



Analytical Methods

Suitability of hyperspectral imaging for rapid evaluation of thiobarbituric acid (TBA) value in grass carp (*Ctenopharyngodon idella*) fillet

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ABSTRACT

The suitability of hyperspectral imaging technique (400–1000 nm) was investigated to determine the thiobarbituric acid (TBA) value for monitoring lipid oxidation in fish fillets during cold storage at 4 °C for 0, 2, 5, and 8 days. The PLSR calibration model was established with full spectral region between the spectral data extracted from the hyperspectral images and the reference TBA values and showed good performance for predicting TBA value with determination coefficients (R^2) of 0.8325 and root-mean-square errors of prediction (RMSEP) of 0.1172 mg MDA/kg flesh. Two simplified PLSR and MLR models were built and compared using the selected ten most important wavelengths. The optimised MLR model yielded satisfactory results with R^2 of 0.8395 and RMSEP of 0.1147 mg MDA/kg flesh, which was used to visualise the TBA values distribution in fish fillets. The whole results confirmed that using hyperspectral imaging technique as a rapid and non-destructive tool is suitable for the determination of TBA values for monitoring lipid oxidation and evaluation of fish freshness.

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1. Introduction

It is well-known that fish is always considered as a significant source of high quality animal proteins for human nutrition and consumption. Particularly, fish is also an important and nutritional seafood due to the natural and high concentrations of polyunsaturated ω -3 fatty acids (PUFA), such as eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3), which have been proved to have beneficial and special health effects to prevent cardiovascular disease, lower cholesterol levels and blood viscosity, and strengthen memory and thinking ability for humans (Karlsdottir et al., 2014; Iglesias & Medina, 2008). However, owing to the high content of PUFA, coupled with extremely active existing pro-oxidants, fish is very vulnerable to lipid oxidation. Degradation of PUFAs caused by self-acting or enzymatic oxidation during diverse storage conditions and processing operations can easily result in the formation of undesirable oxidation products such as peroxides, hydroperoxides, conjugated dienes/trienes, aldehydes, ketones, and others (Alishahi & Aider, 2012; St. Angelo, Vercellotti, Jacks, & Legendre, 1996). They are able to modify fish

muscle components and to generate rancidity and hazardous substances (de Abreu, Losada, Maroto, & Cruz, 2011). For this reason, lipid oxidation has been acknowledged as a leading cause of freshness loss and quality deterioration in fish muscle. Therefore, measurement and evaluation of lipid oxidation of fish is an obviously important task.

There are various analytical methods and techniques available for the determination and assessment of lipid oxidation in muscle foods. Traditionally, the methods currently used for monitoring lipid oxidation are based on chemical analysis to determine some important oxidative parameters that are able to provide useful and accurate information on reflecting lipid oxidation degree (Klaypradit, Kerdpiroon, & Singh, 2010; Karoui & Blecker, 2011). One of the most important products of lipid oxidation is malondialdehyde (MDA), which is considered to be a carcinogenic initiator and mutagen (Halliwell & Chirico, 1993). MDA has been used as an indicator of oxidative damage in biological samples and muscle foods (Guillen-Sans & Guzman-Chozas, 1998) and the pink fluorescent MDA-thiobarbituric acid (MDA-TBA) complex produced after reaction with 2-thiobarbituric acid (TBA) at low pH and high temperature is commonly measured (Fernández, Pérez-Álvarez, & Fernández-López, 1997). Although this TBA value evaluation method can provide relatively precise analysis and valuable measurement, it is usually time-consuming, work-intensive and requires the use of large amounts of chemical solvents and analytical

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reagents that might be hazardous and harmful to analysts and the lab environment (Balasubramanian & Panigrahi, 2011; Iñón, Garrigues, Garrigues, Molina, & de la Guardia, 2003). Therefore, it is very difficult to be employed on-line in a rapid and non-destructive manner.

Recently, hyperspectral imaging technique as an emerging and innovative tool has been increasingly used to non-destructively and rapidly determine and evaluate food quality and safety (Cheng et al., 2013; Cheng & Sun, in press; Barbin et al., 2012; ElMasry et al., 2011; ElMasry, Sun, & Allen, 2011; ElMasry, Sun, & Allen, 2012; Kamruzzaman, ElMasry, Sun, & Allen, 2011; Kamruzzaman, ElMasry, Sun, & Allen, 2012). The hyperspectral imaging system integrates the traditional spectroscopy and imaging or computer vision (Du & Sun, 2005; Jackman, Sun, Du, & Allen, 2008; Sun & Brosnan, 2003; Valous, Mendoza, Sun, & Allen, 2009) technique into one system and provides a hypercube $I(x, y, \lambda)$ including spatial (x and y) and spectral (λ) information simultaneously, which presents a three-dimensional (3D) dataset that contains many images of the same object, and each of which is measured at a different wavelength (ElMasry, Kamruzzaman, Sun, & Allen, 2012; Sun, 2010). In addition, grass carp (*Ctenopharyngodon idella*) as one kind of popular freshwater fish is widely farm-cultured in China and has also been introduced and accepted in Europe and America due to its rapid growth rate, easy cultivation, high yield, and low price as well as high nutritional values (Cheng, Qu, Sun, & Zeng, 2014). Accordingly, some exploratory investigations about using hyperspectral imaging have been successfully conducted for quality evaluation of grass carp fillets based on some important parameters such as colour (Cheng, Sun, Pu, & Zeng, 2014), textural firmness (Cheng et al., 2014), and total volatile basic nitrogen (TVB-N) value (Cheng, Sun, Zeng, & Pu, in press). Based on these studies, it has been proved that hyperspectral imaging technique shows the potential for rapid and non-destructive assessment and analysis of fish quality and safety. However, to the best of our knowledge, no investigation on determination of TBA value in grass carp fillet using hyperspectral imaging technique has been reported until now.

