

## Hyperspectral imaging technique for offal quantification in minced meat

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### Abstract

Spectral imaging is a new technique that combines conventional imaging and spectroscopy in a single system to obtain both spatial and spectral information simultaneously from an object. In this study, potential of hyperspectral imaging in the spectral range of 910-1700 nm was investigated for detecting adulteration in minced lamb meat. Spectral data were extracted to develop a partial least squares regression (PLSR) model to predict the level of adulteration in minced lamb. Good prediction model was obtained using the whole spectral range with a coefficient of determination ( $R^2_{cv}$ ) of 0.97 and root-mean-square errors estimated by cross validation (RMSECV) of 1.80%. Successive projection algorithm (SPA) was employed for optimal waveband selection. The PLSR model using only 7 optimum wavelengths (930, 1067, 1396, 1460, 1658, 1668, and 1702 nm) resulted in a coefficient of determination ( $R^2_{cv}$ ) of 0.97 and RMSECV of 1.84%. The study demonstrated the ability of the hyperspectral imaging as a rapid and alternative to the time-consuming and conventional methods to detect adulteration in minced lamb meat.

**Keywords:** Adulteration, Hyperspectral imaging, Wavelength Selection, Minced meat, Offal, PLSR

### Introduction

Meat products play important role in our daily meals by providing protein for helping body movement and metabolism. But, adulterations of meat products mixing with cheaper substances (such as animal offal, flour and gelatin) were frequently reported (Wu *et al.* 2013, Morsy and Sun 2013) and thus pose a great concern to consumers. Such adulteration is not only a commercial malpractice but also a health risk as certain consumers may be allergic to the adulterant material (Zhao *et al.*, 2014). Several methods such as chromatography, electrophoretic separation of proteins, immunological procedure and DNA based techniques have been utilized for qualitative and/or quantitative analysis of adulteration for authentication of minced meat. These techniques are often not suitable for a fast-paced processing environment because these methods are slow, destructive and also require sophisticated laboratory procedures with monotonous sample preparation. Consequently, a rapid, reliable and non-destructive method is required. Recently, hyperspectral imaging (HSI) has emerged as a rapid and effective tool for non-destructive inspection of various agro- food products. The HSI technology was originally developed by NASA in the field of remote sensing (Goetz *et al.*, 1985). However, the technology is now passing the point of scientific curiosity and is currently under active evaluation by industry and academia in many different applications (ElMasry *et al.*, 2012). This technology mingles the advantages of both spectroscopy and imaging techniques in one system to provide both spectral and spatial information for better inspection. The power of spectroscopy is used to detect or quantify chemical constituents based on their spectral signature and imaging transforms this information into chemical maps in the form of concentration profiles. Recently, this technique has received a considerable attention for assessing fruits (ElMasry *et al.*, 2008), vegetables (Ariana and Lu, 2010), red meat (Naganathan *et al.*, 2008, Qiao *et al.*, 2007), poultry (Nakariyakul and Casasent, 2008), Turkey (Iqbal *et al.*, 2013), fish (Grau *et al.*, 2011; Segtnan, *et al.*, 2009; Sone *et al.*, 2012). However, only one study reported the use hyperspectral imaging to quantify the level of adulteration i.e. pork in minced lamb meat (Kamruzzaman *et al.*, 2013). The objective of this research is to utilize hyperspectral imaging coupling with PLSR analysis for predicting the adulteration level (i.e. heart) in minced lamb meat.

## Materials and Methods

### Sample preparation

Minced lamb meat and lamb heart were purchased from local retail stores and transported to the laboratory of Biosystems Engineering, University College Dublin (UCD) for image acquisition. Firstly, lamb heart was cut into small pieces and then fully minced. Then, the minced lamb was weighed and evenly mixed with minced heart at a certain proportion to guarantee the adulteration percentage ranged from 2% to 36% with around 2% increment. Finally, the mixture was placed on a round metal lid for image acquisition. One sample for each adulteration level was prepared. Therefore, a total number of 18 samples were used in the study.

### Imaging acquisition and correction

The line-scanned hyperspectral imaging system in the reflectance mode was used in the experiment as shown in Fig. 1. The imaging unit was composed of a 12-bit CCD camera, a high performance spectrograph (ImSpector N17E, Specim, Spectral Imaging Ltd., Finland), an illumination unit of two 500-W tungsten halogen lamps (V-light, Lowell Light Inc, USA), a translation stage (MSA15R-N, AMT-Linearways, SuperSlides & Bushes Corp., India), a data acquisition software (SpectralCube, Spectral Imaging Ltd., Finland). Each sample was placed on the translation stage and a hyperspectral image of the sample was acquired line by line. The acquired images were stored in a raw format before being processed. The image acquisition process was controlled by the SpectralCube data acquisition software (Spectral Imaging Ltd., Finland). Each image was recorded in the NIR region of 910-1700 nm with a spectral increment of about 3.34 nm between the contiguous bands, thus producing a total of 237 bands. After image acquisition, reflectance calibration was firstly performed on the obtained hyperspectral images of adulterated minced lamb using white/dark reference, as described by Kamruzzaman *et al.*, (2013).

