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Inspection of harmful microbial contamination occurred in edible salmon flesh using imaging technology

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ABSTRACT

The total counts of *Enterobacteriaceae* and *Pseudomonas* spp. (EPC) appeared on edible salmon flesh were determined by using near-infrared (NIR) (900–1700 nm) hyperspectral imaging. Three-dimensional hyperspectral images (x,y,λ) of salmon samples were acquired at different storage time. Spectra (λ) extracted in reflectance (R) unit and two other transformed spectra units, absorbance (A) and Kubelka–Munck (KM), were prepared to relate to the measured EPC data by using partial least square (PLS) regression. Based on the three spectra parameters, three full wavelength PLS models defined as FR-PLS, FA-PLS and FKM-PLS were developed with all correlation coefficients of prediction (R_P) over 0.900. To simplify these models, wavelengths holding the most important information were selected by executing competitive adaptive reweighted sampling (CARS) algorithm. Better performance was found in the resulting simplified R-PLS model (defined as FR_S-PLS model) which was established with only nine important wavelengths (931, 1138, 1175, 1242, 1359, 1628, 1641, 1652 and 1655 nm) selected from R spectra. The absolute difference between root mean square errors of calibration (RMSEC) and prediction (RMSEP) in the FR_S-PLS model was 0.063, less than half (44%) of that of the original FR-PLS model. By applying the FR_S-PLS model to the 2-D images (x,y), EPC distribution maps were generated to visualize the spatial variation of EPC and the adaptability of the FR_S-PLS model for EPC evaluation was further demonstrated with these distribution maps in which different colors indicated different degrees of EPC contamination. To sum up, NIR hyperspectral imaging technology shows a great potential to predict the EPC contamination in salmon flesh. In view of the results obtained from this study, a multi-spectral imaging system could be developed and further refined for online detection applications in fish industry.

1. Introduction

Salmon products are highly perishable, and efforts should be made to ensure their quality and safety, for example, common techniques used in the agri-food industry such as drying (Sun, 1999; Sun and Byrne, 1998; Delgado and Sun, 2002; Sun and Woods, 1997), cooling (Wang and Sun, 2001), freezing (Delgado et al., 2009) and edible coating (Xu et al., 2001) can be used to preserve their quality. However, unpleasant microbial contamination can still happen in salmon products, many of these types of contamination are the results of growth and reproduction of some harmful spoilage microorganisms. *Enterobacteriaceae* and *Pseudomonas* spp. as two main aerobic Gram-negative microorganisms are considered responsible for the microbial spoilage of salmon flesh (Diaz et al., 2011; Mace et al., 2012; Pettersen et al., 2011). With the increasing contamination caused by the two specific microorganisms, the sensory properties of fish flesh such as appearance, colour, odour, flavour and taste deteriorate gradually (Dondero et al., 2004; Fagan et al., 2003; Hansen et al., 1996; Joffraud et al., 2001). Many methods are currently available to detect the spoilage bacteria loads such as standard colony-counting method (Álvarez et al., 2012; Botsoglou et al., 2010), molecular biological technique such as polymerase chain reaction (PCR) (Doulgeraki and Nychas, 2012; Liu et al., 2013), immunological technique such as enzyme-linked immunosorbent assay (ELISA) (Dwivedi and Jaykus, 2011; Kitaguchi et al., 2005) and molecular biology combined with immunological techniques such as PCR-ELISA (Kuo et al., 2010), however, they are still time-consuming, destructive, labour-intensive and inefficient, which cannot meet the requirements of rapid, non-destructive and real-time detection. Although real-time PCR and redox potential measurement have been developed to toward high-speed detection, sample preparation still require longer time (Biassoni and Raso,

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2014; Reichart et al., 2007). On the other hand, development of novel technologies for online real-time microbial detection is necessary and attracting increased attentions by food researchers and producers.

Optical techniques have been introduced as one option for fast and non-invasive applications in food quality evaluation. Among the various optical methods, near-infrared (NIR) spectroscopy has been investigated and its ability of assessing food quality in terms of physical (Ribeiro et al., 2011), chemical (Balabin and Smirnov, 2011) and microbial indices (Alexandrakis et al., 2012) has been demonstrated. In the application of NIR spectroscopy, the detailed spectral information related with tested quality parameters are provided and used to build suitable models to predict the parameters. In spite of this, quality distribution with visual observation still cannot be achieved and that is however very helpful in exhibiting the concentration of these parameters in food products. Although imaging or computer vision (Valous et al., 2009; Costa et al., 2011; Jackman et al., 2008; Sun, 2004) provides spatial information to visualize the distribution of quality parameters, spectral information cannot be achieved and that is critical to quantitatively analyze the food products.

