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Developing a multispectral imaging for simultaneous prediction of freshness indicators during chemical spoilage of grass carp fish fillet

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ABSTRACT

This study investigated the feasibility of developing a multispectral imaging method using key wavelengths from hyperspectral images for modeling and simultaneously predicting total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS) and K value in grass carp fillet during chemical spoilage. The established least-squares support vector machine (LS-SVM) and multiple linear regression (MLR) models using five successive projection algorithm (SPA)-selected and six genetic algorithm (GA)-selected wavelengths showed excellent performances for predicting TVB-N and K value with $R^2_P > 0.900$ and RPD > 3.000, and poor results for TBARS value prediction. The LS-SVM model using six GA-selected wavelengths showed good reliability and was considered the best for simultaneous determination of TVB-N, TBARS and K value. The distribution maps of chemical spoilage changes were generated using image processing algorithms. The results demonstrated the feasibility of developing a rapid and on-line multispectral imaging system using the feature wavelengths and chemometrics analysis.

1. Introduction

Fish muscle is especially attractive to health conscious consumers due to its lower fat and cholesterol content and higher polyunsaturated fatty acids compared to other types of meats. However, fish is an extremely vulnerable and perishable seafood, which is easy to cause the spoilage processes and the freshness loss, therefore reservation techniques and methods such as cooling (Sun and Wang, 2000; Desmond et al., 2000; Zheng and Sun, 2004; Wang and Sun, 2004), freezing (Kiani et al., 2011) and drying (Delgado and Sun, 2002; Cui et al., 2008) are used to ensure product quality and safety. The spoilage of fish is a multifaceted process that is implicated in physical, chemical and microbiological mechanisms, which is related to color changes, texture collapse, protein deterioration, lipid oxidation, ATP degradation and microbial spoilage (Ghaly et al., 2010; Alishahi and Aïder, 2012). Among them, chemical spoilage plays an important role in indicating fish freshness degree. As one of the important chemical indicators, the value of total volatile basic nitrogen (TVB-N) is usually used for evaluation of fish protein degradation during cold storage (Castro et al., 2006; Dhaouadi et al., 2007). TVB-N mainly contains ammonia, trimethylamine and dimethylamine and its levels increase with spoilage by either bacterial or enzymatic activities (Özoğul and Özoğul, 2000). Thiobarbituric acid reactive substances (TBARS) index is normally used to determine the secondary oxidative products for interpreting further lipid oxidation degree (de Abreu et al., 2011). Malondialdehyde (MDA) is commonly used as an indicator of oxidative damage in biological samples and muscle foods (Guillen-Sans and Guzman-Chozas, 1998; Costa et al., 2011). The most used

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method for measurement of MDA is the spectrophotometric determination of the pink fluorescent MDA-TBA complex produced after reacting with 2-TBA at low pH and high temperature (Fernández et al., 1997). K value is an important biochemical index, which has been widely used for fish chemical spoilage and freshness assessment based on ATP degradation. The K value evolves from the quantification of adenosine-5′-triphosphate (ATP) and its breakdown products, namely adenosine-5′-diphosphate (ADP), adenosine-5′-monophosphate (AMP), inosine-5′-monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx) (Lowe et al., 1993). On the basis of the obtained concentrations of ATP and ATP-related breakdown compounds, K value is generally defined as the percentage rate of HxR and Hx to the sum of ATP and corresponding degradation products.

Based on the importance of these mentioned chemical indicators, their measurement methods and techniques used for evaluating freshness loss and quality deterioration in fish muscle are obviously significant. In the currently used traditional methods, TVB-N value is determined by a stream distillation method, TBARS value is usually measured at 532 nm by a spectrophotometer, while ATP-related breakdown products are commonly determined by high performance liquid chromatography (HPLC) analysis. These above-mentioned methods provide an effective and accurate measurement of these chemical indicators for fish freshness evaluation and inspection. Even some of them have been used as excellent standards for freshness control. However, they are usually time-consuming, tedious and destructive. Some of them need high-skilled operators and precise control of the experimental conditions. Obviously, these methods are not suitable for on-line and real-time monitoring in a rapid and non-destructive manner. Therefore, development of rapid and non-destructive inspection imaging system for simultaneous determination of chemical indicators for comprehensive evaluation of fish freshness loss is of great importance for the industry.

In recent years, by combining computer vision (Wu and Sun, 2013a; Jackman et al., 2009a,b; Wang and Sun, 2002) and spectroscopic techniques into one system, hyperspectral imaging (HSI) as an innovative and powerful tool has been investigated for rapid and non-invasive determination and evaluation of food quality and safety (Cheng et al., 2013; Liu et al., 2014; Menesatti et al., 2010; Wu et al., 2012; Barbin et al., 2012, 2013; ElMasry et al., 2012a,b; Wu and Sun, 2013b; Kamruzzaman et al., 2012; Feng and Sun, 2012, 2013; Liu et al., 2014; Feng et al., 2013). In our previous studies, the value of TVB-N (Cheng et al., 2014c), K value (Cheng et al., 2015a) and TBARS (Cheng et al., 2015b) were successfully and effectively determined and estimated using the HSI technology, showing the feasibility and potentiality of using the HSI method to monitor the fish chemical spoilage during cold storage. However, these previous studies (Menesatti et al., 2010; Cheng et al., 2014c, 2015a, 2015b; Liu et al., 2014; Lorente et al., 2012) only focused on measurement of single chemical indicator using the full and selected wavelengths. In addition, the mentioned successful determination of the single chemical indicator only proved the feasibility and suitability of application of hyperspectral imaging for the evaluation of fish freshness quality. However, the accuracy and reliability of the previous prediction models is not high and it is obviously difficult to understand and evaluate the changes of fish freshness during postmortem spoilage based on a single quality attribute, as freshness is a complicated quality phenomenon. It has been shown that TVB-N, TBARS and K values are all freshness indicators for fish, it will thus be obviously more useful and meaningful to develop a rapid method that can predict these chemical indicators at the same time. Therefore, the current study aimed to simultaneous determination of several chemical parameters including TVB-N, TBARS and K values using key wavelengths from hyperspectral images selected by successive projection algorithm (SPA) and genetic algorithm (GA) in tandem with least square support vector machines (LS-SVM) and multiple linear regression (MLR) for assessing fish freshness, thus overcoming the limitation of the previous studies. To the best of our knowledge, this is the first study in the area.

Table 1
The main composition content of grass carp fish muscle.

Moisture content/%	Crude protein/%	Crude fat/%	Crude ash/%
77.26 ± 0.75	17.39 ± 0.18	3.60 ± 0.15	1.03 ± 0.07

2. Materials and methods

2.1. Preparation of fish fillet samples

A total of thirty fresh farmed grass carp from the same batch with the similar breeding environment and weight (2.0–2.5 kg) were purchased from a local aquatic products market in Guangzhou, China, and were transported to the laboratory alive in water 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 instantly beheaded, skinned, and filleted and then washed with cold water. Table 1 shows the main composition content of grass carp fish fillet muscle. In order to acquire more experimental samples, the fresh fillets were segmented into many cuboids with similar size (3.0 cm × 3.0 cm × 1.0 cm, length × width × thickness). Consequently, a total of 280 subsamples were obtained from different locations of the tested grass carp fillets. And then the whole subsamples were labeled and packaged into the sealed plastic bags and divided into four groups and stored at 4 ± 1 °C for 0, 2, 4, and 6 days that reflected a complete process of fish from freshness to chemical spoilage. Among the 280 subsamples, 180 samples from each group (45 samples in each group) were used for calibration purpose and the remaining 100 samples from each group (25 samples in each group) were used for prediction role.

2.2. Traditional determination of chemical index

2.2.1. Determination of TVB-N value

TVB-N value was determined by a stream distillation method according to Cai et al. (2011) with some modifications. Ten grams of each grass carp fillet was minced and mixed with 90 ml of perchloric acid (0.6 M), the mixture was centrifuged at 3000 rpm for 10 min, its filtrate was made alkaline by adding 50 ml of 30% sodium hydroxide and distilled for 5 min in a 8100 Kjeltec distillation unit (FOSS Tecator, Hillerød, Denmark), with 50 ml distilled water being used as control. The distillate was then collected in a conical flask containing a 50 ml aqueous solution of boric acid (40 g/L) and a mixed indicator created from dissolution of 0.1 g of methyl red and 0.1 g of bromocresol green to 100 ml of 95% ethanol. Afterward, the obtained boric acid solution was titrated with a 0.01 M of hydrochloric acid solution. The TVB-N value was finally measured and expressed as mg N/100 g fish muscle according to the consumption of hydrochloric acid.

2.2.2. Determination of TBARS value

Lipid oxidation was monitored by measuring TBARS value according to the procedure of Salih et al. (1987) with few modifications. Five grams of grass carp fillet muscle was minced and then mixed with 25 mL of trichloroacetic acid (20%) and 20 mL of