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Multispectral image analysis approach to detect adulteration of beef and pork in raw meats

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ABSTRACT

The aim of this study was to investigate the potential of multispectral imaging supported by multivariate data analysis for the detection of minced beef fraudulently substituted with pork and vice versa. Multispectral images in 18 different wavelengths of 220 meat samples in total from four independent experiments (55 samples per experiment) were acquired for this work. The appropriate amount of beef and pork-minced meat was mixed in order to achieve nine different proportions of adulteration and two categories of pure pork and beef. After an image processing step, data from the first three experiments were used for partial least squares-discriminant analysis (PLS-DA) and linear discriminant analysis (LDA) so as to discriminate among all adulteration classes, as well as among adulterated, pure beef and pure pork samples. Results showed very good discrimination between pure and adulterated samples, for PLS-DA and LDA, yielding 98.48% overall correct classification. Additionally, 98.48% and 96.97% of the samples were classified within a ±10% category of adulteration for LDA and PLS-DA respectively. Lastly, the models were further validated using the data of the fourth experiment for independent testing, where all pure and adulterated samples were classified correctly in the case of PLS-DA, while LDA was proved to be less accurate.

1. Introduction

Nowadays European consumers are increasingly demanding information and reassurance not only on the origin but also on the content of their food. Protecting consumer rights and preventing fraudulent or deceptive practices such as food adulteration are important and challenging issues facing the European food industry, as manufacturers are required to provide and confirm the authenticity and point of origin of food products and their components. Furthermore, adulterants can be revealed with great difficulty in the context of methods commonly applied in laboratories. Several standard analytical techniques, such as immunological and enzymatic techniques, DNA and protein based assays and triacylglycerol analysis have been applied to authenticate food commodities (Ballin, 2010; Soares, Amaral, Mafra, & Oliveira, 2010). However, while these methods are usually capable of detecting low levels of adulteration (Ballin, 2010) they are expensive, invasive, sophisticated, laborious, and technically demanding (Ding & Xu, 1999).

Indeed meat adulteration is a growing challenge for EU meat manufacturers since most adulterants are unknown and unpredictable (e.g., horse meat). For this reason attention should also be paid to the safety and authenticity of meat and meat products, as they can be attractive targets for adulteration in many ways, including substitution or partial substitution of high commercial value meat with cheaper, such as pork or offal or by adding proteins from several origins (Kamruzzaman, Sun, ElMasry, & Allen, 2013; Tian, Wang, & Cui, 2013). With minced meat being the basic ingredient for burgers, adulteration of beef minced meat is a current problem, involving economic, quality, safety and socio-religious issues (Alamprese, Casale, Sinelli, Lanteri, & Casiraghi, 2013). Thus, the meat industry urgently needs methods that will screen non-targeted food samples for contaminants in order to provide proof of origin and prevent deliberate or accidental undeclared admixture to food samples, in a rapid and cost efficient way.

Hyperspectral and multispectral imaging spectroscopy have been used as rapid techniques to monitor quality attributes of food products (Wu & Sun, 2013). The former has been used for the rapid detection of total viable counts in pork (Barbin, Sun, & Su, 2013; Huang, Zhao, Chen, & Zhang, 2013) and of the water-holding capacity of fresh beef (ElMasry, Sun, & Allen, 2011) and pork (Prevolnik, Čandek-Potokar, & Škorjanc, 2010). Meanwhile, multispectral image analysis has high potency for the evaluation of food quality systems during handling, processing and storage (Løkke et al., 2013), and it has been previously used for the conversion of meat color in L*, a*, b* values (Sharifzadeh, Clemmensen, Borggaard, Støier, & Ersbøll, 2014) and for quality

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assessment of beef (Dissing et al., 2013; Panagou, Papadopoulou, Carstensen, & Nychas, 2014). Despite the fact that hyperspectral imaging has been used for the detection of minced lamb adulteration (Kamruzzaman et al., 2013) and gelatine adulteration in prawn (Wu, Shi, He, Yu, & Bao, 2013), to the best of our knowledge the use of multispectral image analysis for meat adulteration, especially in the case of minced beef with pork, has never been previously explored.

Surface chemistry, such as multispectral image spectroscopy, is introduced in the present study as a new approach in tandem with advanced statistical approaches, for the discrimination of raw minced beef meat, which has been fraudulently substituted or combined with raw minced pork. Thus, the objective of this study was to (a) evaluate the potential use of multispectral imaging to discriminate pork from beef, (b) identify if possible, the lowest percentage of minced pork adulteration in minced beef that can be safely detected and (c) establish a rapid and non-invasive technique that can potentially give results in a few minutes.