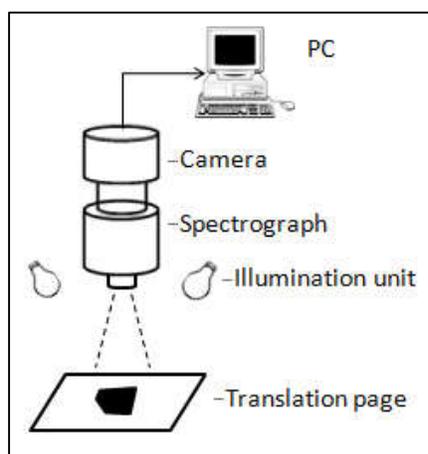


Fig.1. Components of a line-scan hyperspectral imaging system

### Extraction of spatial data

Spectral data were extracted from the minced meat by ignoring background of the sample. A binary mask image was created by thresholding the image at 1300 nm with a value of 0.25. The mask was used as the main region of interest (ROI) to extract spectral data from the calibrated hyperspectral image. The reflectance spectrum from the ROI was computed by averaging the spectral value of all pixels in the ROI to produce only one mean spectrum for each sample. The same procedure was repeated to obtain the spectrum for all the tested samples. The extracted spectral data of each subsample were then arranged in spectral matrix (X) where the columns of this matrix represent the wavelengths (237 variables) and the rows of this matrix represent samples (18 samples).

## Data analysis

Partial least squared regression (PLSR) was applied on the full-wavelength range (910-1700 nm) for calibration and leave-one-out cross-validation (LOOCV). Partial least squares regression (PLSR) is a method for constructing predictive models where the factors are many in number and highly collinear. The core emphasis of this method is to predict the responses rather than identifying the underlying relationship between variables. PLSR method employs linear algorithm as there is a linear relationship between spectra and object properties (Kamruzzaman *et al.*, 2013). Thus PLSR builds a linear model to predict a set of dependent variables,  $y$  (i.e. concentration of chemical attributes) from a large set of independent variables,  $X$  (i.e. predictors or wavelengths). Among many variables, there may be only a few underlying or latent factors that account for best predictive response (Wu *et al.*, 2013). These orthogonal factors are called latent variables (LVs). These LVs are designed appropriately to capture most information in  $X$  as well as  $y$ . The PLSR analysis was carried out using the Unscrambler software v9.5 (CAMO AS, Trondheim, Norway). Performance of the regression models was evaluated using the root mean square errors of calibration (RMSEC), the coefficient of determination in calibration ( $R^2_c$ ), the root mean square errors estimated by cross-validation (RMSECV), and the coefficient of determination in cross-validation ( $R^2_{cv}$ ).

## Optimum wavelengths selection

To reduce data redundancy and speed up analysis, the most pertinent wavebands were selected using successive projection algorithm. Successive projections algorithm (SPA) was proposed in (Araújo *et al.*, 2001) for the optimum wavelengths selection. This method is a novel variable selection algorithm designed to solve the co-linearity problems by selecting variables with minimal redundancy. Nowadays SPA method has found its way with different modelling (Wu *et al.*, 2012). Basically, SPA comprises three main stages. Initially, the algorithm builds candidate subsets of variables with minimum colinearity as the result of projection operations applied to the columns of the spectral matrix available for the training data. The second stage involves the evaluation of candidate subsets of variables based on the value of root mean square error (RMSE) obtained from the resulting multi linear regression (MLR) model assessed by cross-validation or by applying a separate validation set. The final stage consists of a variable elimination procedure aimed at removing uninformative variables without significant loss of prediction ability. Though SPA's simple projection operations is quite effective, there are some drawbacks also. Variables selected by SPA may have a low signal-to-noise ratio (S/N) or be insufficient for multivariate calibration which can affect the precision of model prediction. When the developed model is optimized by selecting several important wavelengths/variables, the spectra can be applied in a pixel by pixel manner to obtain the distribution map where the components of the samples are clearly visualized and easily interpretable. The distribution map can be visually improved by employing post-processing routines.

