Analysis of pork adulteration in minced mutton using electronic nose of metal oxide sensors

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ABSTRACT

The aims were to detect the adulteration of mutton by applying traditional methods (pH and color evaluation) and the E-nose, to build a model for prediction of the content of pork in minced mutton. An E-nose of metal oxide sensors was used for the collection of volatiles presented in the samples. Feature extraction methods, Principle component analysis (PCA), loading analysis and Stepwise linear discriminant analysis (step-LDA) were employed to optimize the data matrix. The results were evaluated by discriminant analysis methods, finding that step-LDA was the most effective method. Then Canonical discriminant analysis (CDA) was used as pattern recognition techniques for the authentication of meat. Partial least square analysis (PLS), Multiple Linear Regression (MLR) and Back propagation neural network (BPNN) were used to build a predictive model for the pork content in minced mutton. The model built by BPNN could predict the adulteration more precisely than PLS and MLR do.

1. Introduction

Adulteration of meat, involving the replacement of selected breeds, particular geographical region or particular traditional method with other cheaper animal proteins and even none meat proteins (soy proteins), has attracted increasing attention. The choice of one meat over another can reflect the lifestyle, religion, diet and health concerns. For example, lard, pork and meats not ritually slaughtered are forbidden for Muslims and Jews (Bonne and Verbeke, 2008). However, cheaper animal protein, take pork as an example, has been fraudulently used to substitute more expensive animal proteins, like mutton and beef. It requires reliable methods for the authentication of meat adulteration.

Techniques that have been used in the detection of meat adulteration include molecular biology-based methods, enzyme linked immunological methods, chromatographic methods and spectroscopy methods. Molecular biology-based methods, such as polymerase chain reaction (PCR), real-time PCR (Rodriguez et al., 2005), restriction fragment length polymorphism analysis (RFLP) (Chen et al., 2010), multiplex PCR (Ghovvati et al., 2009) and species–specific PCR (Man et al., 2007) have been used in the identification of species and adulteration of meat. Enzyme linked immunological methods used in meat and meat products had been reviewed by Asensio et al. (2008). These methods are the most specific and sensitive for species identification. However, they require

expensive laboratory equipments, high degree technical expertise and also suffer from higher false-positive rates. Chromatographic methods, such as gas chromatography mass spectrometer (GC/MS), high performance liquid chromatography (HPLC) have been reported in the differentiation (Nurjuliana et al., 2011) and adulteration of meat (Chou et al., 2007). The requirement for tedious extractions and long analysis times significantly limited the widespread use of the chromatographic methods. For spectroscopy methods, mid-infrared spectroscopy combined with soft independent modeling of class analogies (SIMCA), visible (VIS) and near infrared reflectance spectroscopy (NIRS), Nuclear Magnetic Resonance (NMR) spectroscopy and Fourier transform infrared (FTIR) spectroscopy were shown to be useful for meat in the detection and quantification of adulterants (Meza-Marquez et al., 2010; Rohman et al., 2011), origin traceability (Sacco et al., 2005; Zhang et al., 2008), authentication and identification of meat muscle species (Cozzolino and Murray, 2004). However, the complex analysis of testing data requires specialized software and algorithms and it is difficult for ordinary inspectors to master it. However, there were few studies on meat adulteration in the view of aroma of the sample.

Electronic noses are devices with several advantages over other techniques for analyzing food aroma, such as the small amount of sample required, speed, simplicity, high sensitivity and good correlation with data from sensory analyses. E-nose is comprised of a sensor array with broad and partly overlapping selectivity for the measurement of volatile compounds within the headspace on a sample, combined with computerized multivariate statistical data

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processing tools to give an odor fingerprint of the sample. Although this technique does not allow the identification of compounds and has a high detection limit in comparison with GC–MS, it has been successfully used in processing monitoring, shelf-life investigation, freshness evaluation and authenticity assessment in a wide range of food products, including meat products. In the previous works on adulteration detection, the electronic nose was shown to be able to discriminate adulteration of oil (Cosio et al., 2006; Haddi et al., 2011; Hai and Wang, 2006; Man et al., 2005), wines (Penza and Cassano, 2004) and meat. For meat discrimination, studies were done on freshness evaluation (Musatov et al., 2010), processing methods evaluation (Limbo et al., 2010), meat products differentiation and authenticity assessment (Nurjuliana et al., 2011). For the identification and differentiation of pork for halal authentication, pork and pork sausage from beef, mutton and chicken meat were studied by E-nose (García et al., 2006).

However, most of the research on meat adulteration is mainly focused on the differentiation and classification of species of meat, with few studies performed on aroma differentiation. In addition, for most studies on authentication using E-nose, data used for analysis were the sensor conductance at a particular time, for example, 15 s, 30 s, 42 s, 45 s, 60 s, etc. (Gómez et al., 2006, 2007; Yu and Wang, 2007). In this study, sensors conductance at different collections times were analyzed and feature extraction methods were used to optimize data set.

