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Rapid and real-time prediction of lactic acid bacteria (LAB) in farmed salmon flesh using near-infrared (NIR) hyperspectral imaging combined with chemometric analysis

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ABSTRACT

In this study, hyperspectral imaging working in near-infrared (NIR) region (900–1700 nm) was applied to evaluate surface lactic acid bacteria (LAB) spoilage of farmed salmon flesh during cold storage. Hyperspectral images of salmon samples were acquired at different storage times. Spectral information within regions of interest (ROIs) of images were extracted to relate to reference LAB values measured by standard pour plate method. Least-squares support vector machine (LS-SVM) algorithm was used to calibrate the full NIR range spectral data, resulting in regression coefficients of prediction (R_P) of 0.929 with root mean square error of prediction (RMSEP) of 0.515. Competitive adaptive reweighted sampling (CARS) algorithm was employed to reduce the spectral redundancy and identify the most informative wavelengths (MIWs) most related with LAB prediction across the whole wavelength range. Eight individual MIWs at 1155 nm, 1255 nm, 1373 nm, 1376 nm, 1436 nm, 1641 nm, 1665 nm and 1689 nm were finally selected from the full 239 wavelengths. Based on the selected MIWs, a new optimised model named CARS-LS-SVM was established, leading to R_P of 0.925 with RMSEP of 0.531. At last, the CARS-LS-SVM model was transferred to each pixel of hyperspectral images of samples and colour maps were generated for visualising the LAB spoilage process in salmon flesh. The overall results indicated that NIR hyperspectral imaging is very potential and could be used as a rapid, non-destructive and efficient technique for LAB evaluation in salmon flesh.

1. Introduction

Food quality assurance plays an important role in the food industry as the industry needs to be able to guarantee the quality of the product it produces. Therefore significant efforts have been made by the industry to enhance the quality of agricultural and food products by new techniques and methods such as novel cooling (Sun, 1997; Sun and Brosnan, 1999; Sun and Hu, 2003; Wang and Sun, 2001; Hu and Sun, 2000), freezing (Delgado et al., 2009; Zheng and Sun, 2006), drying (Sun, 1999; Sun and Byrne, 1998; Sun and Woods, 1993, 1994a, 1994b, 1994c, 1997; Delgado and Sun, 2002; Cui et al., 2004) and edible coating (Xu et al., 2001). In particular, microbial evaluation is one of the critical steps for food quality and safety control. Several spoilage microorganisms like *Pseudomonas* spp., *Enterobacteriaceae*, hydrogen sulfide-producing bacteria, *Brochothrix thermosphacta* and Lactic acid bacteria (LAB) are always used as important microbial indexes for food safety assessment, in particular in perishable food products such as fish fillets (Amanatidou et al., 2000; Briones, Reyes, Tabilo-Munizaga, & Pérez-Won, 2010; Mace et al., 2012). LAB is a general name of a clade of Gram-positive, non-motile, non-spore-forming microorganisms, which are rod-shaped bacillus or spherical coccus and characterised by an increased tolerance to acidity. LAB as one group of predominant bacteria is frequently present in salmon flesh. By producing organic acids and ethanol as fermentation end products with LAB group, salmon flesh is easily suffered from sensory quality degradation, mainly referring to flesh souring (Du et al., 2002; Stohr, Joffraud, Cardinal, & Leroi, 2001). When stored under aerobic conditions, salmon flesh is spoiled faster with the increasing growth of LAB colony. Hence, it is very important to conduct LAB evaluation to inspect LAB spoilage in salmon products.

Conventional methods used for LAB determination mainly contain standard pour plate method (Shobharani & Agrawal, 2010), molecular biological technique (Marty, Buchs, Eugster-Meier, Lacroix, & Meile, 2012) and immunological technique (Wagar, Champagne, Buckley, Raymond, & Green-Johnson, 2009). Although these approaches are well-known to be very useful and effective, they are still time-consuming, tedious, invasive and inefficient, and thus not satisfying the increasing requirements of rapid and real-time online inspection. Moreover, these traditional techniques are not suitable for application in the situation where a large number of samples are required to be

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measured. Therefore, novel technology is needed to be developed to meet the demands of both producers and consumers.

