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Developing a machine vision system for simultaneous prediction of freshness indicators based on tilapia (*Oreochromis niloticus*) pupil and gill color during storage at 4 °C

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ABSTRACT

The study assessed the feasibility of developing a machine vision system based on pupil and gill color changes in tilapia for simultaneous prediction of total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA) and total viable counts (TVC) during storage at 4 °C. The pupils and gills were chosen and color space conversion among RGB, HSI and L*a*b* color spaces was performed automatically by an image processing algorithm. Multiple regression models were established by correlating pupil and gill color parameters with TVB-N, TVC and TBA (R^2 = 0.989–0.999). However, assessment of freshness based on gill color is destructive and time-consuming because gill cover must be removed before images are captured. Finally, visualization maps of spoilage based on pupil color were achieved using image algorithms. The results show that assessment of tilapia pupil color parameters using machine vision can be used as a low-cost, on-line method for predicting freshness during 4 °C storage.

1. Introduction

Tilapia (*Oreochromis niloticus*) is one of the most popular cultivated fish species in the world. The Food and Agricultural Organization of the United Nations ranked the yield of tilapia (3,670,260 tons) as the fifth highest among fish produced by aquaculture in 2014. Tilapia has relatively high nutritional value and are rich in protein and enzymes. Recent changes in consumer life style have increased the demand for safe, high-quality fish products. However, production, processing, transportation, retailing, domestic storage, and final meal preparation can produce negative effects on fish quality (Cheng & Sun, 2015). Therefore, freshness and safety are the most important quality characteristics of fish products for consumers and food industry. Microbial and enzyme activities play important roles in tissue deterioration during fish storage, which is characterized by protein denaturation, changes in texture, lipid oxidation, microorganism growth, and production of biogenic amines (Zhang, Qin, Luo, & Shen, 2015; Hong et al., 2013). Chemical and microbiological metrics such as total volatile basic nitrogen (TVB-N), thiobarbituric acid value (TBA) and total viable counts (TVC) are commonly used to determine the quality of fish during storage; however, these traditional determination methods are time consuming, laborious, destructive, and harmful to the experimenter and environment potentially because of the analytical reagents utilized in the assays (Chen, Hui, Zhao, & Ouyang, 2014). The development of a rapid and nondestructive predictive method of evaluating the freshness of fish would be of significant interest to the fish industry because of its potential economic benefits.

Recently, most studies evaluating the freshness of fish have focused only on measurement of a single quality index. Cheng & Sun (2015) measured only changes in TVC based on visible and near infrared hyperspectral imaging to evaluate grass carp spoilage during storage; Huang et al., 2016 used only TVB-N to evaluate fish freshness by correlating TVB-N values with data from computer vision analysis and NIR spectroscopy. Due to the complexity of the changes in fish tissue quality that occur between harvest and meal preparation, accurate and reliable prediction of freshness must be based on more than one quality index. Thus, a rapid, nondestructive and accurate predictive method for simultaneous determination of chemical decomposition and microbiological spoilage in fish during storage is urgently needed by the fish industry.

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Color perception is closely associated with fish freshness and influences the purchasing decisions of consumers (O'Sullivan et al., 2003). Therefore, machine vision, a rapid, non-destructive and economic method of product evaluation, has been utilized in many studies of food quality assessment (Huang, Zhao, Chen, & Zhang, 2014; Korel, Luzuriaga, & Balaban, 2001). Generally, two or three color spaces and several color parameters, such as $L^*a^*b^*$, RGB, HSI, and HSV, are used to create, represent and visualize colors (Zhou et al., 2015). The most often used color models are the RGB and $L^*a^*b^*$ models, and the HSI color space is also used to describe color characteristics and perception (Valous, Mendoza, Sun, & Allen, 2009). Many studies have reported that machine vision can be utilized to establish the freshness of whole fish, fillets and skin. However, there is a lack of studies on the application of machine vision for fish pupil and gill assessment during chilled storage.

The objective of this study was to quantify and analyze changes in pupil and gill color (R, G, B, L^*, a^*, b^* and H, S, I) in tilapia stored at 4 °C using a machine vision system and to identify the feasibility of using online and automated predictive methods based on pupil and gill color parameters for freshness assessment via correlation with chemical and microbiological indicators.

2. Materials and methods

2.1. Materials

A total of 120 tilapia (weight 759 ± 53 g and length 31.7 ± 3.7 cm) were purchased from a market (Beijing, China) and transported to the laboratory over a period of 20 min while alive. In order to transport the fish to the laboratory alive, water and fish were packed and sealed in polythene bags with overladen oxygen. Tilapia were killed by a blow to the head with a stick, packaged, and sealed in polythene bags. All fish were stored in refrigerated incubators at 4 ± 1 °C. Twenty randomly selected samples were selected for the experiments every 2 days.

