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Monitoring of bacterial contamination on chicken meat surface using a novel narrowband spectral index derived from hyperspectral imagery data

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ABSTRACT

This study presents a novel narrowband spectral index for monitoring bacterial contamination on chicken meat surface. Fresh chicken meats were prepared and stored aerobically in a refrigerator at 4 °C for 11 d. Hyperspectral images and the total viable count (TVC) of bacteria for meat samples were obtained every 24 h. A new two band freshness index (TBFI) method was proposed for developing the bacteria prediction models. Results indicated that the model with the TBFI based on the wavelengths 650 and 700 nm achieved the optimal estimation of TVC ($R^2 = 0.6833$). The TBFI value for each image pixel was calculated using the above two wavelengths, and then used to predict the TVC for the corresponding pixel on the image. Finally, the predicted TVC were visualized to illustrate the temporal variation and spatial distribution of viable bacteria on meat surface over storage. The results demonstrate the promising potential of the developed TBFI for the detection of viable bacteria contamination on chicken meat surface.

1. Introduction

Microbial spoilage of meats is one of the major concerns in food industry. Meat spoilage may be caused by a wide range of reactions, including some that are mainly physical or chemical, others due to action of enzymes or microorganisms (Huis in't Veld, 1996). Hence, meat spoilage is practically unavoidable because the enzymes are endogenous to meat (Kakouri & Nychas, 1994), and the infectious microorganisms may be borne by the animal itself, the people handling the meat, and/or the implements (Oshita, Al-Haq, Kawagishi, Makino, Kawagoe, Ye, Shinozaki, & Hiruma, 2011).

Currently, the shelf life of meat is usually defined by an arbitrary best before date (Van Boxstael, Devlieghere, Berkvens, Vermeulen, & Uyttendaele, 2014), and thus some meat may be disposed of as garbage even though the meat may be still unspoiled after the best before date has lapsed. Therefore, it is necessary to conduct a timely and accurate assessment of the meat quality prior to consumption or disposal.

Sensory attributes such as odor, color, tenderness, texture, etc. are considered as the main quality attributes that influence consumers' overall evaluation of meats (Xiong, Sun, Zeng, & Xie, 2014). Ye, Iino, and Zhang (2015) investigated the changes of odor intensity and total viable counts (TVC) of chicken meats under refrigerated (4 °C) aerobic storage conditions. They found that both the odor intensity and the TVC increased rapidly during storage, and the two parameters were significantly correlated ($R^2 = 0.8617$, $p < 0.001$). This suggests that the increased intensity of odors emitted from the meat might be caused by the increased TVC during storage. Ercolini, Russo, Torrieri, Masi, and Villani (2006) reported that the beef color turned from red to brown when stored under MAP conditions, and the viable counts of the targeted microbial groups (including TVC, Psychrotrophic bacteria, *Pseudomonas* spp., *B. thermosphacta*, *Enterobacteriaceae*, LAB) from beef samples all increased under the storage conditions. Ntzimani, Giatrakou, and Savvaidis (2010) conducted a microbiological and sensory evaluation for the cooked coated chicken fillets by a panel of seven trained assessors on each day of sampling, and found that the populations of TVC, *Pseudomonas* spp., and *Brochothrix thermosphacta* all increased while the scores of sensory evaluation decreased with the storage time, regardless of the different treatment that the samples actually received. Thus, they concluded that the shelf-life of the samples could be determined using both microbiological and sensory analyses. Moreover, Biswas, Chakraborty, Patra, and Dhargupta (2011) found a significant ($p < 0.01$) positive correlation of TVC values with sensory properties like juiciness, texture, flavor and overall acceptability of duck patties (a local food produced with duck meat). Therefore, the bacterial loads including TVC values might indicate the sensory attributes of meats, and hence can be potentially used as the effective parameters to assess the quality of meats.