Spectroscopic techniques such as Raman spectroscopy (Gaus et al., 2006; Lu, Al-Qadiri, Lin, Rasco, 2011; Sowoidnich, Schmidt, Maiwald, Sumpf, Kronfeldt, 2010) and infrared (IR) spectroscopy (Amiel, Mariey, Curk-Daubié, Pichon, & Travert, 2000; Dziuba, Babuchowski, Nałęcz, & Niklewicz, 2007; Alexandrakis, Downey, Scannell, 2012; Lu, Al-Qadiri, Lin, Rasco, 2011) have been developed as rapid and non-invasive tools for LAB evaluation. However, such optical technologies provide only spectral information related to LAB prediction, but cannot offer spatial information which is especially useful when detailed LAB analysis is required. In recent years, hyperspectral imaging has emerged to integrate spectroscopy and imaging or computer vision technique (Du and Sun, 2005; Valous, Mendoza, Sun, Allen, 2009) into one system, which enables hyperspectral imaging to offer spectral and spatial information of a target object simultaneously. With this technique, a so-called "hypercube" or "datacube" (x, y and λ) described as one-dimensional spectrum (λ) at every two-dimensional pixel (x, y) was created (ElMasry & Sun, 2010; He, Wu, & Sun, 2013b). A target object can be evaluated by analysing the information extracted from the "hypercube", in which spectrum is used for predictive model calibration while spatial pixel is used for image formation.

Many studies on the use of hyperspectral imaging for quality evaluation have been reported in agricultural and food products, for example, determination of chemical attributes such as fat, protein and moisture in red meats (Barbin, ElMasry, Sun, & Allen, 2013; ElMasry, Sun, & Allen, 2013; Kamruzzaman, ElMasry, Sun, & Allen, 2012), compositional analysis in dairy products (Gowen, Burger, O'Callaghan, & O'Donnell, 2009), embryo development evaluation in eggs (Liu & Ngadi, 2012), internal quality assessment in fruits and vegetables (Cen, Lu, Ariana, & Mendoza, 2013; Lorente et al., 2012; Menesatti et al., 2009), infection detection in poultry (Park et al., 2011) and cereals (Bauriegel, Giebel, Geyer, Schmidt, & Herppich, 2011).

As for fish products, in particular salmon fillets, some physical and chemical attributes such as moisture (He, Wu & Sun, 2013a; Zhu, Zhang, Shao, He, & Ngadi, 2014), texture profile (Wu & Sun, 2013b), tenderness (He, Wu, & Sun, 2014a), fat content (Segtnan, Høy, Lundby, Narum, & Wold, 2009), ice fraction (Ottestad, Høy, Stevik, & Wold, 2009), pH (He, Wu, & Sun, 2014b), salt content (Segtnan, Høy, Sorheim, et al., 2009) and water-holding capacity (Wu & Sun, 2013a) as well as fresh classification (Zhu, Zhang, He, Liu, & Sun, 2012) have been studied using hyperspectral imaging technique. However, application of hyperspectral imaging for LAB assessment in salmon fillets or other fish products has never been reported. Given the usefulness and advantage of hyperspectral imaging, the main objective of this study was to investigate the feasibility of hyperspectral imaging in the wavelength range of 900–1700 nm for LAB evaluation in farmed salmon fillets during cold storage. Hyperspectral images containing spectral and spatial information of salmon samples were acquired and used for quantitative analysis. LAB-related spectral information was used for modelling and spatial information was used for LAB visualisation. The specific aims were to: (1) develop a hyperspectral imaging system to assess LAB values in salmon fillets during spoilage process, (2) acquire hyperspectral images of salmon samples which were used as a basis for spectral and spatial analysis, (3) apply chemometric algorithms to build quantitative relationship between spectral data and reference LAB values measured by standard pour plate method, (4) conduct wavelength selection to obtain the most informative wavelengths (MIWs) for reduction of redundant information among continuous wavelength bands, (5) build optimised model based on the selected MIWs, (6) compare the predictive abilities of the original model developed with full wavelengths and the optimised model built with MIWs, and (7) at last, form distribution maps for visualising the LAB spoilage process at different storage times by transferring the best model to hyperspectral images.

2. Materials and methods

2.1. Sample preparation

Thirty-three fresh farmed Atlantic salmon fillets (*Salmo salar*) with length 17 ± 1.5 cm and width 3.0 ± 1.5 cm were originated from Norway and supplied by local fish supermarkets in Dublin, Ireland. The fillets were vacuum-packed and transported in ice boxes to laboratory of Food Refrigeration and Computerised Food Technology (FRCFT), University College Dublin (UCD), Ireland, and then immediately sampled for further experiments. Sampling was conducted through cutting each fillet into several cubes which had size of 3 cm × 3 cm × 1 cm (length × width × thickness, approximate 10 g). One hundred and eighteen samples in total were finally obtained from the tested 33 fillets. The samples were repacked using cling film, labelled and then stored at 4 °C in a lab refrigerator for 0, 2, 4, 6, 8, 10 and 12 days.

2.2. Hyperspectral imaging system and image acquisition

In this study, a line-scan pushbroom hyperspectral imaging system was used for image acquisition of salmon samples in reflectance mode. The components of the hyperspectral imaging system mainly contain a Specim ImSpector N17E spectrograph (Spectral Imaging Ltd., Oulu, Finland) covering a near-infrared (NIR) range of 897–1753 nm (256 spectral bands), a CCD camera with a 12-bit high performance (320 spatial × 256 spectral), a C-mount lens (Xeva 992, Xenics Infrared Solutions, Belgium), an illumination unit that consists of two tungsten halogen lamps (V-light, Lowel Light Inc., USA), a moving table (MSA15R-N, AMT-Linearways, SuperSlides & Bushes Corp., India) driven by a stepping motor (GPL-DZTSA-1000-X, Zolix Instrument Co., China) and a SpectralCube software (Spectral Imaging Ltd., Oulu, Finland) for image acquisition. Detailed information about this system can be found in the study of He, Wu, and Sun (2013a).

In order to obtain hyperspectral images of salmon samples at different storage times for further data analysis, about 17 samples were taken out of refrigerator on each test day. After reaching around room temperature (25 °C), the samples were placed on the moving table and then scanned by the hyperspectral imaging system line by line. The acquired hyperspectral images (n = 118) were three-dimensional 'hypercubes' (x, y and λ), in which (x, y) represents two-dimensional images (spatial information) of salmon samples and λ is one-dimensional wavelength bands (spectral information) from 897 to 1753 nm. The spectral data were used for model calibration to predict LAB values while the spatial information were used as a basis for LAB visualisation.

2.3. Image calibration and spectral extraction

Because of the signal intensity rather than the reflectance spectra first collected by the CCD camera of the hyperspectral imaging system, image calibration was required to calibrate the raw images into reflectance images. To conduct the calibration, two reference images, white and dark, were also required. The white image (about 99.9% reflectance) was obtained by scanning and recording an image of a white ceramic tile. The dark image (about 0% reflectance) was collected by turning off the illumination unit and covering the camera lens with cap. The calibration procedure was carried out by using the Specim Tools attached in the ENVI v4.6 software (Research Systems Inc., Boulder, CO, USA). The raw hyperspectral images were calibrated using the following equation:

$$I_C = \frac{I_R - I_D}{I_W - I_D} \times 100 \quad (1)$$

where I_C, I_R, I_W and I_D represented calibrated image, raw image, white image and dark image, respectively.

After image calibration, the region of interest (ROI) of hyperspectral image of each sample was identified using the ROI function of ENVI