2. Materials and methods

2.1. Sample preparation

Different levels of adulteration of minced beef and pork were prepared as follows; fresh beef and pork fillets *Longissimus* muscle of normal pH (5.6–5.8) were purchased from central butcher shops in Athens and transported under refrigeration to the laboratory within 30 min. The fillets were cut into smaller pieces and grinded separately one at a time, using a domestic meat-mincing machine. The machine parts coming in contact with the meat were initially disinfected by washing with detergent and hot water, and rinsing with pure ethanol. To achieve different levels of adulteration, ranging from 10 to 90% with a 10% increment, the appropriate amount of each type of meat was used and mixed in conditions that simulate industrial processing. From each level of adulteration, five different portions of ca. 75–80 g were placed in Petri dishes, and snapshots were taken using VideometerLab vision system (Videometer A/S, Hørsholm, Denmark). For every level of adulteration (nine categories of mixed meat and two categories of pure pork and beef), each Petri dish was considered as a replicate in the experiment (5×11 samples in total per experiment).

All experimental procedure took place aseptically and was repeated four times. One hundred and sixty five (165) samples from three independent experiments (i.e., 55 samples per batch) were used to develop the model, and 55 samples from the fourth experiment were employed for the purpose of external validation. It should be noted that 220 samples from different batches were analyzed in total. From this point on, meat samples from the previously mentioned independent experiments will be referred to as samples from batches 1, 2, 3 and 4.

2.2. Image acquisition and analysis

Images from every sample were captured using VideometerLab, a system which acquires multispectral images in 18—non-uniformly distributed—different wavelengths ranging from 405 to 970 nm. Analytically, the wavelengths are 405, 430, 450, 470, 505, 565, 590, 630, 645, 660, 850, 870, 890, 910, 920, 940, 950 and 970 nm. The system has been developed by the Technical University of Denmark and commercialized by "Videometer A/S" (Carstensen & Hansen, 2003; http://www.videometer.com). A detailed description of the instrument has been reported elsewhere (Panagou et al., 2014). The advantage of this instrument is that it not only uses the information of visible and short-NIR spectral regions, but moreover uses the spatial information of each pixel.

The system was first calibrated radiometrically and geometrically using well-defined standard targets, followed by a light setup based on the type of object to be recorded (Folm-Hansen, 1999) called "autolight". In autolight, it is always the brightest sections in the image that dictate the final result. Petri dishes (75–80 g meat portions) were placed inside an Ulbricht sphere in which the camera is top-mounted. For every random dish in each level of adulteration, a different autolight procedure was employed.

The resulting image includes redundant information, such as the Petri dish and its surrounding background, as well as the fat and connective tissue of the meat. For this reason an image-processing step is needed that will result in an image mask where only meat tissue is included. This step, which includes transformation and segmentation procedures, was implemented using the respective routines of the VideometerLab software (version 2.12.39) that 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 (Daugaard, Adler-Nissen, & Carstensen, 2010). Following this transformation, the separation was distinct, and a simple threshold was enough to separate meat from non-meat pixels. The result of this processing is a segmented image for each meat sample with the isolated part of the meat tissue as the main region of interest (ROI) to be used for the extraction of spectral data that were further employed in statistical analysis. The procedure is graphically presented in Fig. 1.

2.3. Data analysis

For each image, the mean reflectance spectrum was calculated by averaging the intensity of pixels within the ROI at each wavelength. Furthermore, the standard deviation of the pixels' intensity per wavelength was extracted. The resulting data consisted of 18 mean values and 18 standard deviations of the reflectance, as it was recorded by the camera for the pixels that were included in each image's ROI, and were further analyzed with various classification methods.

Two methods, partial least squares discriminant analysis (PLS-DA) (Barker & Rayens, 2003; De Jong, 1993) and linear discriminant analysis (LDA) (Fisher, 1936), were performed in order to discriminate among all adulteration classes (11 in total), as well as among adulterated, pure beef and pure pork samples.

As both methods are supervised, the data were partitioned in two sets: the training set used for model calibration and the test set used for validation. A 60–40% stratified partition was applied on the first three batches, meaning 60% of the dataset was chosen in a random way for calibration (99 samples out of 165) as long as all classes and batches were included and equally represented. The fourth batch was also reserved for independent model validation.

Model performance was measured in terms of recall (sensitivity) and precision, as well as overall correct classification (OCC) (Sokolova & Lapalme, 2009). Especially in the case of PLS-DA, the optimum number of PLS components was estimated using stratified three-fold cross-validation.

Lastly, hierarchical cluster analysis—HCA (Everitt, Landau, Leese, & Stahl, 2011) was performed per batch as an unsupervised technique to explore the relationship between variables and adulteration classes, using Euclidean Distance and Ward's minimum variance agglomeration method. Then, principal component analysis—PCA (Jolliffe, 2002) was performed per batch, as well as with all three batches so as to visualize whether there were significant differences among samples from different batches, as well as among different classes.

The partitioning algorithms of the dataset and the LDA algorithm were implemented in MATLAB, 2012a (The MathWorks, Inc., Natick, Massachusetts, United States), while HCA, PCA and PLS-DA were implemented in R v.3.0.2 (RStudio, n.d.), using the "plsgenomics" package (Boulesteix, 2004; Boulesteix & Strimmer, 2007; De Jong, 1993). Lastly, a heatmap was created using the MetaboAnalyst 2.0 software (Xia, Mandal, Sinelnikov, Broadhurst, & Wishart, 2012).