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Integration of classifiers analysis and hyperspectral imaging for rapid discrimination of fresh from cold-stored and frozen-thawed fish fillets

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ABSTRACT

The investigation of visible and near infrared hyperspectral imaging (400–1000 nm) coupled with classifiers and spectral pre-processing techniques was conducted to discriminate fresh from cold-stored (4 °C for 7 days) and frozen-thawed (−20 °C and −40 °C for 30 days) grass carp fish fillets. Four classifiers with four spectral pretreatment methods were applied to establish the classification models. Compared with the original models established using the full wavelengths, the classification models with three classifiers of soft independent modeling of class analogy (SIMCA), least squares-support vector machine (LS-SVM) and probabilistic neural network (PNN) in tandem with the first derivative pretreatment showed the best classification performance and the highest correct classification rate (CCR) of 94.29%. In addition, in order to reduce the high dimensionality of hyperspectral images, seven optimal wavelengths were selected by successive projections algorithm (SPA) and used to simplify the classification models. The simplified model obtained by the LS-SVM classifier coupled with the first derivative pre-processing method also presented good prediction accuracy with the CCR of 91.43%. The results demonstrated that the integration of hyperspectral imaging and classifiers analysis had a great potential for on-line detection and was feasible to rapidly and non-invasively discriminate fresh and frozen-thawed fish fillets.

1. Introduction

Fish and fish products play an important role in human nutrition, being the basis of the diet in many countries. The main existing problem with this kind of muscle food is its high perishability. In addition, fishing grounds and the target aquatic product markets are sometimes far apart. Therefore, efficient storage and transportation techniques and methods are very necessary.

Like drying (Sun and Byrne, 1998; Sun and Woods, 1997; Delgado and Sun, 2002a,b), cooling (Sun, 1997a,b; Sun et al., 1996; McDonald and Sun, 2001; McDonald et al., 2001) and edible coating (Xu et al., 2001), freezing (Kiani and Sun, 2011) as an excellent preservation method has been widely used to ensure agri-food product quality, and thus is often used for reducing the risks linked to microbial growth and extending the shelf-life of the fish (Le Grandois et al., 2013). Especially, the fishery industry heavily depends on the cold chain to ensure the commercial viability of the fishery products. However, most of the consumers prefer fresh fish despite its higher price due to the fact that, during freezing storage and thawing process, fish muscle usually suffers from some irreversible changes of protein degradation and lipid oxidation, which directly influence the sensory and nutritional quality of the product as well as the shelf-life (Ottavian et al., 2013a). With this regard, the storage at different low temperatures is an important authenticity issue. As using cold-stored in particular frozen-thawed fish to substitute the fresh products is an unfair competition behavior in the trade and a commercially fraudulent practice that not only damages consumers from an economical point of view, but can also cause food safety issues (Ottavian et al., 2013b).

The quality of fishery products depends greatly on the freshness of the raw material. Techniques and methods measuring and documenting the quality of fish in production would be of great value to consumers and the fishery industry. Thus, identification and classification of fresh fish from cold-stored and frozen-thawed ones is

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urgently needed. Although analytical methods have been employed such as solid-phase microextraction gas chromatography–mass spectrometry (SPME/GC/MS) for differentiation of fresh and frozen-thawed cultured gilthead sea bream (*Sparus aurata*) (Iglesias et al., 2009) and for recognition of European sea bass (*Dicentrarchus labrax*), gilthead seabream (*S. aurata*), cod (*Gadus morhua*) and salmon (*Salmo salar*) (Leduc et al., 2012), these methods normally suffer from disadvantages such as being time-consuming, tedious, and destructive. As an alternative to the abovementioned techniques, non-destructive spectroscopic technologies have been successfully developed for differentiating the fresh from frozen-thawed fish (Uddin and Okazaki, 2004; Karoui et al., 2006; Dalle Zotte et al., 2014; Fuentes et al., 2013). For example, Uddin and Okazaki (2004) classified the fresh and frozen-thawed horse mackerel with correct separation of 100% using near infrared spectroscopy coupled with principal component analysis (PCA) and stepwise multiple linear regression (MLR). Uddin et al. (2005) developed a visible/NIR spectroscopy system equipped with a surface interactance fiber-optic accessory in tandem with soft independent modeling of class analogy (SIMCA) and linear discriminant analysis (LDA) for classification of fresh or frozen-thawed red sea bream, and a classification accuracy of 100% was achieved based on the original absorbance spectral data. Nevertheless, unlike imaging or computer vision (Sun and Brosnan, 2003; Valous et al., 2009; Jackman et al., 2008; Sun, 2004; Jackman et al., 2009; Wang and Sun, 2002), spectroscopic techniques cannot offer spatial information distribution of the object. As an innovative platform technology, hyperspectral imaging (HSI) has been developed for food quality and safety control and evaluation especially for the quality of meats (Kamruzzaman et al., 2011, 2012b; ElMasry et al., 2011a,b, 2012; Barbin et al., 2012a; Wu et al., 2012). As a rapid, non-destructive, and non-contact tool, this emerging technique integrates the traditional spectroscopy and imaging method into one system and provides the spectral and spatial information simultaneously with a three-dimensional (3D) hypercube (Sun, 2010; Lorente et al., 2012; Liu et al., 2014). In recent years, a number of studies have highlighted the performance of HSI coupled with appropriate chemometric multivariate analysis for the categorization of pork quality (Liu et al., 2010), recognition of fresh and frozen-thawed longissimus dorsi muscles of pork (Barbin et al., 2013), grading and classification of pork muscles (Barbin et al., 2012b), classification of pork fat samples from different subcutaneous layers (Foca et al., 2013), categorization and authentication of pork, beef and lamb (Kamruzzaman et al., 2012a), and detection of expired vacuum-packed smoked salmon based on partial least square discriminant analysis (PLS-DA) (Ivorra et al., 2013). However, very little research work has been reported in rapid discrimination of fresh from frozen-thawed grass carp (*Ctenopharyngodon idella*) fillets using HSI. The only study conducted by Zhu et al. (2013) used the HSI in the spectral region of 380–1030 nm to differentiate between fresh and frozen-thawed halibut (*Psetta maxima*) fillets combined with only one classifier of least squares-support vector machine (LS-SVM), however, the classification performance was strongly affected by the fish species under investigation, the integrity of the product (whole fish or fillet) or by its shelf life that influences the absorbance of substance and the selected characteristic wavelengths.

Therefore, in order to explore the feasibility of hyperspectral imaging on grass carp fish species and to compare the different classifiers and spectral pretreatment methods for contribution to the classification accuracy, the aim of this work was to develop the HSI in the spectral range of 400–1000 nm for rapid discrimination of fresh and thawed grass carp fish fillets coupled with four classifiers of soft independent modeling of class analogy (SIMCA), partial least square discriminant analysis (PLS-DA), least squares-support vector machine (LS-SVM) and probabilistic neural network (PNN).

2. Material and methods

2.1. Fish fillets preparation

Fifteen fresh grass carp fish from the same batch with similar breeding environment and weight (approximately 1.5 kg) were purchased from a local aquatic products market in Guangzhou, China, and directly transported to the laboratory alive in water within 15 min. Upon arrival, the fish samples 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 belly location of the grass carp. The fish samples were then immediately beheaded, filleted, skinned, and washed with cold water. Thirty fresh fish fillets each with similar dimension were acquired. In order to obtain more fish samples, the fresh fillets were rapidly subsampled into a rectangular shape with the same size of 3.0 cm × 3.0 cm × 1.0 cm (length × width × thickness). As a result, a total of 120 subsamples of fish were obtained and randomly divided into four groups (each group 30 subsamples). The first group subsamples (G1) was used as fresh or unfrozen samples soon after subsampling. The second group subsamples (G2) was used for cold-stored at 4 °C for 7 days. The third and fourth group subsamples (G3 and G4) each were frozen-stored at the constant low temperature of −20 and −40 °C for 30 days. The subsamples were put into the plastic bags to prevent drying of the surface during cold storage. After 30 days, the frozen subsamples were thawed at 4 °C for 12 h. All samples were allowed to equilibrate to room temperature before scanning by the HSI system. Among the 120 samples, two thirds of subsamples including 20 G1, 20 G2, 20 G3 and 20 G4 were randomly selected to build the calibration set, and the remaining one third subsamples were used to create the prediction set.

2.2. Image analysis

2.2.1. Hyperspectral imaging system

A laboratory HSI system was used to acquire the hyperspectral images of grass carp fillets in the reflectance model. The system consisted of a line-scanning imaging spectrograph (Imspector V10E, Spectral Imaging Ltd., Oulu, Finland) covering the spectral range of 308–1105 nm with the spectroscopic resolution of 2.8 nm; a charge-coupled device (CCD) camera (DL-604M, Andor, Ireland) with the effective resolution of 1004 × 1002 pixels by 12 bits and its corresponding camera lens (OLE23, Schneider, German) with the focal length of 23 mm; an illumination source including two 150 W halogen lamps (3900-ER, Illumination Technologies Inc., New York, USA) attached to a fiber optical line light located at a certain angle to illuminate the mobile platform monitored by a stepping motor (IRCP0076-1COMB, Isuzu Optics Corp., Taiwan, China); a computer control system (Spectral Image software, Isuzu Optics Corp., Taiwan, China) regulating the exposure time, motor speed, combining mode, wavelength range and hyperspectral image acquisition. In this study, the working wavelength region of HSI system was 308–1105 nm with a spectral increment of approximately 1.58 nm between the contiguous bands, thus generating a total of 501 bands. However, due to the reduced CCD detector sensitivity in the spectral range of 308–399 nm and 1001–1105 nm, there was much observed noise, which carried some useless information and influence the reliability and accuracy of classification models. Therefore, the redundant bands were rejected and the spectral range of 400–1000 nm with a total of 381 variables was used for further analysis.