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Multispectral imaging (MSI): A promising method for the detection of minced beef adulteration with horsemeat

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A B S T R A C T

In recent years, detection of fraudulent and deceptive practices has become a major priority in the food industry and inspection authorities. The aim of this study was to investigate the potential of multispectral imaging coupled with data analysis methods for the detection of minced beef adulteration with horsemeat, as well as to explore model performance during storage in refrigerated conditions. For this reason, multispectral images of 110 samples from three different batches of minced beef and horsemeat in 18 wavelengths were acquired. Images were taken again after samples were stored at 4 °C for 6, 24 and 48 h. Classification models (partial least squares discriminant analysis, random forest, support vector machines) based on the first two batches were developed while the third batch was set aside for external/independent validation. Results showed that freshly-ground and stored samples were clearly distinguishable, whereas classification model performance for detection of adulterated samples was significantly affected by changes in meat color during storage. Using a two-step SVM model however, all pure and freshly-groundsamples were classified correctly and the overall correct classification was equal to 95.31% for independent batch validation.

1. Introduction

In recent years, detection of fraudulent and deceptive practices has become a major priority for food monitoring agencies as well as the food industry worldwide, since such practices can lead to consumer loss of confidence. Meat and meat products are important food commodities that have been targets for adulteration in the past and, whether deliberately or accidentally, undeclared admixtures and previously unknown and unpredictable adulterants have been observed. While some of these cases include substitution or partial substitution of high commercial value food commodities with cheaper ones, such as beef adulteration with pork or offal or by adding proteins from several origins(Kamruzzaman, Makino, & Oshita, 2015; Ropodi, Pavlidis, Mohareb, Panagou, & Nychas, 2015; Tian, Wang, & Cui, 2013; Zhao, Downey, & O'Donnell, 2014), non-compliance to label has not only economic, but also quality, safety and socio-religious consequences(Alamprese, Casale, Sinelli, Lanteri, & Casiraghi, 2013).

Standard analytical techniques (e.g. immunological and enzymatic techniques, DNA and protein based assays, triacylglycerol analysis) have been used in the past for the authentication of food products and have been very effective in detecting low levels of adulteration (Ballin, 2010; Soares, Amaral, Mafra, & Oliveira, 2010). However, these methods are expensive, invasive, sophisticated, laborious, and technically demanding and thus cannot be used in large-scale in-, on- or at-line applications (Ding & Xu, 1999; Ropodi, Panagou, & Nychas, 2016).

Hyperspectral and multispectral imaging (HSI and MSI) have been proposed as non-invasive rapid methods for monitoring quality, safety and authenticity of foods and in particular meat and meat products (Ropodi et al., 2016; Wu & Sun, 2013). Specifically, minced meat adulteration has been explored in previously published articles using imaging or spectroscopic techniques. Ropodi et al. (2015) investigated the case of minced beef adulteration with pork using MSI, whereas HSI was used for the detection of minced lamb adulterated with pork (Kamruzzaman, Sun, ElMasry, & Allen, 2013). In terms of minced beef adulteration with horsemeat, Raman spectroscopy has been applied recently with promising results(Boyacı et al., 2014; Zając, Hanuza, & Dymińska, 2014). To our knowledge, MSI has not been used previously in the case of minced beef adulteration with horsemeat. Furthermore, no comparison has been performed so far between freshly-ground meat

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and meat stored in refrigerated conditions where changes in meat color naturally occur.

Thus, the objective of this study was to (a) evaluate the potential of multispectral imaging in tandem with data analysis techniques to identify and/or quantify horsemeat in minced beef, and (b) explore model performance under refrigerated storage of both type meat samples.

2. Materials and methods

2.1. Experimental design

The experimental procedure consisted of three distinct stages. In the first stage, different levels of adulteration with a 20% step were prepared based on the procedure described by Ropodi et al. (2015). Briefly, fresh beef and horsemeat *Longissimus* muscle fillets were purchased, cut into smaller pieces and ground separately using a domestic meat-mincing machine. The appropriate portions of each meat were mixed in order to achieve fourlevels of adulteration, 20–80%, 40–60%, 60-40% and 80-20% (w/w), as well as pure beef and horsemeat. From each level of adulteration, five different portions of ca.75–80 g ($5 \times 6 = 30$ samples in total) were placed in Petri dishes and snapshots were taken using VideometerLab vision system(Videometer A/S, Hørsholm,Denmark). After preliminary analysis of the data, it was decided to create more samples per category and focus on the pure samples, and the levels of 60–40% and 80–20%(w/w) for beef and horsemeat, respectively, where beef is adulterated with horsemeat. A 90–10% level for the second stage was added in order to explore smaller levels of adulteration. Additionally, the opposite case of adulteration horsemeat with beef would create similar models as shown by Ropodi et al. (2015). Furthermore, eight samples per level ($8 \times 5 = 40$ samples) were prepared and multispectral images were acquired at the time, as well as after the samples were stored in high-precision incubators at 4 °C for 6, 24 and 48 h. A graphical representation of the experimental design is shown in Fig. 1. Lastly, in the third stage, the previous procedure was repeated for validation purposes. From now on, meat batches from each experimental stage will be referred to as batch 1, 2 or 3 (b1, b2 or b3).

