S. (2011). Meat species specifications to ensure the quality of meat: A review. *International Journal of Meat Science.*, 1, 15–26.

Soares, S., Amaral, J. S., Mafra, I., & Oliveira, M. B. P. (2010). Quantitative detection of poultry meat adulteration with pork by a duplex PCR assay. *Meat Science*, 85, 531–536.

Soares, S., Amaral, J. S., Oliveira, M. B. P., & Mafra, I. (2013). A SYBR green real-time PCR assay to detect and quantify pork meat in processed poultry meat products. *Meat Science*, 94, 115–120.

Syazana, A. L., & Minhaz, U. A. (2016). A label free electrochemical immunosensor for sensitive detection of porcine serum albumin as a marker for pork adulteration in raw meat. *Food Chemistry*, 206, 197–203.

Tanabe, S., Miyauchi, E., Muneshige, A., & Kazuhiro, M. I. O. (2007). PCR method of detecting pork in foods for verifying allergen labeling and for identifying hidden pork ingredients in processed foods. *Bioscience, Biotechnology, and Biochemistry*, 71, 1663–1667.

Thienes, C. P., Masiri, J., Benoit, L. A., Barrios-Lopez, B., Samuel, S. A., Cox, D. P., … Samadpour, M. (2017). ~~Quantitative Detection of Pork Contamination in Cooked Meat Products by ELISA~~Quantitative detection of pork contamination in cooked meat products by ELISA. *Journal of AOAC International*. doi:10.5740/jaoacint.17-0036.

Trivedi, D. K., Hollywood, K. A., Rattray, N. J. W., Ward, H., Trivedi, D. K., Greenwood, J., … Goodacre, R. (2016). ~~Meat, the metabolites: an integrated metabolite profiling and lipidomics approach for the detection of the adulteration of beef with pork Analyst~~Meat, the metabolites: An integrated

metabolite profiling and lipidomics approach for the detection of the adulteration of beef with pork analyst (141, pp. 2155–2164). doi:10.1039/c6an00108d.

Walker, M. J., Burns, M., & Burns, D. T. (2013). Horse meat in beef products species substitution. *Journal of the Association of Public Analysts*, 41, 67–106.

Warris, P. D., & Brown, S. N. (1998). The relationships between initial pH, reflectance and exudation in pig muscle. *Meat Science*, 20, 65–74.

William, P. G. (2007). Nutritional composition of red meat. *Nutrition and Dietetics*, 64, 113–119.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., … Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, 78, 343–358.

Yang, L., Wu, T., Liu, Y., Zou, J., Huang, Y., Sarath, B. V., & Lin, L. (2018). Rapid identification of pork adulterated in the beef and mutton by infrared spectroscopy. *Journal of Spectroscopy*, 6, 1–10.

~~Appendix A.~~Appendix A Supplementary data

Multimedia Component 1

Supplementary material

Queries and Answers

Query: Your article is registered as a regular item and is being processed for inclusion in a regular issue of the journal. If this is NOT correct and your article belongs to a Special Issue/Collection please contact t.sivakumar@elsevier.com immediately prior to returning your corrections.

Answer: Yes

Query: Please confirm that given names and surnames have been identified correctly and are presented in the desired order, and please carefully verify the spelling of all authors' names.

Answer: Abdulaziz Y. Alkindi affliation is department of Biological Sciences and Chemistry that is (a)

Query: The author names have been tagged as given names and surnames (surnames are highlighted in teal color). Please confirm if they have been identified correctly.

Answer: Yes

Query: Please check if the captured country name for third and fourth affiliations if correct and amend as necessary.

Answer: correct

Query: Uncited references: This section comprises references that occur in the reference list but not in the body of the text. Please position each reference in the text or, alternatively, delete it. Thank you.

Answer: Please remove

Query: Correctly acknowledging the primary funders and grant IDs of your research is important to ensure compliance with funder policies. We could not find any acknowledgement of funding sources in your text. Is this correct?

Answer: The authors acknolwedge DARIS Centre for Research and Technology Department, University of Nizwa, Oman, Natural and Medical Sciences Research Center, University of Nizwa, PC 616, Nizwa, Oman Prof. reoger Purchas from Massy University, New Zealand is highly apprecaired