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Analytical Methods

Quantitative determination of total pigments in red meats using hyperspectral imaging and multivariate analysis

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ABSTRACT

This study investigated the potential of hyperspectral imaging (HSI) for quantitative determination of total pigments in red meats, including beef, goose, and duck. Partial least squares regression (PLSR) was applied to correlate the spectral data with the reference values of total pigments measured by a traditional method. In order to simplify the PLSR model based on the full spectra, eleven optimal wavelengths were selected using successive projections algorithm (SPA). The new SPA-PLSR model yielded good results with the coefficient of determination (R_p^2) of 0.953, root mean square error (RMSEP) of 9.896, and ratio of prediction to deviation (RPD) of 4.628. Finally, distribution maps of total pigments in red meats were developed using an image processing algorithm. The overall results from this study indicated HSI had the capability for predicting total pigments in red meats.

1. Introduction

Red meats commonly refer to darkly colored meat, such as beef, lamb, horsemeat, goose, duck, etc. (Cheng, & Sun, 2008; Kamruzzaman, Barbin, ElMasry, Sun, & Allen, 2012; Xiong, Sun, Zeng, & Xie, 2014). Red meats play an important role in daily life, as they can provide a variety of nutrients to maintain human health, such as protein, iron, zinc and vitamins (McAfee et al., 2010). Currently, consumers are more concerned about food quality, therefore measures and techniques such as drying (Delgado, & Sun, 2002a,b; Sun, & Byrne, 1998; Sun, & Woods, 1997), refrigeration (Kiani, & Sun, 2011; McDonald, & Sun, 2001; Sun, 1997; Sun, Eames, & Aphornratana, 1996) and edible coating (Xu et al., 2001) are used to enhance the quality. For meats, their quality is generally affected by various factors, such as external factors (storage conditions, pre-slaughter stress, ageing time and temperature, etc.) and internal factors (breed, sex, age, compositions, etc.) (Xiong, Xie, Sun, Zeng, & Liu, 2014). Before making a purchase, consumers use indexes such as flavor, color, and tenderness to evaluate meat freshness and wholesomeness. Among these, color is the first attribute for assessing meat freshness, and consumers prefer to buy products with a satisfactory flesh color. There is no standard definition for a satisfactory color as it is related to consumers' buying habits in different countries. In fresh red meats, a bright red color is usually desired (Bekhit & Faustman, 2005; Kamruzzaman, ElMasry, Sun, & Allen, 2012). However, red meats with a pale color are preferred by consumers in some parts of the world (Carrasco, Panea, Ripoll, Sanz, & Joy, 2009). Meat color is related to the level of protein pigments in the muscles. Commonly, there are three protein pigments in meat: myoglobin, hemoglobin, and cytochrome C, and myoglobin is the principal protein responsible for meat color (Elmasry, Barbin, Sun, & Allen, 2012). There are two traditional methods for determining meat color: instrumental measurement using colorimeters and chemical measurement of protein pigments (Wu, Sun, & He, 2012b). Both methods can provide objective results, but they are tedious and/or destructive and, thus, unsuitable for on-line industrial applications. Consequently, rapid, non-destructive and accurate analytical methods for evaluating meat quality are required by the meat industry.

As a further development of non-destructive computer vision technology (Jackman, Sun, Du, & Allen, 2009; Jackman, Sun, Du, Allen, & Downey, 2008; Sun, 2004; Valous, Mendoza, Sun, & Allen, 2009; Wang, & Sun, 2002), hyperspectral imaging (HSI) has

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been developed in recent years (Sun, 2010). Originally, HSI was used for remote sensing, but due to the merits of providing spectral and spatial data simultaneously, HSI has found potential in other fields, such as agriculture (Vermeulen et al., 2013) and food quality and safety (Feng & Sun, 2013b; Gowen, Taghizadeh, & O'Donnell, 2009; Singh, Choudhary, Jayas, & Paliwal, 2010). The large amount of data in hyperspectral images is expressed in a three-dimensional "hypercube". Through processing and analyzing the data in the "hypercube", not only external characteristics (size, shape, marbling, defects, contaminants, etc.) but also internal characteristics (protein, fat, water, tenderness, firmness, etc.) of food can be predicted.