Therefore, the purpose of this study was first to investigate the suitability of using hyperspectral imaging (400–1000 nm) for non-destructive and rapid determination of TBA value for evaluation of lipid oxidation in farmed grass carp fillets and to generate the distribution map of TBA value in grass carp fillets with multivariate analysis.

2. Materials and methods

2.1. Fish samples preparation

Eighteen farmed fresh grass carps with similar age of three months, approximately weight of 1.5 kg, and feeding environment from the same freshwater aquaculture ponds were purchased in a local market in Guangzhou, China, and directly transported to the laboratory alive in water using a big plastic bucket within 15 min. Upon arrival, the fishes were stunned by a sharp blow to the head with a wooden stick and then gill cutting. The internal organs were removed at the same time with bloodletting from the fish belly location. Then they were immediately beheaded, skinned, and filleted and then washed with cold water. In order to acquire more fish samples, the fresh fillets were instantly subsampled into a cuboid shape with analogous sizes of 3.0 cm × 3.0 cm × 1.0 cm (length × width × thickness). Consequently, a total of 180 subsamples of fish fillets were obtained from different locations of tested fish fillets. In order to acquire a practical and full range of TBA values indicating the fish flesh from fresh (fully acceptable) to badly oxidative (totally unacceptable) for further establishing better cal-

ibration and prediction models, all the subsamples were labelled and packaged into the sealed plastic bags and randomly divided into four groups subjected to cold storage for 0, 2, 5, and 8 days at 4 ± 1 °C in a refrigerator (Haier Company, Qingdao, China). Among these 180 subsamples, two thirds samples ($n = 120$) were used to create the calibration models and the remaining one third samples ($n = 60$) were used to establish the prediction model. Each group having 45 subsamples were first scanned by the hyperspectral imaging system and then these subsamples were immediately used to measure the reference TBA values using the traditional method described below.

2.2. Evaluation of TBA value

In this study, lipid oxidation was monitored by the evaluation of thiobarbituric acid reactive substances (TBA-RS) according to the procedure of Salih, Smith, Price, and Dawson (1987) with some modifications. Five grams of grass carp fillet muscle was minced and then mixed with 25 mL of trichloroacetic acid (20%) and 20 mL of distilled water for centrifuging for 10 min with the revolving speed of 8000 rpm, and the filtrate was diluted with ultrapure water to 50 mL. The mixture of 10 mL of diluent and 10 mL of thiobarbituric acid solution was heated in a boiling water bath (95–100 °C) for 15 min to develop a pink colour, and then cooled with running tap water for 5 min. The absorbance of the cooled supernatant was measured at 532 nm by a spectrophotometer (UV-1800, Shimadzu, Instruments of Mfg. Co. Ltd., Suzhou, China). A standard curve was prepared using 1,1,3,3-tetramethoxypropane at a concentration ranging from 0 to 10 ppm, and the amounts of TBA-RS were expressed as mg of MDA/kg sample.

2.3. Hyperspectral imaging system

A typical lab push-broom hyperspectral imaging system was used to acquire hyperspectral images of grass carp fillets in reflectance mode. This system is mainly composed of a line-scanning imaging spectrograph (Inspector V10E, Spectral Imaging Ltd., Oulu, Finland) covering the spectral range of 308–1105 nm, a high performance charge-coupled device (CCD) camera (DL-604M, Andor, Ireland) attached with the effective resolution of 1004×1002 pixels, a camera lens (OLE23, Schneider, German), an illumination unit consisting of two 150 W halogen lamps (2900-ER, Illumination Technologies Inc., New York, USA) equipped with a fiber optical line light situated at an angle of 45° to light the moving platform controlled by a stepping motor (IRCP0076-1COMB, Isuzu Optics Corp., Taiwan, China), and a computer control system with hyperspectral image data acquisition software (Spectral Image software, Isuzu Optics Corp., Taiwan, China). The software could regulate the exposure time, motor speed, combining mode, wavelength range, and image acquisition. In this study, the actual working spectral range of this hyperspectral imaging system was 308–1105 nm with a spectral increment of about 1.58 nm between the contiguous bands, thus generating a total of 501 bands. However, according to the visual inspection of spectral information of the acquired hyperspectral images, there is a low signal-to-noise at both ends of the spectral range that would influence the further reliability and prediction ability of models. Therefore, the spectral range of 308–399 nm and 1001–1105 nm were removed and the effective spectral range of 400–1000 nm with a total of 381 wavebands (variables) was considered and used for further analysis.

2.4. Image acquisition and calibration

For each group fish samples, 45 cubed subsamples were placed on the moving platform and then conveyed to the field of the view

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