2.2. Traditional determination of chemical and microorganism indicators

The biochemical state of samples during 4 °C storage was assessed based on total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA) and total viable counts (TVC), which were measured according to the methods of Hu et al. (2013), Shi, Cui, Yin, Luo, and Zhou (2014) and Wang et al. (2014). TVC were estimated by the pour plate method. Five grams of fish muscle were homogenized with 45 ml of sterile 0.9% physiological saline for 1 min. The homogenized samples were diluted in sterile 0.9% saline serially (1:10), and then used for microbial analysis. The incubation condition used for total aerobic counts was 30 ± 1 °C for 72 h.

2.3. Image analysis

The average color of fish pupils and gills was measured and analyzed automatically in a program in MATLAB R2014a in three steps. First, the images of eyes and gills were captured by a camera; second, the region of interest in the image was chosen and R (red), G (green) and B (blue) values were measured automatically using an image preprocessing method; third, conversion of three color spaces and color parameter analysis were performed using image analysis algorithms.

2.3.1. Image acquisition

The implemented machine vision system had two components: a digital camera and an illumination setup.

Images of the eyes and gills were captured for color assessment at a resolution of 4160 × 3120 pixels in PNG format using a digital camera in the indoor temperature (25 ± 1 °C). The camera characteristics were as follows: no flash, 28 mm focal length, ISO-100 sensitivity, manual operation mode, Av F/2.0 aperture, and 1/15 s shutter speed. The white balance was set using fluorescence. The vertical distance between the camera and fish was 30 cm. Test fish were placed on the object stage individually for sample imaging in the RGB color space. The images were transmitted to the computer via a USB port.

To ensure uniform illumination conditions, four daylight fluorescent lamp assemblies were placed below the fish. One lamp assembly consisted of four 36-W daylight fluorescent lamps (brick, 41.1 mm long, 6700 K color temperature). PVC sheets were located above the lamps to provide diffuse illumination, avoid specular reflections of the samples, and improve the quality of the images (Misimi, Mathiassen, & Erikson, 2007). To ensure a stable light source, the lamps were turned on 30 min before use.

2.3.2. Image pre-processing and image segmentation

The captured images were processed by reducing the resolution to 400 × 400 pixels. In order to improve image quality, image processing techniques were used to remove background noise and enhance important features of the samples before computational analysis. Median filtering is commonly used as an image processing tool to remove low-frequency background noise (Moore & Jorgenson, 1993). In this study, median filtering removed the peaks of interest and left the edges.

The process of image segmentation is used to divide an image into related sections or regions, and the accuracy of data extracted from segmented images is highly dependent on this step (Osuna-Enciso, Cuevas, & Sossa, 2014). In this study, images of the eyes and gills were segmented by the hill climbing and region growing methods; the algorithms were implemented in MATLAB R2014a software. After image pre-processing, color images were converted to binary images and the appropriate threshold of image segmentation was selected. Accordingly, pixels comprising the pupil and gill were labeled 1 (white), while all other pixels were classified as background pixels and labeled 0 (black). Finally, the pixels comprising the segmented images of the pupils and gills were extracted and automatically represented in RGB.

2.3.3. Conversion of RGB images into $L^*a^*b^*$ and HSI

In this study, color data for tilapia pupils and gills were assessed using three color models: RGB, HSI (hue, saturation and intensity) and $L^*a^*b^*$ (lightness, redness and greenness, and yellowness and blueness).

2.3.3.1. Conversion of RGB images into $L^*a^*b^*$. The RGB to $L^*a^*b^*$ transformation was performed in two steps as described by León, Mery, Pedreschi, and León (2006) with some modifications. In the first step, nonlinear RGB values were transformed to XYZ values:

$$R\gamma = \left(\frac{R + \alpha 1}{\alpha 2}\right)\gamma \tag{1}$$

$$G\gamma = \left(\frac{G + \alpha 1}{\alpha 2}\right)\gamma \tag{2}$$

$$B\gamma = \left(\frac{B + \alpha 1}{\alpha 2}\right)\gamma \tag{3}$$

In order to correct the RGB values from the digital camera, a better calibration of the transformation model was obtained, where $\gamma = 2.2$ was a parameter that corresponded to $\alpha 1$ and $\alpha 2$.

The values of (X, Y, Z) were acquired as follows:

$$\begin{bmatrix} X \\ Y \\ Z \end{bmatrix} = (\mathrm{MAT}) \begin{bmatrix} R\gamma \\ G\gamma \\ B\gamma \end{bmatrix} \tag{4}$$

MAT was a linear transformation matrix between color spaces RγGγBγ and XYZ using coefficients recommended by Valous et al. (2009).

The MAT parameters vector for the model: