Meat Science 119 (2016) 118–122

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Identification of meat species by using laser-induced breakdown spectroscopy

Gonca Bilge [a], Hasan Murat Velioglu [b], Banu Sezer [a], Kemal Efe Eseller [c], Ismail Hakki Boyaci [a,d,*]

[a] Department of Food Engineering, Hacettepe University, Beytepe, 06800 Ankara, Turkey
[b] Department of Agricultural Biotechnology, Namık Kemal University, 59030 Tekirdag, Turkey
[c] Department of Electrical and Electronics Engineering, Atilim University, 06836 Ankara, Turkey
[d] Food Research Center, Hacettepe University, Beytepe, 06800 Ankara, Turkey

ARTICLE INFO

Article history:
Received 2 November 2015
Received in revised form 25 April 2016
Accepted 26 April 2016
Available online 27 April 2016

Keywords:
Laser-induced breakdown spectroscopy (LIBS)
Multivariate data analysis
Meat identification
Meat adulteration

ABSTRACT

The aim of the present study is to identify meat species by using laser-induced breakdown spectroscopy (LIBS). Elemental composition differences between meat species were used for meat identification. For this purpose, certain amounts of pork, beef and chicken were collected from different sources and prepared as pellet form for LIBS measurements. The obtained LIBS spectra were evaluated with some chemometric methods, and meat species were qualitatively discriminated with principal component analysis (PCA) method with 83.37% ratio. Pork-beef and chicken-beef meat mixtures were also analyzed with partial least square (PLS) method quantitatively. Determination coefficient (R^2) and limit of detection (LOD) values were found as 0.994 and 4.4% for pork adulterated beef, and 0.999 and 2.0% for chicken adulterated beef, respectively. In the light of the findings, it was seen that LIBS can be a valuable tool for quality control measurements of meat as a routine method.

1. Introduction

Meat products have an important role in human diet because of its high nutritional content. However, availability of meat products is limited due to its high price, and thus meat is an attractive product for adulteration to make profit. Adding cheaper meat species such as pork and chicken to the costlier ones such as beef is the most commonly applied meat adulteration (Kamruzzaman, Sun, ElMasry, & Allen, 2013; Tian, Wang, & Cui, 2013). However, this adulteration type not only leads to financial, ethical, and health problems, but also raises concerns about religious beliefs and may cause certain allergies that limit the allowable intake of certain species. (Ballin, Vogensen, & Karlsson, 2009; Ong, Zuraini, Jurin, Cheah, Tunung, Chai, Haryani, Ghazali & Son, 2007). The most commonly used analytical methods for detection of meat adulteration are polymerase chain reaction (PCR) (Calvo, Rodellar, Zaragoza, & Osta, 2002), real time PCR (Rodriguez, Garcia, Gonzalez, Hernandez, & Martin, 2005), gas chromatography mass spectrometer (GC/MS), high performance liquid chromatography (HPLC) (Nurjuliana, Man, Hashim, & Mohamed, 2011), isoelectric focusing (Skarpeid, Kvaal, & Hildrum, 1998), capillary gel electrophoresis (Vallejo-Cordoba & Cota-Rivas, 1998) and ELISA (Gonzalez-Cordova, de la Barca, Cota, & Vallejo-Cordoba, 1998; Koppelman, Lakemond, Vlooswijk, & Hefle, 2004). Although DNA and protein based methods are most widespread and reliable methods, spectroscopic methods have also recently come to the fore. In this context, mid-infrared spectroscopy combined with soft independent modeling of class analogies (SIMCA) (Meza-Marquez, Gallardo-Velazquez, & Osorio-Revilla, 2010), Fourier transform infrared (FTIR) spectroscopy (Rohman, Sismindari, Erwanto, & Man, 2011), near infrared reflectance spectroscopy (NIR) (Alamprese, Casale, Sinelli, Lanteri, & Casiraghi, 2013) and Raman spectroscopy (Boyaci et al., 2014a; Boyaci et al., 2014b) were found to be useful for detection of meat adulteration. However, these methods have some problems for identification of resembling meat species due to similar molecular structures. Analysis of DNA and protein is the most common practice to identify meat species. Although genetic methods are quite sensitive and reliable, they are expensive methods which require specialists and DNA and protein extraction steps. Chemical methods are also time-consuming. Due to cross reactions caused by antibody biomarkers, immunological methods may give false results, as well (Kumar et al., 2015). Although there are many methods for determination of meat adulteration, they are insufficient and meat industry needs a more rapid, accurate and sensitive method.

