Fibreoptic fluorescence spectroscopy for monitoring fish freshness

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ABSTRACT

In this study, a portable Y-type fibreoptic fluorescence spectroscopy measurement system was used to evaluate the freshness of eight cobias (Rachycentron canadum). The results showed that the ratio of fluorescent intensity, which F_{480} nm/$F_{exci+50}$ nm was belong with the range of collagen type I and type V characteristic spectra, was positive correlated to the frozen time by hours. It was a strong approach to be a potential index for differentiating the fish freshness during delivery process. Besides, the different pattern results of dorsum and abdomen were shown in this study. In further, fibreoptic fluorescence spectroscopy could be a way not only to measure and quantify the freshness of different fish body but also to verify the level of taste.

Keywords: Fresh fish, fluorescence, spectroscopy, collagen

1. INTRODUCTION

In general, the fish freshness is the major concern for consumer purchasing. The quality of fish freshness is strongly affected by delivery process and variant environment between consumers and suppliers. For example, temperature and humidity can change the ripe time and affect freshness directly. In literature, researchers had found the degree of fish deterioration during freezing and thawing increased as dehydration, protein denaturation, lipid oxidation, autolysis, and enzyme degradation [1-2]. The mechanism of chemical compounds interaction also led into some differences in the various properties of fish meat. For examples, the different properties mainly contained pH value, flavors, appearance, microstructure, color, and luster. The determination methods of fish meat in the laboratory are complex course and time-consuming issue. It is worthy to investigate the possible monitoring system for detecting all transporting chains in delivery process and storage process.

In food industries, researchers had been applied different sensing technologies to develop a low-cost and easy-to-use system for monitoring freshness [3]. For example, Gil developed an electronic tongue using metallic potentiometric electrodes for fish freshness analysis [4, 5]. Yapar used refractive index of eye fluid to determine changes of fish freshness [6]. Okuma and Nanjyo combined enzyme electrodes sensors with an injection flow device to a discriminable system for fish deterioration [7-8]. Abbas used pH devices to be remarkable correlation between the pH and the fish freshness during storage [9]. Barbri produced a portable electronic nose system for the fish peculiar smell produced by time and evaluate the difference of fish freshness [10]. Kroeger adopted machine vision for analysis of whole fish and fillets with respect to freshness [11]. Nilsen had shown that visible and near infrared spectroscopy were used to analyze the quality of fish [12].

Due to amino acids, protein, and enzyme being the well-known fluorescence molecules, fluorescence spectroscopy is higher potential to be served as analytical tools for chemical, biological, biomedical, and food sciences in recent years [13]. For example, the fluorescence spectra of tryptophan (amino acids), collagen (protein), and NADH (coenzyme) with linear multivariate analysis methods were adopted for human normal and dyplasia tissues differentiation [14]. It is also known that the change of intrinsic fluorophores can be measured in fish muscle [15]. Besides, the amount of fluorescence spectra can be described during frozen storage. It implied the destruction of amino acids aromatic, the

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deposition of protein, and the action of metabolic enzyme can be treated as biomarkers for fish freshness.

Many studied groups have been distinguished the stages of fish freshness from fluorescence spectra [13]. For example, Aubourg and Duflos presented the fluorescence spectra of lipid oxidation products were measured as an index of fish freshness [16-17]. Dufour found the fluorescence spectra of tryptophan and NADH as fingerprints of fish [18-19]. Karoui adopted principle component analysis and detrended fluctuation analysis methods to extract NADH fluorescence information for getting a precise classification [20]. In addition, Andersen presented that autofluorescence of collagen type I and type V were found from fluorescence spectra of salmon and cod muscle [21].

In order to develop a portable fluorescence measurement system for monitoring fish freshness during delivery process with variant environment, the objective of this study focused on fibreoptic fluorescence spectroscopy for monitoring fish freshness during frozen time. The academic investigation could provide a successful roadmap with an unsophisticated, selective excitation light and portable device to understand the quality of the fish freshness for both consumers and suppliers.

2. MATERIALS AND METHODS

2.1. Instrument

The Y-type fibreoptic measurement system was integrated as fluorescence spectroscopy in this study (figure 1). This system included a CERMAX xenon lamp (ILC, US), a H10 monochromator (HORIBA Jobin Yvon, France), a MicrHR180 spectrometer (HORIBA Jobin Yvon, France), a R928 photomultiplier tube (PMT) (Hamamatsu, Japan), a Y-type optical fiber (Oriel, US), and a general commercial desktop (ASUS, Taiwan). At first, the broadband light was produced by xenon lamp and was passed through the H10 monochromator to generate specific wavelength and narrowband light. The samples were excited by specific wavelength of light and were emitted respective fluorescence signals. Since the surface of fiber bundle was vertical to investigated samples, the emission signals were sent back through the fiber bundle and were split by the MicrHR180 spectrometer. The intensities of specific emission signals were acquired by PMT. The wavelength range of excitation light is from 320 nm to 350 nm with 10 nm interval, and the wavelength range of emission light is from 350 nm to 600 nm with 2 nm resolution. The whole controlling and acquiring processes were designed and implemented by LabVIEW 8.5 (National Instruments, US).

