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Efficient integration of particle analysis in hyperspectral imaging for rapid assessment of oxidative degradation in salmon fillet

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ABSTRACT

This study investigated the potential of using hyperspectral imaging technology (900–1700 nm) to determine the thiobarbituric acid (TBA) value and pH for evaluation of lipid oxidation in Atlantic salmon (*Salmo salar*) fillets during cold storage for 0, 3, 6, 9 and 12 days at 1 ± 1 °C. Good results were achieved for both parameters by using partial least square regression (PLSR) calibration models with full spectral region. Two simplified models were then built by using forward stepwise-multiple linear regression (MLR) variable selection method to select 18 and 10 most important wavelengths for TBA value and pH, respectively. The optimised stepwise-MLR model for TBA value yielded satisfactory results with correlation coefficient (r_C) of 0.921 and root mean square error of calibration (RMSEC) of 1.840 μmol MDA/kg fish. This model was used to visualise the TBA value distributions during different storage days. Further improvements were achieved by applying particle analysis on the images to extract only the spectra from white stripes in salmon fillet. When in tandem with detrend pre-processing technique, the calibration model based on particle analysis demonstrated the best performance for TBA value prediction ($r_C = 0.957$ and RMSEC = 1.449 μmol MDA/kg). In addition, a novel chemometric strategy accomplished by the use of hyperspectrograms was also proposed in this work and satisfactory results were obtained. The overall results confirmed the capability of near-infrared hyperspectral imaging as a rapid and non-invasive technique to monitor lipid oxidation in salmon fillets.

1. Introduction

Among fish products, salmon has always been regarded as a gourmet fish species with beneficial and special health effects. Fresh salmon is extremely perishable (Alishahi and Aïder, 2012) and therefore techniques such as refrigeration (Sun and Eames, 1996; Wang and Sun, Nov 2002, 2004; Kiani and Sun, 2011; Zheng and Sun, 2004) and drying (Cui et al., 2008; Delgado and Sun, 2002) can be used to preserve its quality. In particular, low temperature storage is one of the primary methods to maintain fish freshness. Due to the large amount of polyunsaturated fatty acid in fish lipids, salmon is highly susceptible to undergoing lipid oxidation during cold storage. Traditionally, lipid oxidation can be monitored by using chemical analysis to measure some critical oxidative parameters. One of the most significant products from secondary lipid oxidation in food is malondialdehyde (MDA), which has shown to be carcinogenic and mutagenic (Halliwell and Chirico, 1993). Thiobarbituric acid (TBA) test, which is based on spectrophotometric quantitation of the pink MDA-TBA complex, has been widely used to measure MDA in food products and biological samples. In spite of its relatively precise result, this method is normally time-consuming, labour-intensive and requires a large amount of hazardous analytical reagents and chemical solvents.

Recently, hyperspectral imaging (HSI) technique has emerged as a state-of-the-art tool to non-invasively and rapidly assess and control food quality (Sun, 2010; Barbin et al., Jan 2012; Wu et al., 2012; Barbin et al., Mar 2012; Wu and Sun, 2013b; ElMasry et al., 2012a, b; Kamruzzaman et al., 2012). Cheng et al. (2015) investigated the use of HSI technique for rapid determination of TBA value in farmed grass carp fillets. A PLSR calibration model was established and it showed good performance. In this study (Cheng et al., 2015), the spectral range of 400–1000 nm was used and two chemometric methods, namely PLSR and MLR, were applied to build models. To further improve this research, different spectral regions and more analysis strategies should be investigated. In addition, it

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is useful and valuable to explore more parameters for evaluating oxidative changes during cold storage based on HSI system.

Unlike grass carp, Atlantic salmon is a fat-rich fish with a large proportion of lipids congregated in white stripes of connective tissue (myocommata), segmenting the red-coloured muscle tissue in vertical blocks and presenting a zebra-like appearance (Borderías et al., 1999). Previous studies have shown that the proportion of myocommata in a salmon fillet correlated well with its fat content (Segtnan et al., 2009). Stien et al. (2007) developed a method for the automatic measurement of fat content in salmon fillets using computer imaging analysis that is often used for food quality evaluation (Costa et al., 2011; Sun, 2004; Jackman et al., 2009; Wang and Sun, May 2002), and confirmed that fat content could be estimated based on image analysis to determine the area of the white stripes visible on the surface compared to the total area of the fillet. In light of the research (Stien et al., 2007), it can be inferred that the spectra extracted from white stripes could be highly correlated with TBA values of salmon fillets. However, to the best of our knowledge, no investigation has been reported to determine TBA value in salmon fillet by general HSI technique so far, not to mention the investigation of using the spectra extracted only from the pixels of the white stripes in hyperspectral image to predict TBA value.

