Meat Science 90 (2012) 851–857

Contents lists available at SciVerse ScienceDirect

Meat Science

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

Simultaneous determination of tenderness and *Escherichia coli* contamination of pork using hyperspectral scattering technique

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ARTICLE INFO

Article history:
Received 6 August 2010
Received in revised form 7 October 2011
Accepted 14 November 2011

Keywords:
Pork
Tenderness
Escherichia coli contamination
Hyperspectral imaging
Light scattering
Lorentzian distribution function

ABSTRACT

A rapid nondestructive method based on hyperspectral scattering technique for simultaneous determination of pork tenderness and *Escherichia coli* (*E. coli*) contamination was studied in the research. The hyperspectral scattering images of thirty-one pork samples were collected in 400–1100 nm, and the scattering profiles were then fitted by Lorentzian distribution function to give three parameters *a* (asymptotic value), *b* (peak value) and *c* (full width at *b*/2). The combined parameters of (*b*-*a*), (*b*-*a*)×*c*, (*b*-*a*)/*c* and "*a*&*b*&*c*" were used to develop multi-linear regression (MLR) models for prediction of pork tenderness and *E. coli* contamination. It was shown that MLR models developed using parameters *a*, *b*, (*b*-*a*) and (*b*-*a*)/*c* can give high correlation coefficients of 0.831, 0.860, 0.856 and 0.930 respectively for pork tenderness prediction. For *E. coli* contamination of pork, MLR models based on parameters *a* and "*a*&*b*&*c*" can give high R_{CV} of 0.877 and 0.841 respectively.

1. Introduction

Pork is a commercially important and widely consumed muscle food, so the assurance of its quality and safety is of utmost importance. Meat quality includes many attributes, such as tenderness, juiciness, cooking lose, color and so on, while tenderness is regarded as one of the most important quality attribute (Boleman, 1995). That is because tenderness directly relates to the eating quality of meat and is one key factor for consumer's acceptance. Some studies also have shown that variations in meat tenderness will affect consumer's decision to repurchase (Fonseca, Wilson, Horgan, & Maltin, 2003). However, the conventional methods for evaluating meat tenderness are mainly via shear force apparatus which is destructive and can only be applied to a small portion of meat samples (Shackelford, Wheeler, & Koohmaraie, 1999). As such an efficient sensing technology that can nondestructively determine meat tenderness will be of great value for meat sorting and grading industries.

Serious outbreaks of food borne disease and recalls due to the bacterial contamination of raw meat and products highlight a great risk on public health and security in the meat supply system (Doyle, 1991; Stock & Stolle, 2001; Yoda & Uchimura, 2006). It is sure that producing safe meat and products is the priority for meat industries. However, during slaughter and subsequent processing operations, strains of enteric *Escherichia coli*, *Salmonella* and *Shigella* etc may taint meat products. Current methods for detecting bacterial contamination of meat are based on plate culturing, microscopy, ATP bioluminescence, electrical phenomena, enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) (Ellis & Goodacre, 2001). These methods may give precise results in laboratory, but the information mostly is retrospective for requiring cumbersome pretreatment and bacterial incubation (Dainty, 1996; Ellis & Goodacre, 2001, Ellis, Broadhurst, Kell, Rowland, & Goodacre, 2002; Nychas, Skandamis, Tassou, & Koutsoumanis, 2008). That is to say the current methods for detecting bacterial of meat are unsuitable for rapid screening application or online analysis (Ellis, Broadhurst, Clarke, & Goodacre, 2005).

Among several emerging technologies, optic-based methods have the greatest potential for online application because they are rapid, nondestructive (Shackelford et al., 1999; Swatland, Ananthanarayanan, & Goldenberg, 1994; Vote, Belk, Tatum, Scanga, & Smith, 2003). A number of studies have been reported on using near infrared spectroscopy (NIR) to predict beef tenderness, while the coefficient of determination (R) yielded only reached to 0.61–0.81 which was also with considerable inconsistency (Park, Chen, Hruschka, Shackelford, & Koohmaraie, 1998; Prieto, Andrés, Giráldez, Mantecon, & Lavín, 2006; Rødbotten et al., 2000; Zhao, Zhao, & Liu, 2006). While for the detection of bacteria using optical technique, some studies have demonstrated its ability in the fields of bacteria discrimination, classification, identification and detection of meat spoilage (Ellis & Goodacre, 2001; Ellis et al., 2002; Mariey, Signolle, Amiel, & Travert, 2001, Zhao, Rachel, David, Gareth, & Royston, 2006). Hyperspectral imaging is an important technique that

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doi:10.1016/j.meatsci.2011.11.028

combines traditional imaging with spectroscopy to attain both spatial and spectral information from an object. Considerable studies have been reported on application of hyperspectral imaging technique to evaluate the quality and safety properties of agricultural products and food (Cheng et al., 2004, Kim et al., 2002; Kim et al., 2002, Park, Windham, Lawrence, & Smith, 2005a, Park, Yoon, Lawrence, & Windham, 2005b; Park, Lawrence, Windham, & Buhr, 2002, 2004, Park, Yoon, Lawrence, & Windham, 2004). Hyperspectral image contains three-dimensional "hypercube" information of the object and so if resolved spatially, the scattering characteristics obtained can be used to quantify attributes of samples. Previous studies have demonstrated that the spatially-resolved hyperspectral imaging technique is capable to predict fruits firmness and soluble solids content, fat content of milk and beef tenderness (Peng and Lu, 2007, Peng and Wang, 2008b, Peng and Wu, 2008a; Qin and Lu, 2007).