Many methods are currently available to detect the bacterial loads such as the standard colony-counting method (Sieuwerts, de Bok, Mols, de Vos, & van Hylckama Vlieg, 2008), and several other modern molecular biological and immunological techniques (Liu, Yeung, Chen, Yeh, & Hou, 2013a; Dwivedi & Jaykus, 2011). However, these techniques

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still depend on destructive samplings and need follow a series of complicated chemical procedures, which are not only costly but also time-consuming. Recently, various electronic nose instruments have also been used a non-destructive tool to measure and monitor the quality of meat (Mason, Mukhopadhyay, Jayasundera, & Bhattacharyya, 2014; Ye et al., 2015). However, specific instruments should be used for the specific applications, and this necessitates careful selection of sensor arrays and sampling system (Loutfi, Coradeschi, Mani, Shankar, & Rayappan, 2015). Therefore, there is a need for more alternative effective methods to assess meat quality through estimating the bacterial loads.

Recently, hyperspectral imaging has been widely investigated as a new tool to evaluate the quality and safety of various food and agricultural products (He & Sun, 2015; Barbin, Elmasry, Sun, & Allen, 2012; Cheng & Sun, 2014; Costa, Antonucci, Menesatti, Pallottino, Boglione, & Cataudella, 2013; Elmasry, Iqbal, Sun, & Allen, 2011; Wu, Sun, & He, 2012a; Wu, Peng, Li, Wang, Chen, & Dhakal, 2012b; Jackman, Sun, Du, & Allen, 2008). By combining the digital imaging with conventional spectroscopy into one system, the hyperspectral imaging system can provide both spatial and spectral information on the observed sample simultaneously (Ye & Sakai, 2015; Wu et al., 2012a, b). Hyperspectral imaging has been used to estimate the total viable counts (TVC) of microbes on various poultry, meat and fish products. For example, Grau, Sanchez, Giron, Iborra, Fuentes, and Barat (2011) investigated the use of visible hyperspectral imaging to study the freshness of chicken fillets during storage at 4 °C. Feng and Sun (2013a) carried out a similar study with hyperspectral imaging in the near-infrared wavelength range. Barbin, Elmasry, Sun, Allen, and Morsy (2013) used near-infrared hyperspectral imaging to quantify TVC loads and classify pork samples into fresh and spoiled categories in terms of their bacterial loads. Wu and Sun (2013a, b) studied visible and near-infrared hyperspectral imaging for prediction of TVC on salmon fillets. In addition, various hyperspectral imaging applications have also been employed as a non-destructive tool to evaluate the sensory attributes of meats, such as color (Qiao, Wang, Ngadi, Gunenc, Monroy, Gariepy, and Prasher, 2007; Wu et al., 2012a, b), tenderness (Tao, Peng, Li, Chao, & Dhakal, 2012), texture (Brooks, Belew, Griffin, Gwartney, Hale, Henning, Johnson, Morgan, Parrish, & Reagan, 2000; Kim & Lee, 2003), showing their great potential for assessing the quality of meats.

Due to the high dimensionality of hyperspectral imagery data, a variety of chemometric methods, such as principal component analysis (PCA) (Abdi & Williams, 2010), partial least squares (PLS) regression (Abdi, 2003), least squares support vector machines (LS-SVM) (Suykens & Vandewalle, 1999), etc., have been developed for hyperspectral image processing and data mining. These techniques have proven useful in a wide range of studies by reducing the dimensionality of hyperspectral data (Lorente, Aleixos, Gómez-Sanchis, Cubero, García-Navarrete, & Blasco, 2012; Quevedo & Aguilera, 2010; Liu et al., 2013a; Liu, Zeng, & Sun, 2013b). However, the algorithmic complexity and the necessity of full spectral data in the analysis make these chemometric methods difficult to use in practical application. Particularly, when it comes to the development of instrument for practical use, a simple method that uses a simple algorithm and few spectral channels is more desirable.