The potential use of E-nose for detection of pork adulteration in minced mutton was investigated in this work. The objectives of this study were: (1) to investigate the use of an E-nose combined with pattern recognition methods to detect the presence of pork in minced mutton, (2) to build a model for the prediction of pork content in minced mutton, (3) to optimize the feature extraction methods, and (4) to develop a rapid method for detection of pork adulterated in minced mutton.

2. Materials and methods

2.1. Meat samples

All the mutton samples detected by E-nose and used for determination of physical properties were obtained from logistics center for agricultural products of Hangzhou, and the pork samples were obtained from Wal-Mart Stores in Hangzhou, China, at the day they were slaughtered. Before experimental process, fat and connective tissue were removed, and the meat samples were frozen at −18 °C.

The adulterated mutton was made by blending the frozen mutton with pork at levels of 0%, 20%, 40%, 60%, 80% and 100% by weight, respectively. The adulterated meat was minced for 2 min by mincer. The mixed meat was brought to room temperature before detection.

2.2. Physiochemical analysis

2.2.1. pH measurement

pH was measured by a pH meter (PB-10, Sartorius, Germany) using the method of GB/T9695.5 (2008). The experiment was completed by three duplicates for each sample. The pH was expressed as the mean of three replicates.

2.2.2. Color analysis

Three samples from each treatment were randomly selected to evaluate their color. Color (CIE tristimulus system, L, a and b values) of the minced meat samples was measured using a Minolta CM-700d/600d spectrophotometer (Konica Minolta Sensing, Inc., Osaka, Japan) with 10° standard observer and D65 daylight

illuminant and calibrated with a white plate. The means were used for analysis.

2.3. The electronic nose (E-nose)

A PEN2 E-nose (portable electronic nose II, Airsense Corporation, Germany) was used to obtain the chemical fingerprint of the samples. The basic system, which has been described in previous researches (Hai and Wang, 2006; Zhang et al., 2007), consisted of a sampling apparatus, a detector unit containing the sensor array and pattern recognition software (Win Muster v.1.6) for data recording and processing. The sensor array was composed of 10 different metal oxide sensors positioned into a small chamber. Each sensor has a certain degree of affinity towards specific chemical or volatile compounds, and the nomenclature and characteristics of the sensors used are as follows: W1C (S1), sensitive to aromatics; W5S (S2), sensitive to nitrogen oxides; W3C (S3), sensitive to ammonia, aromatic molecules; W6S (S4), sensitive to hydrogen; W5C (S5), sensitive to methane, propane, and aliphatic non-polar molecules; W1S (S6), sensitive to methane; W1W (S7), sensitive to sulfur-containing organics; W2S (S8), sensitive to broad alcohols; W2W (S9), sensitive to aromatics, sulfur- and chlorine-containing organics; W3S (S10), sensitive to methane and aliphatic.

The experimental conditions for E-nose are given as follows: 10 g of the minced mixed meat was placed in a beaker of 250 ml at the temperature of 25 °C ± 3 °C, and the beaker was sealed by plastic for a headspace generation time of 30 min. The headspace generation was carried out to increase the volatile compounds from the meat sample. Before one sample was detected by E-nose, the sensors were cleaned with the flow of fresh dry air, so that the sample can be tested. Thereafter, the sensors were exposed to sample volatiles and the changes in sensors' responses were acquired by the data acquisition system (Winmuster). During the sampling process, the sample gas was transferred into the sensor chamber at a flow rate of 200 ml min^{-1} and the collection time was 80 s at an interval of 1 s.

2.4. Optimization of sensor array and signal processing

Containing 10 sensors with different sensitivity, the E-nose gives a data set of 800 (10 sensors × 80 s of detecting time) for each, with a total of 120 samples. The multidimensional signals of the E-nose required some data pretreatment before statistical analysis was performed. Feature extraction and selection was done by method of Stepwise discriminant analysis (Step-LDA), Principle component analysis (PCA) and loading analysis, the effects were reviewed by comparison with the original data and data set containing one particular time using three discriminant analysis methods.

The ability of E-nose in identification of adulteration was analyzed by multivariate data analysis. As a supervised method, Canonical discriminant analysis (CDA) and Bayes discriminant analysis (BDA) were used for data visualization and identification of adulteration according to the content of pork. Partial least square analysis (PLS), using cross-validation, was performed to study the predictive capacity of E-nose for the content of pork Multiple Linear Regression (MLR) is one kind of statistical technique that use several explanatory variables to predict the outcome of a response variable. The goal of MLR is to model the relationship between the E-nose signals and the content of pork. The model creates a relationship in the form of a straight line (linear) that best approximates all the individual data points. Back propagation neural network (BPNN), famous for its finer and more complex classifications, a commonly employed and most intensely studied neural network, was employed to study the predictive capacity of E-nose