2.2. Samples and statistics

Nowadays, the cobias are the main type of fish in offshore cage aquaculture in the world. The fishes are the most famous for sashimi preparation in Asia. Actually, cobia is fed in a net/box at warm water in Taiwan. Since Taiwan is a desirable environment to exploit cobia offshore cage aquaculture, a cobia with 5-7 months old which is suitable for sashimi preparation is chosen as monitoring target in this study.

Total eight cobias with 5±1 kg weight were captured from the cages in this study. The dorsum and abdomen of the fishes were sliced into total 64 specimens (4x3x1 cm) for fluorescence spectroscopic measurements. Total specimens were frozen at a 4 ^{o}C refrigerator. The one specimen of dorsum and abdomen were taken with 6 hours internal from 0 to 18 hours until measurement. The results of these experiments were analyzed with analysis of variance (ANOVA) using tests of within-subjects effects, and post-hoc analysis in SPSS v.15 (SPSS Inc., US) to evaluate the variation of fluorescence with time.

3. RESULTS AND DISCUSSION

It had been investigated that fluorescence spectroscopy was the high potential technique to detect the variation of matters by mean of concentration of fluorescent molecules. Important fluorophores as collagen type I, type V, and NADH were related to the changes of fish products.

The fluorescence spectra, excited by different wavelengths, of 64 fish dorsum and abdomen specimens were recorded by fibreoptic measurement system in our study. Performing fluorescence measurements exciting from 330 nm to 420 nm with 10 nm interval and recoding the intensity of corresponding emission spectra at each 2 nm were result in an excitation-emission matrix (EEM) [21] of a specimen. The EEM contour plots used by peak intensity normalization from a dorsum specimen at 0-18 hours frozen time were presented in Fig.2. Fluorescence was observed throughout the whole

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collection range, with two peaks located at (350 nm, 400 nm) (excitation wavelength, emission wavelength) and (350 nm, 480 nm). A ridge extends from (360nm, 400 nm) to (350 nm, 600 nm). A valley was seen at between 440 and 460 nm emission wavelengths. These peaks and valleys were the well known appearance of the fluorescence intensities which were also agreed with major contributors from collagen type I and type V **[21]**.In Fig.2(a) and (b),the changes were observed between 330-360 nm excitation wavelength so their emission spectra was extracted to analyze the relationship between fluoresce intensities and fish freshness. In addition, according to collagen type I and type V were degraded with time, the ratio of fluorescence intensity $F_{480\ nm}/F_{exci+50nm}$ (exci=excitation wavelength) was calculated for each specimen.

Figure 3 presents that the frozen time (0, 6, 12, and 18 hours) was related to the average ratio of fluorescence at 330 nm, 340 nm, 350 nm, and 360 nm excitation wavelength. The vertical error bars labeled indicated the standard deviations of the mean. The dorsum and abdomen specimens of cobia outcomes were shown in figure 3(a) and figure 3(b). The results illustrated that the ratio decreased by increasing frozen time unless abdomen specimens at 320 nm excitation wavelength. In addition, the large drop heights were appeared between 6 and 12 hours at different excitation wavelengths.

The statistical comparison of ratio values at different frozen times was also calculated through the tests of within-subjects effects. The compared results indicated that the ratio values at 0, 6, 12, and 18 hours were significant difference ($P < 0.0001$) under same excitation wavelength. Furthermore, the post-hoc analysis was used to investigate detail discriminate relation between frozen times. The results presented that the ratio values of abdomen specimens at 0 hour were significant difference ($P < 0.0001$) with the ratio values of abdomen specimens at 12, 18 hours under same excitation wavelength of 320, 330, 340, and 350 nm. The ratio values of abdomen specimens at 6 hour were significant difference ($P < 0.0001$) with these at 12, 18 hours too. The same results were occurred in the dorsum specimens under same excitation wavelength of 330, 340, and 350 nm. According to the results at dorsum and abdomen specimens, the calculated values of $F_{470\ nm}/F_{exci+50\ nm}$ are proved to be an index of fish freshness since Collagen type I and type V being a biomarker of fish **[21]**.

4. CONCLUSIONS

In this study, we used conventional components to integrate a portable Y-type fibreoptic measurement system for fluorescence spectroscopy. The results showed that the ratios of fluorescence intensity, $F_{470\ nm}/F_{exci+50\ nm}$, is a potential index to differentiate the fish freshness by hours. It indicated that the fibreoptic fluorescence spectroscopy could be applied for monitoring fish freshness by hours. The different results of dorsum and abdomen were also shown in this study. It provided a way to diagnose different parts of fish body for sashimi preparation. In further, the system would be adopted to measure and quantify other parts of fish body. It was not only to determinate the degrees of fish freshness but also to verify the level of fish taste.