Optical scattering within meat was reported to be subject to the effects of muscle structural properties such as sarcomere length and collagen content (Xia et al., 2008a, 2008b). These same meat structural properties are also two primary mechanisms controlling meat tenderness, and so the scattering information within pork samples can be expected to give better prediction for meat tenderness. As to bacteria detection, previous work has revealed the possibility of employing the spatially-resolved hyperspectral imaging technique to quantify total viable counts during meat spoilage (Peng et al., 2009). However, an early detection of bacteria contamination of meat is still a not reached challenge.

The purpose of the present study was to explore the correlation between light scattering information of pork and the attributes of pork tenderness, *E. coli* contamination, and so to develop a valid tool for simultaneous determination of pork tenderness and bacterial contamination that is appropriate for on-line or real-time use.

2. Materials and methods

2.1. Hyperspectral imaging system

The hyperspectral imaging system was set up in our lab to acquire the hyperspectral images of pork. It mainly consisted of a high-performance back-illuminated CCD camera (Sensicam QE, Germany), an imaging spectrograph (ImSpector V10E, Spectral Imaging Ltd., Finland) which covered the spectral range of 400–1100 nm and a light unit (Oriel Instruments, USA). The imaging system was operated by camera control software (Camera control Kit V2.19, the Cooke Corp., Germany). Hyperspectral images were acquired from objects by performing line scans at the surface of samples. The diameter of the light beam incident was 5 mm, and the scan line was collected at a distance of 3 mm from the light beam incident center to avoid signal saturation on CCD detector. The resolution of the system is spectrally 2.8 nm and spatially less than 9 μm. The whole imaging system was enclosed in a duralumin shield box to avoid the interference from external light.

2.2. Experimental procedure

2.2.1. Sample preparation and microbiological test

The generic *E. coli* was provided by College of Food Science & Nutrition Engineering, China Agricultural University. Bacteria activation was first performed, and then different loads of *E. coli* were removed using sterilized incubation loop to 0.85% saline solution to achieve varying concentrations of bacteria suspension.

Fresh pork was purchased from a supermarket and transported to the lab under refrigeration in 30 min. Test samples were chopped into pieces of 9 cm × 5 cm × 2.5 cm (length × width × thickness) uniformly to minimize the influence from varying experiment conditions. To prepare uncontaminated samples for the following microbiological experiments, surface piece of 1 cm thickness was chopped off from each pork sample under sterile procedure. After that, the pork was dipped into prepared bacteria suspensions to get the samples of different contaminated levels. The reference plate count method was conducted at the same time to quantify the live number of *E. coli* suspension and the result was expressed in log CFU/mL. At last, the prepared samples for subsequent collection of hyperspectral images were left at room temperature for a certain time to insure the adhesion of bacteria on pork surface.

2.2.2. Collection of hyperspectral images

Flat surface with no fat or connective tissue from samples was chosen for the collection of hyperspectral images. During experiments, all scan lines were kept parallel to the longitudinal orientation of pork. Four images were acquired for each sample and then the four images were averaged to one which was saved for further analysis. Additionally, to improve the signal-to-noise ratio, 2 × 2 binning was performed during the acquisition of images. The final hyperspectral image was of 520 × 688 pixels.

2.2.3. Measurement of pork tenderness

After collection of hyperspectral images, the measurement of pork tenderness was performed. Digital meter with Warner-Bratzler Shear accessory (C-LM3B, Northeast Agricultural University, China) was used to measure the shear force which was regarded as the reference value of meat tenderness (Honikel, 1998). The testing method was referred to NY/T 1180–2006. The speed for apparatus probe was set at 5 mm/s. Repeats of tenderness for one sample were averaged and the value of pork tenderness was expressed in the unit of Newton (N).

2.3. Data analysis method

2.3.1. Lorentzian function fitting for scattering profiles

Lorentzian distribution function which is commonly used to describe the laser profiles and light distribution patterns in optics research (Davis, 1996) can be mathematically expressed by Eq. (1). Peng and Lu (2005) proposed using the 3-parameter Lorentzian distribution function to describe the spatial scattering profiles of apples and concluded that the function can give excellent fitting result for fruits. Lorentzian function was applied to fit the scattering profiles of pork in the study.

$$I_{w_i} = a_{wi} + \frac{b_{w_i}}{1 + \left(x/c_{w_i}\right)^2} \tag{1}$$

where I is the light intensity, in CCD count; x is the scattering distance from the center of beam incident, in mm; a is the asymptotic value of the light intensity; b is the peak value of the scattering profile at $x = 0$, in CCD count; c is the full width of the scattering profile at $b/2$ (FWHM), in mm. The subscript w_i represents one individual wavelength of the whole spectral range with $i = 1, 2, 3, \ldots N$; and N is the total number of wavelengths.

Nonlinear curve-fitting algorithm was performed using the function to fit the scattering profiles of pork in the spectral range of 400–1100 nm. Thus, the extracted parameters which can represent the features of scattering profiles were determined.

2.3.2. Calibration and validation of MLR models

The extracted three individual parameters a, b and c were used to establish MLR models in the study. Moreover, as considering that the combined parameters of $(b\text{-}a)$, $(b\text{-}a) \times c$ and $(b\text{-}a)/c$ may represent the maximum attenuation of scattering, scattering area and attenuation ratio respectively in a simple sense, MLR models were also tried to be developed using these combined parameters for prediction of pork tenderness and *E. coli* contamination.