This study presents a new method for rapid and non-destructive assessment of meat freshness using hyperspectral imaging. The study chose to study the freshness of chicken meat, which is one of the most commonly consumed meat products in Japan (Koizumi, Kobayashi, Pan, Takaku, Nishino, & Nagano, 2000). Experiments were designed to obtain the hyperspectral data and bacterial contamination levels on meat surface at different storage times. A novel spectral index based on two narrowband wavelengths was developed for estimating the bacterial contamination levels on meat surface. The visualized representation of the predicted bacterial contamination successfully illustrated the temporal variation and spatial distribution of viable bacteria contamination on meat surface over storage. The results suggest the feasibility of the developed simple spectral index for the detection of viable bacteria contamination on meat surface.

2. Materials and methods

2.1. Meat material and sample preparation

Fresh chicken breast meats from one batch (identical origin and packing date) were purchased from a local super market. 44 samples (each weighs approximately 50–60 g) were prepared by cutting and removing the skin, and stored aerobically in a refrigerator at 4 °C for 11 d. Preparation and manipulation of the samples were under aseptic conditions during the experiment.

2.2. Hyperspectral image acquisition

During the storage, hyperspectral images were acquired for 4 of the meat samples (including both the cut surface and the surface after the removal of skin) every 24 h with a hyperspectral imaging system in the laboratory (Fig. 1). The system is composed of an ImSpector V10E spectrograph (Spectral Imaging Ltd., Finland), a 14-bit IMPERX B1410 CCD camera (IMPERX, Inc., USA), a motor-driven mechanical system, a computer and Scanner Unit software (JFE Techno-Research Corporation, Japan). The ImSpector V10E is connected to the computer, and the hyperspectral images are captured by the CCD camera when the sample moves beneath the camera operated by the software controlled motor-driven mechanical system. The hyperspectral images acquired by the system cover the effective wavelength range from 400 to 1000 nm with a spectral resolution of 5 nm. The whole imaging system is under the control of the software, and the recorded hyperspectral images can be further analyzed with the software. The distance between the lens and meat sample is set to 50 cm, and two halogen lamps are positioned at 45–degree angles to the right and left of the stage. The spatial resolution depends on the distance between the sensor and the object to capture, which slightly differs among the samples due to their different thicknesses.

Fig. 2a illustrates the hyperspectral data cube obtained for one of the meat samples. The data cube is a typical three-dimensional form including two spatial dimensions and one spectral dimension. Each frame corresponds to the image of the object at one spectral wavelength. Compared to the common digital camera which usually contains three major broadband wavelengths (red, green and blue) in the visible region, the hyperspectral camera captures several tens to hundreds of narrowband wavelengths spreading from the ultraviolet to the near-infrared wavelength regions (Ye & Sakai, 2015). Therefore, the hyperspectral camera has the advantage of providing a large amount of spectral data that could not be obtained by the common digital camera, and this information may have significant relationships with various properties of the object under inspection. A RGB color image can

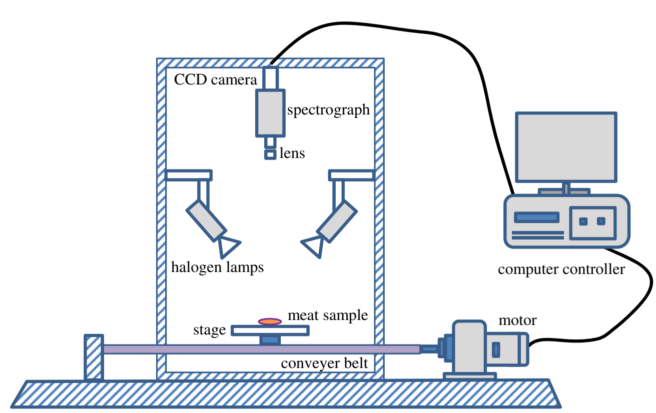

Fig. 1. The schematic diagram of the hyperspectral imaging system.