## Results and Discussion

### Spectral features of heart adulteration

The mean relative reflectance spectra representing different heart adulteration levels in the minced lamb meat are shown in Fig. 2. Although the spectral curves show a similar pattern for different adulteration levels, the reflectance values throughout the entire spectral regions decreased with increasing adulteration percentage, indicating a close relationship between the adulteration content and the reflectance spectra. In the spectra, the major absorption bands were observed at 974, 1211 and 1440 nm. Absorption peaks identified at 974 nm and 1440 nm are due to water absorption bands related to O–H stretching second and first overtones respectively (Barlocco *et al.*, 2006), and absorption bands around 1211 nm are related to C–H stretching second overtone which is attributed to fat content (Andrés *et al.*, 2008).

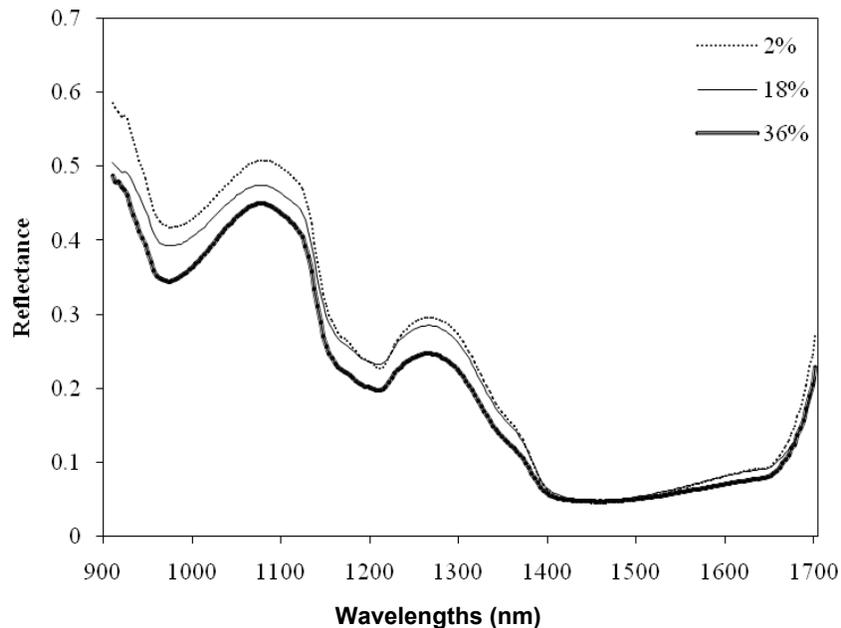


Fig. 2. The mean NIR spectra of different adulteration percentage extracted from ROI in hyperspectral images for heart adulteration

#### PLSR model development with full wavelength range (900-1700 nm)

Regression model was developed using PLSR to correlate the spectral data with the level of adulterations. Full cross validation was utilized to validate the calibration model. The critical step for an accurate PLSR model is to select the correct number of latent variables (LVs) needed to obtain the best prediction. In this study, it was selected at the lowest value of predicted residual error sum of squares (PRESS). PLSR prediction results are presented in the scatter plots shown in Fig. 3. It was possible to detect adulteration in minced lamb with high performance in both calibration and cross-validation conditions using five LVs. The level of adulteration in minced lamb was predicted with a coefficient of determination in calibration ( $R^2_c$ ) of 0.99, standard error of calibration (RMSEC) of 1.21%, coefficient of determination in cross-validation ( $R^2_{cv}$ ) of 0.97 and standard error from cross-validation (RMSECV) of 1.80%. The model had good prediction ability as accentuated by the high values of the coefficients of determination as well as the small difference between RMSEC and RMSECV. The results obtained in this study were higher than those reported by Ding & Xu (2000) but were similar to those mentioned by Meza-Márquez *et al.* (2010) for predicting adulteration in minced beef using NIR spectroscopy. Meza-Márquez *et al.* (2010) reported  $R^2_p$  of 0.99 for quantifying horse adulteration in minced beef, whereas Ding & Xu (2000) obtained lower values of  $R^2$  in predicting adulteration in beef hamburgers with mutton ( $R^2_{cv}=0.86$ ) and pork ( $R^2_{cv}=0.89$ ).

#### Optimum wavelength selection

Wavelength selection is a critical step in spectral data analysis to reduce the high dimensionality of spectral data. In essence, the selection of informative variables could produce better prediction and simpler models. In this study, SPA was used to select the optimum wavelengths. The technique has been used to solve the co-linearity problems by selecting variables with minimal redundancy with different modelling techniques such as SPA-PLS, SPA-least square support vector machine (LS-SVM) SPA-artificial neural network (ANN), SPA-linear discriminate analysis (LDA) (Wu *et al.*, 2012). By using SPA method, seven wavelengths (930, 1067, 1396, 1460, 1658, 1668, and 1702 nm) were selected as optimum wavelengths for detecting adulteration in minced lamb as shown in Fig. 4. The selected optimum wavelengths are indicated by circles on top of a representative spectrum in Fig. 4.

