



# A comparative study for improving prediction of total viable count in beef based on hyperspectral scattering characteristics



Feifei Tao<sup>a</sup>, Yankun Peng<sup>a,\*</sup>, Carmen L. Gomes<sup>b</sup>, Kuanglin Chao<sup>c</sup>, Jianwei Qin<sup>c</sup>

<sup>a</sup> College of Engineering, China Agricultural University, 17 Qinghua East Road, Haidian, Beijing 100083, China

<sup>b</sup> Department of Biological & Agricultural Engineering, Texas A&M University, College Station, TX 77843-2117, USA

<sup>c</sup> Environmental Microbial and Food Safety Laboratory, Agricultural Research Service, United States Department of Agriculture, 10300 Baltimore Avenue, Beltsville, MD 20705, USA

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## ABSTRACT

The objectives of this study were to investigate the feasibility of hyperspectral scattering imaging to predict the bacterial contamination in meat nondestructively, and propose an optimal approach for detecting low levels of total viable count (TVC) contamination in beef. Fresh beef samples were obtained from a commercial slaughtering plant, and stored at 4 °C for 0–12 days. The visible/near-infrared (VIS/NIR) hyperspectral images in the backscattering mode were acquired from 3–5 beef samples on each day of the experiment, in parallel with microbiological analysis to enumerate the TVC population. Lorentzian function was used to resolve the light scattering information within the hyperspectral image and consequently Lorentzian parameters, which represented different hyperspectral scattering characteristics were extracted. In this study, not only the individual Lorentzian parameters but also the parameter combinations were used to establish the multivariate statistical models for predicting beef TVC, based on the modeling methods of principal component regression (PCR), partial least squares regression (PLSR), and back propagation neural network (BPNN), respectively. The models established using individual Lorentzian parameters did not perform well in predicting low levels of TVC contamination in beef, and the best prediction result could only achieved with the correlation coefficient of prediction set ( $R_p$ ) and root mean squared error of prediction set (RMSEP) of 0.81 and 1.27 log CFU/g, respectively. Based on the parameter combinations, the best modeling results were achieved with  $R_p$  and RMSEP of 0.86 and 0.93 log CFU/g, 0.87 and 0.79 log CFU/g, 0.90 and 0.88 log CFU/g by PCR, PLSR, and BPNN methods, respectively, which confirmed the superiority of the parameter combination method. The results of this study demonstrated for the first time that hyperspectral scattering imaging combined with Lorentzian function and the proposed parameter combination method could be used to detect low levels of bacterial contamination in beef nondestructively.

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## 1. Introduction

Food quality and safety are directly related to the consumers' health and social progress, and thus are important issues throughout the world (Cen and He, 2007). There is an expectation throughout the society that the food supplied for human consumption is safe and nutritious to eat (Frewer et al., 2005; de Jonge et al., 2004). Beef is a commercially important and widely consumed muscle food, due to that it is a good source for proteins and other essential nutrients. However, it is also an ideal substrate for the growth of both spoilage and pathogenic microorganisms (Schirmer and

\* Corresponding author at: P.O. Box 102, College of Engineering, China Agricultural University, 17 Qinghua East Road, Haidian, Beijing 100083, China. Tel.: +86 10 62737703; fax: +86 10 62737997.

E-mail address: [ypeng@cau.edu.cn](mailto:ypeng@cau.edu.cn) (Y. Peng).

Langsrud, 2010). As reported, microbial hazard is still considered to be one of the major challenges to meat safety in the 21st century (Sofos, 2008). The microbiological quality of meat mainly depends on the physiological status of the animal at slaughter, the spread of contamination during slaughter and processing, the temperature and other conditions of storage and distribution (Nychas et al., 2008). In general, the metabolic activity of microorganisms which prevails in a meat ecosystem leads to the manifestation of changes or spoilage of meat. Nychas et al. (2007) clarified that it was the microbial growth *per se*, rather than the activity of microbial enzymes and as a consequence, the accumulation of metabolic by-products of microorganisms that characterized food spoilage. Currently, the practice to assure microbial safety of meat still relies on regulatory inspection and sampling regimes. This approach, however, seems inadequate because it cannot sufficiently guarantee consumer protection, since 100% inspection and sampling is

technically, financially and logistically impossible (Kodogiannis et al., 2014). In addition, most of the related microbiological detection/measurement methods are destructive, time-consuming, and giving retrospective information (Ellis and Goodacre, 2001), thus are not suitable to fast-paced processing environment in meat plants. In a modern food-processing environment, monitoring procedures need to give results in real-time so that corrective actions can be taken as soon as possible when implementing Hazard Analysis and Critical Control Point (HACCP) plan (Ellis et al., 2002).

A promising way to overcome these limitations is to apply non-destructive optical technology (Sowoidnich et al., 2012). Among current emerging technologies, the optic-based methods were reported to have the greatest potential for online application (Shackelford et al., 1999; Vote et al., 2003). The “point” detection spectroscopic methods, such as Fourier transform infrared spectroscopy (FTIR), Near-infrared (NIR) spectroscopy and Raman spectroscopy, which can only detect small portions of the samples, commonly require the tested meat samples to be minced instead of meat chops or need many detected positions to ensure the representativeness of the obtained spectra, and such nature has significantly limited their widespread applications. Hyperspectral imaging is a new rapidly growing technique that integrates spectroscopic and imaging techniques together to provide both spectral and spatial information simultaneously, thus it could provide more adequate and comprehensive information of the object. Recently, studies have been reported on assessing the microbial contamination of meat, in terms of TVC, psychrotrophic plate count (PPC) and *Enterobacteriaceae* by using a NIR hyperspectral system (900–1700 nm) in the reflectance mode or a visible/NIR (VIS/NIR) hyperspectral scattering system (400–1100 nm) in the backscattering mode (Barbin et al., 2013; Feng and Sun, 2013; Feng et al., 2013; Peng et al., 2011; Tao et al., 2012; Tao and Peng, 2014, 2015). Different from the common hyperspectral imaging system, hyperspectral scattering system is based on the light-backscattering imaging (LBI) in which point light is applied as the illuminant source, and thus could capture the backscattering image of the object. During the process of meat spoilage, not only the bacterial load and chemical ingredients undergo changes, but also the microstructure of meat can be different, and these changes could be reflected by the derived light absorption and scattering features from hyperspectral scattering image. Light scattering is commonly reported to be due to physical characteristics (e.g., particle size, cellular structure, and density) of the tissue, whereas light absorption is related to the chemical constituents. And these two optical properties can be characterized by the reduced scattering coefficient,  $\mu_s'$ , and the absorption coefficient,  $\mu_a$  (Tuchin, 2007).

Despite of our previous encouraging results (Peng et al., 2011; Tao et al., 2012; Tao and Peng, 2014, 2015; Wu et al., 2012), further studies are still necessary to fully explore the potentials and limitations of hyperspectral scattering imaging. Therefore, the objectives of the present study were to: (1) exploit the capabilities of the improved hyperspectral scattering imaging system, which was enhanced by introducing a laser displacement detector for accurate measurement of the object distance in this study for detecting low levels of TVC contamination in beef stored at 4 °C, to best simulate the real cold chain condition; (2) propose a data processing method for comprehensively utilizing the information within the three individual Lorentzian parameters, and explore its superiority on predicting low levels of TVC contamination in beef to individual parameters; (3) apply different chemometric methods for quantitative prediction of beef TVC and compare their modeling performances. Methods used in this study consisted of the linear regression methods of principal component regression (PCR) and partial least squares regression (PLSR), and the non-linear regression method of back propagation neural network (BPNN).

## 2. Materials and methods

### 2.1. Preparation of beef samples

Two batches of beef (*Longissimus dorsi* muscle) were purchased from a local commercial slaughtering plant (Yuxiangyuan Husbandry Co., Ltd., Beijing, China) on two different days and immediately transported to the lab under refrigeration. All beef samples were from *Simmental* cattle breed. Totally, 47 beef samples were prepared aseptically by trimming into an uniform size of 9 cm × 5 cm × 2.5 cm (length × width × thickness), and packed separately in commercial food grade polyethylene bags. The samples were placed orderly in the refrigerator and stored at 4 °C for 0–12 days. On each day of the experiment, 3–5 samples were withdrawn randomly for hyperspectral imaging and reference microbiological analysis. Day 0 samples were used immediately before storage.

### 2.2. Hyperspectral scattering imaging system

A laboratory VIS/NIR hyperspectral scattering imaging system in the spectral range of 400–1100 nm was used in this study. Compared to the previous studies on evaluating beef quality and safety attributes in our lab (Peng et al., 2011; Wu et al., 2012), the system used in this study was improved by introducing a laser displacement detector for accurate measurement of the object distance. Briefly, the enhanced hyperspectral imaging system consisted of a high-performance back-illuminated 12-bit charge coupled device (CCD) camera (Sensicam QE, PCO AG, Kelheim, Germany), an imaging spectrograph (ImSpector V10E, Spectral Imaging Ltd., Oulu, Finland), an illumination unit (Oriol Instruments, Stratford, USA) equipped with optical fiber, a laser displacement sensor (GV-H45, Keyence Corp., Shanghai, China) and a computer supported with a data acquisition and control software. The optical fiber was used to form point light in the scattering imaging system, and the diameter of the light beam formed was 5 mm. In addition, in order to minimize the effect of ambient light, the imaging system was enclosed in a shield box.

The system worked in a line scanning mode, and all scans were obtained at a position of 3 mm (from the incident light center to the scanned position) off the incident light center in order to avoid the signal saturation on CCD detector. The resolution of the imaging system was spectrally 2.8 nm with a 0.74 nm interval, and spatially less than 9 μm. The original image generated by this system was of 1376 × 1040 (spatial × spectral) pixels, with the CCD camera binning variable in the horizontal and vertical directions of 1, 2, 4, 8 and 1, 2, 4, 8, 16, respectively.

### 2.3. Image acquisitions

In order to eliminate the dark current effect of the imaging system, the dark image was first obtained by covering the camera lens before imaging for each beef sample. Sample surface with no visible fat or connective tissue was selected for imaging. Before each imaging, the object distance was first measured by the laser displacement sensor, and then kept to the set distance by adjusting the vertical translation stage. For each sample, 5 positions were selected for imaging, and for each imaging, 4 images were averaged to 1 automatically by setting the camera working parameter, which meant that actually 20 hyperspectral images were acquired for each sample.

In order to improve the signal-to-noise ratio (SNR), 2 × 2 binning was performed on the original images in this study, thus the resulting images were of 688 × 520 (spatial × spectral) pixels.

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