In total, 110 samples were prepared and 350 images were acquired (i.e., 30 images from batch 1, $40 \times 4 = 160$ images from batch 2, and $40 \times 4 = 160$ images from batch 3).

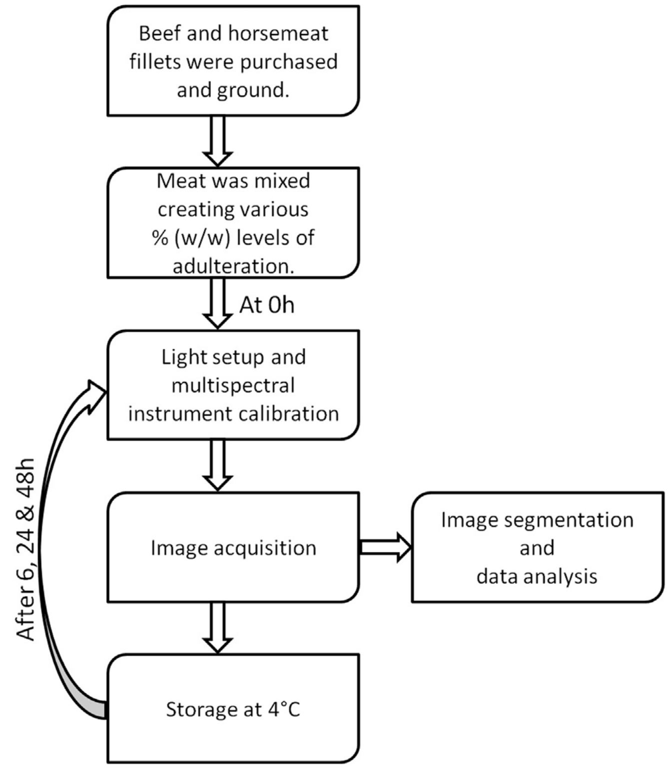

Fig. 1. Graphical representation of experimental design.

2.2. Image acquisition and segmentation

Multispectral images were captured in 18 non-uniformly distributed different wavelengths ranging from 405 to 970 nm. The VideometerLab instrument used was commercialized by "Videometer A/S" (Carstensen & Hansen, 2003) and a more detailed description can be found elsewhere (Panagou, Papadopoulou, Carstensen, & Nychas, 2014; Ropodi et al., 2015). Instrument calibration is a two stage procedure, where a light setup called "autolight" based on the type of object to be recorded and a geometrical and radiometrical calibration using well-defined standard targets are performed (Folm-Hansen, 1999).

While the resulting images provide spectral as well as spatial information, they also include redundant information (e.g.sample background, Petri dish). Image segmentation is an image-processing step applied in order to removeimage background and the Petridishfrom the actual sample, as well as separate adipose from lean tissue. Using the respective routines of the VideometerLab software (version 2.12.39) which controls the operation of the instrument, canonical discriminant analysis (CDA) was employed as a two-step supervised transformation building method to divide the images into regions of interest and using a simple threshold to separate between pixels of lean tissue and other pixels.

2.3. Data analysis

Following image segmentation, the average reflectance values and their standard deviation per wavelength based on pixel intensity values were extracted, resulting in 36 variables (18 mean values and 18 standard deviations) and various data analysis techniques were employed.

Specifically, the unsupervised method of principal component analysis (PCA) (Jolliffe, 2002) was used in order to visualize and interpret data compared to previous works as well as among different experimental stages. Furthermore, various supervised classification techniques were employed in order to discriminate among different levels of adulteration and other classes, such as freshly-ground vs. stored minced meat samples and pure vs. adulterated samples. These techniques are presented below.

(i) Partial least squares discriminant analysis (PLS-DA) (Barker & Rayens, 2003; de Jong, 1993) was employed for various class combinations. The optimum number of PLS components was estimated based on the overall correct classification (OCC) using cross-validation (CV) results of 100 random partitions (80% for training, 20% for testing). Other CV combinations were also performed (results not shown).

(ii) Random forest (RF) (Breiman, 2001) is a supervised learning algorithm which uses an ensemble of classification trees. Ensemble methodologies involve generating multiple classifiers and aggregating their results (e.g. bagging) (Breiman, 2001; Liu, Wang, Wang, & Li, 2013). In RF various parameters were explored and models were chosen based on Out-Of-Bag (OOB) classification error, as a subset of the training