Studies show that Ca, Mg, K, Na, Zn, Cu and Fe compositions vary between meat species (Bodwell & Anderson, 1986; Lombardi-Boccia, Lanzi, & Aguzzi, 2005; Yaralı & Öztan, 2005). These differences were demonstrated through inductively coupled plasma mass spectroscopy (ICP-MS) and atomic absorption spectroscopy (AAS). Recently, laser-induced breakdown spectroscopy (LIBS) has been introduced as a rapid and practical technique for elemental analysis. LIBS is a laser based optical spectroscopy technique used to detect atomic and

* Corresponding author at: Food Research Center, Hacettepe University, Beytepe, 06800 Ankara, Turkey.
E-mail addresses: goncabilge@yahoo.com.tr (G. Bilge), mvelioglu@nku.edu.tr (H.M. Velioglu), efe.eseller@atilim.edu.tr (K.E. Eseller), ihb@hacettepe.edu.tr (I.H. Boyaci).

http://dx.doi.org/10.1016/j.meatsci.2016.04.035
0309-1740/

molecular emission signals of elements. It has been used for qualitative and quantitative measurements of the elemental composition of different matrixes such as solid, liquid and gas (Cho et al., 2001). It is a simple method to perform multi elemental analysis in ppm range without any need for a penetration procedure (Hanafi, Omar, & Gamal, 2000; Pace, D'Angelo, Bertuccelli, & Bertuccelli, 2006). LIBS analysis start with focusing laser energy into a small volume of material within a short period of time. This rapid energy deposition on the object leads to a breakdown into atoms which produce characteristic light. Recording of this emission on a spectrometer provides the LIBS spectrum.

There are few LIBS applications in food technology, some of which are analysis of mineral composition of milk powder (Lei et al., 2011), detection of pesticides in powdered spinach and rice pellets (Kim, Kwak, Choi, & Park, 2012), and identification of *Escherichia coli O157*:*H7* and *Salmonella enterica* in foods on surface (Multari, Cremers, Dupre, & Gustafson, 2013), and Na analysis in bakery products (Bilge, Boyaci, Eseller, Tamer, & Cakir, 2015). However, there are some limitations of LIBS applications for quantitative studies. Chemometric techniques such as partial least square (PLS) and principal component analysis (PCA) are more widely used in order to enhance analytical performance of LIBS. These advanced techniques reduce the complexity of spectra and provide valuable information. Many studies have been conducted by combining LIBS with PLS and PCA (Clegg, Sklute, Dyar, Barefield, & Wiens, 2009; Unnikrishnan et al., 2013).

The aim of this study is to evaluate the potential of LIBS combined with multivariate data analysis techniques such as PLS and PCA as a rapid and in-situ method for identifying meat species for the first time. Discrimination of samples was performed according to LIBS spectra, which indicate the elemental composition differences. For this purpose, pork, beef and chicken samples from different animals were collected and analyzed with LIBS, and the obtained spectra were evaluated with chemometric methods. Elemental compositions of meat samples were also verified with the results of AAS.

2. Material and methods

2.1. Sample preparation

Beef and chicken were obtained from local markets in Tekirdag, Turkey, and pork was imported from local butcher shops in Alexandroupolis, Greece. For the discrimination study, the meat parts, namely sirloin, flank and round taken from six different beef and pork carcasses were used, while chest and leg parts taken from six different chicken carcasses were used. All subcutaneous fat was removed manually from the samples, and lean meats were grounded with a 3 mm plate grinder. The beef meat samples adulterated with pork and chicken were produced using minced meat to improve homogenous mixing efficiency. In this quantitative study, chicken leg, pork round and beef round from three different animals were used at known concentrations between 10% and 50% for adulteration. Minced meat samples were subjected to drying in an oven at 105 °C for 2 h. For solvent extraction, 25 g of the dried minced meat was placed in cellulose paper cones and extracted using hexane in a Soxhlet extractor for 4 h after the removal of water (AOAC, 2005). The dried and defatted samples were ground into powder form by using laboratory mill (M20 Univeral Mill, IKA-Werke, Staufen, Germany). Following this procedure, the powdered samples were sieved using 180 mesh screen. Then, the samples were formed as pellet for LIBS analysis with a Specac pellet press machine (15T Manual Hydraulic Press, Swedesboro, New Jersey) by means of exposing 400 mg of the meat to a 10 ton hydraulic press. For each meat sample, three pellets were prepared.