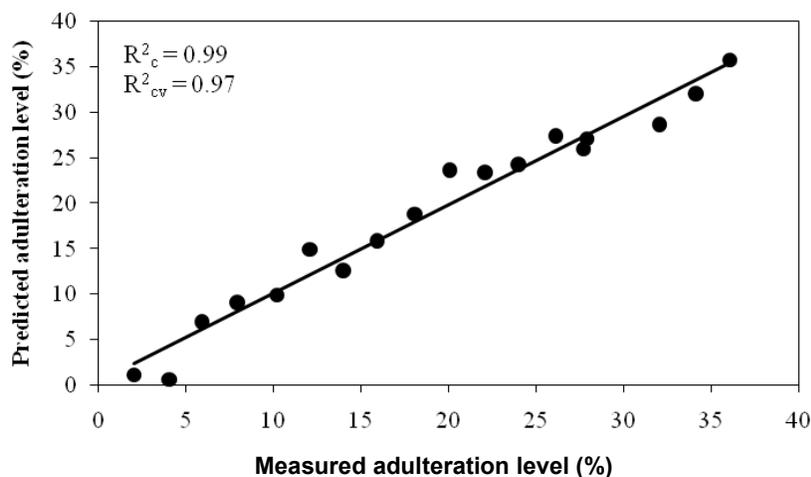


Fig.3. Predicted versus measured adulteration levels of tested samples using PLSR model at full wavelengths range

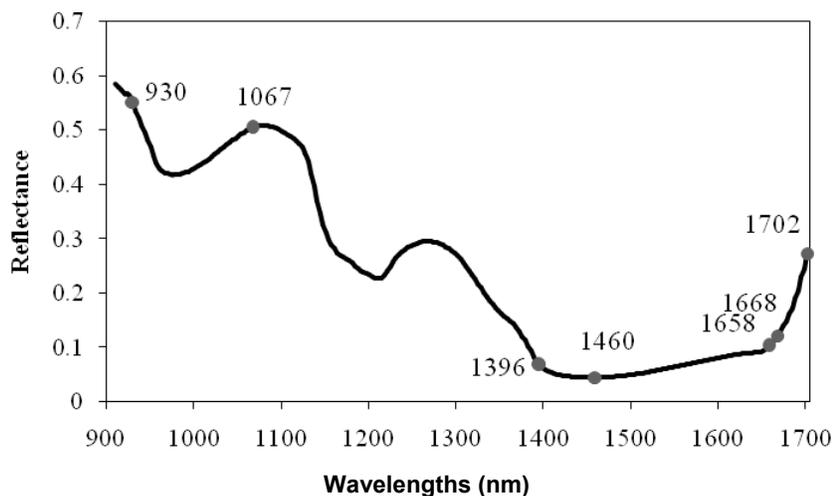


Fig.4. Selection of optimum wavelengths by SPA for predicting adulteration in minced lamb

**Model performance comparison**

Once the important wavelengths were selected, the spectral data were reduced to a matrix of 18 samples × 7 variables by selecting those variables at the identified optimum wavelengths. The number of variables was reduced from 237 to 7. A new PLSR model was also created to verify the potential of selected important wavelengths over the PLSR model developed using the full spectral range. Table 1 displays results of calibration models and cross-validation models performance for heart adulteration in minced lamb using both full wavelengths range and seven selected optimum wavelengths. The prediction ability of PLSR model with selected important wavelength was equivalent to the PLSR model with full spectra, with  $R^2_{cv}$  (0.97 vs. 0.97), and RMSECV (1.80% vs. 1.84%), demonstrating that the selected wavebands were capable enough to represent the full wavebands for the evaluation of heart adulteration.

**Table 1. Comparison of the PLS results for predicting adulteration level on minced lamb meat in full-wavelength and selected-wavelength range**

Model based on	$R^2_c$	RMSEC (%)	$R^2_{cv}$	RMSECV (%)
Full-wavelength	0.99	1.21	0.97	1.80
Selected-wavelength	0.99	1.40	0.97	1.84

## Conclusion

The current study showed that NIR hyperspectral imaging coupled with PLSR analysis was suitable for predicting adulteration in minced lamb meat. Using SPA, seven optimum wavebands were selected to reduce data redundancy without reducing prediction performance. Using these optimum wavelengths, simple, fast and low cost spectral imaging sensor can be designed for adulterate detection in minced meat.

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