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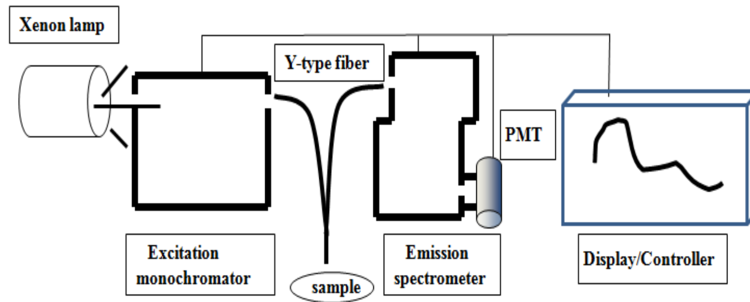

Figure 1. The portable Y-type fibreoptic fluorescence spectroscopy measurement system includes a xenon lamp, a H10 monochromator, a MicrHR180 spectrometer, a R928 photomultiplier tube, Y-type optical fiber and a commercial desktop.

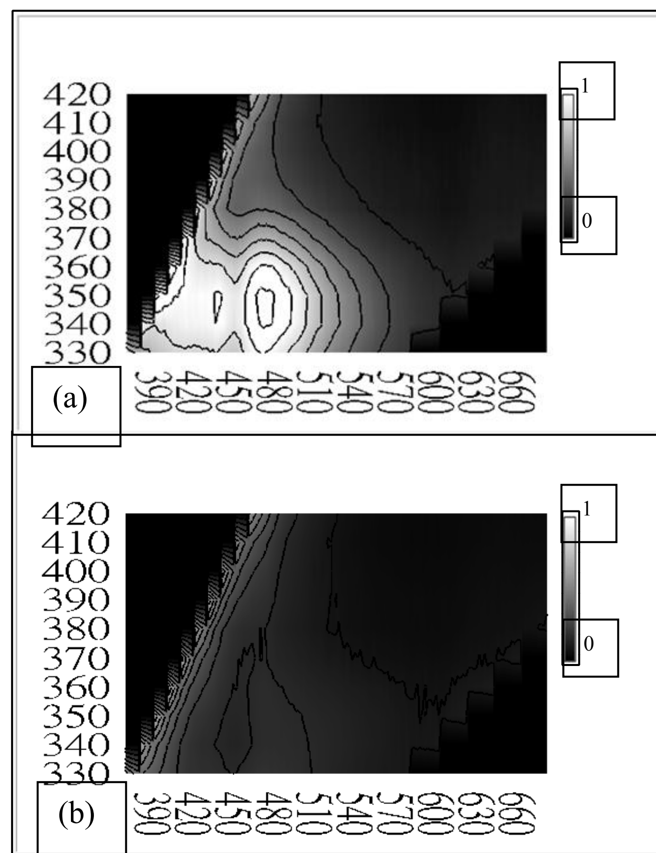

Figure 2. It shows the excitation-emission matrix (EEM) of abdomen specimens for cobia fish.(a)0 hour frozen time(b) 18 hours frozen

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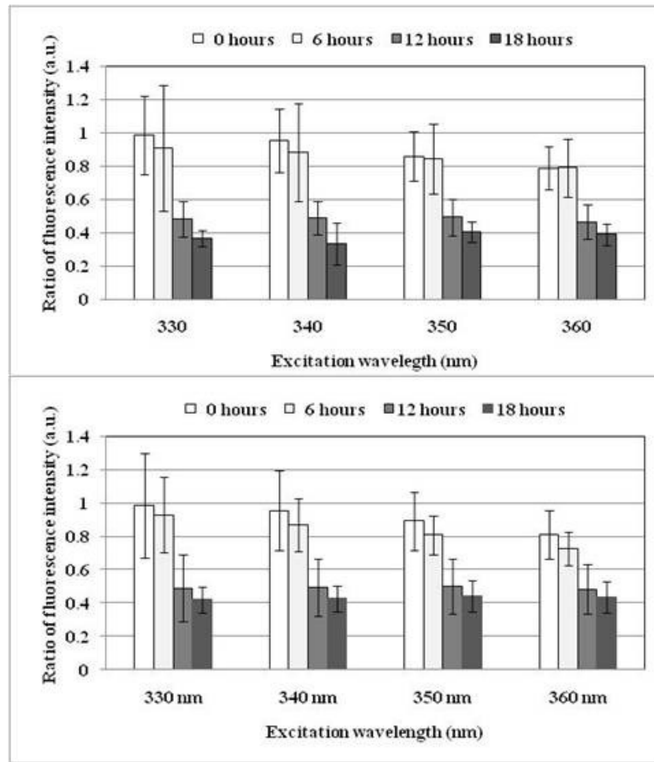

Figure 3. The average ratio of fluorescence intensity produced by each excitation wavelength light (330-360nm) was related to frozen time (0-18 hours) for eight cobias (a) dorsum and (b) abdomen.

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