2.2. LIBS instrumentation

LIBS spectra were recorded using an Applied Spectra 50 mJ 1064 nm Nd:YAG laser (Fremont, CA USA) and Applied Spectra 5 channel Aurora LIBS spectrometer (Fremont, CA USA). The laser was operated at a fundamental wavelength of 1064 nm and used for sample ablation. The laser was operated in the Q-switched mode at a repetition rate of 4 Hz, 300 ns gate delay and 1.05 ms integration time. The laser energy was 38 mJ/pulse. Samples were measured using LIBS technique in triplicate, and scanning seven different locations and fifteen laser shots per location.

2.3. Data analysis

Data analyses were performed by using PCA and PLS methods. Determination of LIBS data is very difficult because of its extremely rich spectra; therefore, multivariate data analysis was performed to obtain qualitative and quantitative data, which also provided the elimination of laser fluctuations from shot to shot and physical/chemical matrix effect. In this study, PCA was used to discriminate the three different meat species. LIBS spectra of meat obtained from 3 parts of 6 animals for each species were investigated with PCA analysis (Version 7.5.2 for Windows 7, Eigenvector Research Inc., Wenatchee, WA, USA). The spectral differences of the data set were analyzed with PCA. The applied preprocessing methods were second derivative, Poisson (Sqrt Mean) scaling and detrend, respectively.

In order to determine the adulteration ratio, PLS (Version 7.5.2 for Windows 7, Eigenvector Research Inc., Wenatchee, WA, USA) was applied to the data set. The data obtained from the pork adulterated beef were divided into a calibration and a validation subset; and normalize, second derivative, detrend and baseline (automatic Whittaker filter) methods were used as pre-processing methods. The same processes were performed for the chicken adulterated beef, but the applied preprocessing methods were orthogonal signal correction (OSC), standard normal variance (SNV) scaling and detrend, respectively. Calibration validity was determined by investigating the value of root mean square error of calibration (RMSEC) and the coefficient of determination (R^2).

2.4. Atomic absorption spectroscopy

Inorganic compositions of meats were analyzed by using AAS as a reference method. Sample preparation step was performed with acid digestion according to EPA Method 3051A (EPA, 1994). Meat samples were weighed as 0.3 g into a fluorocarbon polymer vessel, and 10 ml of concentrated HNO_3 was added. The samples were extracted through heating with CEM Corp. MARS laboratory microwave unit (Matthews, NC, USA). After cooling, the vessel contents were filtered with Whatman No. 1 filter paper and diluted in 100 ml of deionized water. AAS were recorded with the Thermo Scientific iCE 3000 Series Atomic Absorption Spectrometer (Cambridge, UK).

The results of AAS were subjected to Analysis of Variance (ANOVA) using the statistical software SPSS 15.0 for Windows. All data were given as mean ± standard error (SE) and mean were compared by one-way procedure tests at $\alpha = 0.05$.

3. Results and discussion

The selected LIBS spectra of pure beef, pure pork and pure chicken meats are presented in Fig. 1. Elemental composition and concentration differences can be seen in LIBS spectra, which is characteristic for this study to discriminate the meat species effectively. Peaks in LIBS spectra were associated with the most probable elements in Table 1. Furthermore, in Table 2, elemental composition differences between meat species were demonstrated with the results obtained through AAS, which was used as a reference method for pure chicken, pure pork and pure beef. One can see that chicken is richer in mineral composition compared to the others. Parallel with the data in the literature, our results indicate that pure chicken samples are richer in Mg, Na (Ortega-Barrales & Fernández-de Córdova, 2015) and K (Yaralı & Öztan, 2005) in comparison with pure beef and pork. However, its Zn content is