Nowadays, a frequently-adopted method involves extracting spectra from user-defined regions of interest (ROIs) and averaging all extracted spectra to represent one hyperspectral image. This approach sometimes leads to satisfactory results, especially for homogeneous materials, but it also leads to losing the information related to spatial variability (Ferrari et al., 2014). In order to develop a fast and easy-to-use tool able to analyse large datasets of hyperspectral images while maintaining both spectral- and spatial-related information, the use of hyperspectrograms was proposed as an approach, which automatically converted each hyperspectral image into a signal and it was proven the effectiveness of the hyperspectrogram-based approach to address a calibration and a defect detection issue in food samples (Ferrari et al., 2013). However, this novel chemometric method has been scarcely applied in the fish industry.

In order to maintain global competitiveness and obtain cost reduction, rapid and accurate monitoring and control of quality changes during cold storage is of great importance for the salmon industry. Therefore, the objectives in our study were (1) to build hyperspectral imaging (900–1700 nm) calibration models to simultaneously predict TBA value and pH changes in Atlantic salmon (*Salmo salar*) fillets during cold storage; (2) to identify effective wavelengths for these two parameters and to generate distribution maps of TBA value in salmon fillets during storage; (3) to implement particle analysis to explore the possibility of using the spectra extracted from the pixels within the white stripes to establish calibration model for TBA value prediction, and (4) to investigate the suitability of applying hyperspectrogram-based approach to predict TBA value and to compare all the results achieved from different methods mentioned above.

2. Materials and methods

2.1. Samples preparation

A total of 150 fresh Atlantic salmon (*S. salar*) fillets originated from Norway were labelled and then directly transported to laboratories of Food Refrigeration and Computerized Food Technology (FRCFT), University College Dublin (UCD), Ireland. All the samples were packed into the sealed plastic bags and stored at −18 °C for three months. After frozen storage, fillets were thawed and randomly divided into five groups subjected to cold storage for 0, 3, 6, 9 and 12 days at controlled refrigerated conditions (1 ± 1 °C).

2.2. Hyperspectral image system

Spectral images of the prepared samples were acquired in the reflectance mode by employing a laboratory-based pushbroom hyperspectral imaging system shown in Fig. 1. The core components of the system included: an imaging spectrograph (ImSpector, N17E, Spectral Imaging Ltd, Oulu, Finland) collecting spectral images in a wavelength range of 900–1700 nm, a high performance camera with C-mount lens (Xeva 992, Xenics Infrared Solutions, Leuven, Belgium), two tungsten-halogen illuminating lamps (V-light, Lowel Light Inc., New York, USA), a translation stage operated by a stepper motor (GPL-DZTSA-1000-X, Zolix Instrument Co., Beijing, China), and a computer installed with a data acquisition software (SpectralCube, Spectral Imaging Ltd., Oulu, Finland). The spectrograph with a spectral increment of approximately 3.34 nm between contiguous bands producing overall 256 bands had a fixed-size internal slit (30 μm) to denote a field of view (FOV) for the spatial line (horizontal pixel direction).

2.3. Image acquisition and calibration

Each salmon sample was placed on the translation stage and then conveyed to the FOV of camera with a speed of 2.7 cm/s to be scanned line by line. Exposure time of the camera, frame rate and the motor speed were carefully selected to obtain equal pixel resolution of the horizontal and vertical axes and to avoid distortions of images. When each sample moved into FOV, a corresponding three-dimensional image named 'hypercube' with one spectral dimension (λ) and two spatial dimensions (x, y) was acquired and subsequently recorded and stored in a raw format.

Meanwhile, two additional standard images, namely the white Teflon tile (ca. 99% reflectance) and the dark current (ca. 0% reflectance) were also acquired to eliminate the impacts of bright and dark background on the raw images (R_0). The calibrated image (R_S) of the sample was calculated using the following formula with the aid of the two standard images obtained as aforementioned (Kamruzzaman et al., 2015):

$$R_S = \frac{R_0 - R_b}{R_w - R_b} \times 100\% \quad (1)$$

where R_b is the dark current image recorded with the light source all off and the camera lens completely covered with its opaque cap and R_w is the white reference image achieved from a white Teflon tile as reference. All calibration, models extraction routines and multivariable data analysis were programmed in Matlab 7.7 R2008b software (The Mathworks Inc., MA, USA).

2.4. Measurement of pH and TBA values

After being scanned by the hyperspectral imaging system, the reference values of pH and TBA of the fish fillet samples were immediately determined. The pH value was evaluated by a benchtop pH metre (Orion 520Aplus, Thermo Fisher Scientific Inc., Fort Collins, Colorado USA). Three pH readings were averaged and the mean value was recorded as the ultimate pH value for each sample.

Afterwards, TBA value was determined according to the procedure described by Vyncke (1970) with some modifications. In the current study, perchloric acid was used in place of trichloroacetic acid (TCA) as recommended by Salih et al. (1987). Approximately two grams of salmon flesh were minced with 15 ml 5% perchloric