Hyperspectral imaging as another optical technique has emerged to extend the spectroscopy or imaging technology. Hyperspectral imaging for product analysis is based on the acquired hyperspectral image, which is a three-dimensional (3-D) "datacube" (x,y,λ) consisted of a series of 2-D spatial images (x,y) at each individual wavelength (λ) (Wu and Sun, 2013a). The 3-D "datacube" contains a large amount of useful information in which spectral information is used for modelling and spatial information is used for visualisation. With the combination of modelling and imaging functions, a product will be characterized more objectively and reliably. By assembling traditional spectroscopy and computer vision techniques, hyperspectral imaging has been applied as a quick, contactless and reagentless tool in many kinds of products such as fruits and vegetables (Lorente et al., 2012; Wei et al., 2013), meats (Wu et al., 2012c; Kamruzzaman et al., 2011, 2012; ElMasry et al., 2011a, 2011b, 2012), poultry (Feng and Sun, 2013), pharmaceutics (Gendrin et al., 2007), seafood (He et al., 2013b), cereals (Serranti et al., 2013; Shahin et al., 2013), eggs (Abdel-Nour and Ngadi, 2011) and milk (Qin et al., 2012).

As for salmon fish, representing finfish group, quality evaluation using hyperspectral imaging mainly focuses on the physicochemical attributes such as colour (Wu et al., 2012b), fat (Segtnan et al., 2009) pH (He et al., 2014c) and moisture (He et al., 2013a; Zhu et al., 2014). Recently, we presented hyperspectral imaging for prediction of lactic acid bacteria (LAB) growth in salmon fillets with a good performance (He et al., 2014a). To promote the need for further evaluation and control of microbial contamination in salmon industry, this study was conducted to investigate the potential of NIR hyperspectral imaging for determination of EPC presented in edible salmon flesh during the cold storage. The specific objectives of the study were to:

(1) identify the regions of interest (ROIs) of images and isolate the ROIs from the background;
(2) extract the spectral data from the ROIs and transform the reflectance (R) data into absorbance (A) and Kubelka–Munck (KM) data;
(3) analyse the R, A and KM spectra by using multivariate calibration tools to relate to the EPC data measured by standard pour plate method;
(4) develop full wavelength models and examine their performances in EPC prediction;
(5) select the most important wavelengths to simplify the full wavelength models; and
(6) apply the best simplified model to each pixel of 2-D images and produce the EPC distribution maps to visualize the spatial variation of EPC values in salmon flesh.

2. Materials and methods

2.1. Salmon samples and subsampling

Salmon samples were prepared by vacuum-packing fresh edible farmed salmon fillets (*Salmo salar*) ($n = 30$) in plastic trays and transported in an ice chest from a local seafood supermarket to laboratory of Food Refrigeration and Computerised Food Technology (FRCFT), University College Dublin, Ireland, within 30 min. Then, subsampling was conducted by cutting all fish samples into cubes (~10 g) which had the size of 3 cm (length) × 3 cm (width) × 1 cm (thickness). Ninety-four subsamples ($n = 94$) in total were finally obtained, repacked, labelled and then stored in cold condition (4 °C) for further image acquisition and microbiological test.

2.2. Hyperspectral image acquisition

At each testing day (Day 0, 3, 6, 8, 10 and 13), a set number of subsamples (~15) were placed on the moving platform of a lab NIR hyperspectral imaging system and scanned in reflectance mode to acquire the raw hyperspectral images of subsamples. The whole hyperspectral imaging system is mainly composed of a spectrograph (Specim ImSpector N17E, Spectral Imaging Ltd., Oulu, Finland), a CCD camera (SUI Goodrich SU320M-1.7RT, a 12-bit high performance of 320 spatial × 256 spectral), a moving platform (MSA15R-N, AMT-Linearways, SuperSlides & Bushes Corp., India), an illumination unit (V-light, Lowel Light Inc, USA) and a computer installed with an image acquisition software (SpectralCube, Spectral Imaging Ltd., Oulu, Finland). Detailed information on the system can be found in the study of He et al. (2013a). The acquired hyperspectral image of each subsample was comprised of a series of continuous sub-images at wavelength from 897 to 1753 nm. In this work, the hyperspectral image at the wavelength range of 900–1700 nm (239 spectral bands) was used for data analysis because of low signal-to-noise ratio beyond this range.

2.3. Image pre-processing and spectral extraction

The raw hyperspectral images of subsamples were required to be calibrated into reflectance images, as the signal intensity was first collected by the CCD camera of the NIR hyperspectral imaging system. Together with two other reference images, white and black, all hyperspectral images were calibrated using the follow formula:

$$R = \frac{R_0 - R_B}{R_W - R_B} \times 100 \quad (1)$$

where R is the calibrated reflectance image, R_0 is the raw image, R_B is the black image (~0% reflectance) and R_W is the white image (~99.9% reflectance). Among, the R_W was obtained by recording an image of a white tile while the R_B was achieved by covering the lens with cap and then collecting an image after turning off the light source completely.

With the help of ENVI v4.6 software (Research Systems Inc., Boulder, CO, USA), the regions of interest (ROIs) of calibrated hyperspectral images of subsamples were identified and isolated from the background using ROI Tool attached in ENVI software. The spectral profiles of all pixels within each ROI were extracted and averaged into one reflectance spectrum representing the ROI. Then, the reflectance spectra were transformed into A and KM spectra using the following Eqs. (2) and (3), respectively.