In recent years, more and more studies and applications have been reported for quality and safety assessment of food products using HSI, including fruit and vegetables (Hashim et al., 2013; Lorente et al., 2012; Rajkumar, Wang, Elmasry, Raghavan, & Gariepy, 2012), meat (Feng & Sun, 2013a; Tao & Peng, 2014; Pu, Sun, Ma, Liu, & Cheng, 2014), cereals (Fox & Manley, 2014), and egg (Dooley et al., 2013; Zhang, Pan, Tu, Zhang, & Liu, 2014). For meat, in particular, HSI has received considerable attention for predicting quality attributes (Barbin, ElMasry, Sun, & Allen, 2012; ElMasry, Iqbal, Sun, Allen, & Ward, 2011; ElMasry, Sun, & Allen, 2011, 2012; Kamruzzaman, ElMasry, Sun, & Allen, 2011, 2012), in particular chemical attributes (protein, fat, total volatile basic nitrogen (TVB-N), moisture, etc.) (ElMasry, Sun, & Allen, 2013; Liu et al., 2014), sensory attributes (color, tenderness, firmness, marbling, etc.) (Barbin, Valous, & Sun, 2013; Huang, Liu, Ngadi, & Gariepy, 2014), technological attributes (pH and WHC) (ElMasry, Sun, & Allen, 2012; He, Wu, & Sun, 2014b), and microbiological attributes (*Pseudomonas*, total viable bacteria counts (TVC), *Escherichia coli*, etc.) (Huang, Zhao, Chen, & Zhang, 2013; Peng et al., 2011). However, no research endeavors have been reported yet on predicting total pigments in red meats using HSI. Therefore, our study implemented visible and near-infrared (Vis/NIR) HSI for predicting total pigments in red meats. More specifically, the aims of the current study were to (1) acquire hyperspectral images of meat samples in the range of 400–1000 nm and extract spectral data from the hyperspectral images; (2) use the whole spectra to build a partial least squares regression (PLSR) model; (3) select the representative wavelengths by the methods of regression coefficients (RC) and successful projections algorithm (SPA); (4) build simplified PLSR models and compare their performance; and (5) generate distribution maps of total pigments in red meats using an image processing algorithm.

2. Materials and methods

The experimental procedures used in this study included image acquisition and pre-processing, measurement of total pigments by a traditional method, spectral analysis and modeling, image post-processing and generation of distribution maps.

2.1. Sample preparation

A total of 12 animals from three species (Chinese yellow cattle, goose and duck) with four animals representing each were bought in a local market (Guangzhou, China). The cattle were slaughtered at a commercial slaughterhouse (Jinsheng meat industry Co. Ltd., Guangzhou, China) while the goose and duck animals were slaughtered by the butcher in the market. Three muscles (*musculus longissimus dorsi* (LD), goose breast muscles and duck breast muscles) were dissected from the animals using a scalpel and used for experiments. During the 24 h-post-mortem, the muscles were removed and cut into slices (7 mm thickness) using a scalpel and a cutting machine (QX, Lijin Food Machinery Corp., Guangzhou, China). After slicing, all meat fillets were individually vacuum packed and stored at 4 °C for additional 24 h aging, which started during rigor. A total of 168 fillets (56 fillets of each species) were obtained. One third ($n = 56$), the second of each fillet, was used for the prediction and the remaining two thirds ($n = 112$) for calibration and cross-validation. All meat fillets were first scanned by the Vis/NIR HSI system and then cut into ground meat in order to measure the reference values of total pigments using a spectrophotometer method described below.

2.2. HSI system and image acquisition

Meat samples were scanned by a pushbroom Vis/NIR HSI system (spectral range of 328–1115 nm) as described by Xiong, Sun, Pu, Zhu, and Luo (2015). In brief, the system was composed of: a charged couple device (CCD) camera (DL-604M, Andor, Ireland) with C-mount lens (OLE23, Schneider, German, 23 mm focal length), a Imspector V10E spectrograph (Spectral Imaging Ltd., Oulu, Finland), two 150 W halogen lamps as an illumination unit (3900-ER, Illumination Technologies Inc., New York, USA), a translation stage (IRCP0076-1COMB, Isuzu Optics Corp., Taiwan), a data acquisition software (Isuzu Optics Corp., Taiwan), and a computer.

Before image acquisition, surface moisture on meat samples was removed using paper towels because the moisture can generate absorption bands in the Vis/NIR region, which may influence the spectral curves of meat samples. Each meat sample was placed on the translation stage and scanned line by line at a constant speed of 1.2 mm/s. When the entire surface of the sample was scanned, a hyperspectral image with a dimension of (x, y, λ) was created, in which (x, y) represents the spatial data while λ represents the spectral data. More specifically, spatial data (x, y) was used for generation of distribution maps while λ was used for value prediction of quality attributes. There are 501 continuous wavebands in all hyperspectral images in the spectral range 328–1115 nm. However, the spectral images in the ranges 328–400 and 1000–1115 nm were noisy due to low response of the CCD detector in the two ranges. Therefore, only spectral data in the range 400–1000 nm (with 381 wavebands) was used for modeling.

2.3. Total pigments analysis

After image acquisition, all meat samples were immediately used for measurement of reference values of total pigments using a spectrophotometer instrument (UV-1800, Shimadzu Corp., Japan) (Lee, Hendricks, & Cornforth, 1999). Ground meat samples (10 g) were first mixed with acid acetone (40 ml acetone, 2 ml deionized water and 1 ml HCl). Then, the mixture was macerated with a glass rod and allowed to stand for 12 h at room temperature. Finally, the extract was filtered with a Whatman No. 102 filter paper (Whatman–Xinhua Filter Paper Co., Ltd, Hangzhou, China), and the absorbance was recorded at 640 nm against an acid acetone blank. In order to achieve accurate measurement, each extract was measured three times, and their averaged value was taken as the final reference value. The total pigments were calculated as hematin using the following formula:

$$\text{Total pigments (ppm)} = A_{640} \times 680 \quad (1)$$

2.4. Image processing

There were three essential steps in image pre-processing. The first step was to calibrate raw hyperspectral images in order to eliminate the influence of dark current from the CCD camera. The second step was to identify a region of interest (ROI) in the calibrated images, and the third step was to extract spectral data in the ROIs. Image pre-processing was achieved with the aid of the