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Fluorescence fingerprint approach for monitoring the post-mortem changes in frozen horse mackerel fillets

Md. Mizanur Rahman [1,2], Mario Shibata [1], Naho Nakazawa [1], Tomoaki Hagiwara [1], Kazufumi Osako [1] and Emiko Okazaki [1,*]
[1] *Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Tokyo, 108-8477, Japan*
[2] *Department of Fisheries Technology, Patuakhali Science and Technology University, Patuakhali, 8602, Bangladesh*
* Correspondence: eokazaki@kaiyodai.ac.jp; Tel.: +81-03-5463-0618

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Introduction

Frozen fish consumed as 'Sushi' and 'Sashimi' is required high freshness quality due to hygienic and good properties of texture, color, flavor, and taste. The fish frozen at pre-rigor stage retains good quality with better color than the fish frozen at post-rigor stage [1]. However, the traders can't know the exact initial condition of frozen fish instantly without thawing them. Moreover, the chemical methods for tracking the changes in the fish body are destructive and time-consuming. So, the nondestructive monitoring of post-mortem changes in frozen fish fillets without thawing is challenging.

Fluorescence spectroscopy has shown potentiality for rapid, non-contact and noninvasive analysis of fishery and other food products [2]. Recently, the FFs technique was employed for predicting frozen fish freshness indices previously ice stored for 14 days [3-4]. However, these studies were conducted using the fish with long ice storage period (14 days) and did not emphasis the highly fluorescent compounds (e.g. NADH) which are related to the early stage post-mortem changes in fish body. Therefore, the present study was carried out for short ice storage period (48 hours) especially aimed to focus on the changes of nicotinamide adenine dinucleotides (NAD and NADH) which are deeply involved in the progress of glycolysis and expected to be an indicator of the state of rigor mortis in fish body.

Materials and methods

Sample preparation and fluorescence measurement
Fifty-six alive horse mackerel (*Trachurus japonicus*) were sacrificed instantly by spinal cord destruction and kept on ice for 14 different periods (0-48 hour) to prepare samples with different quality, then filleted and frozen. According to ElMasry et al. [3], the FFs of the frozen samples were then acquired using fluorescence spectrophotometer (F-7000 Hitachi High-Tech Science Corp., Japan) aided with an external fiber optic probe at -30°C (Fig. 1) [3]. Then, the samples were repacked and kept frozen at –60°C to maintain their original quality until the chemical analysis of NAD and NADH content. Moreover, standard NAD and NADH solutions were also measured by F-7000 to characterize their fluorescence attributes.

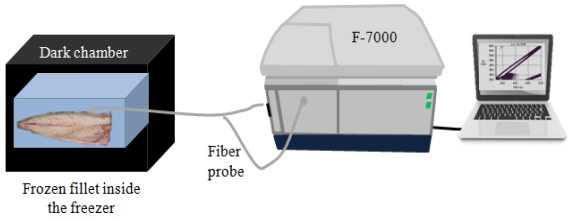

Fig. 1. Fluorescence fingerprint acquisition process.

Chemical analysis of the fish fillets
The fluorescence measured muscle position of the fillet was dissected and used for the chemical analysis. The NAD and NADH content of the frozen samples were determined by enzymatic method and assay kit according to Ehira [5] and Imamura et al. [6], respectively.

Data analysis
According to the relevant literature [3,7], the FFs of the samples were preprocessed and masked. Partial least square (PLS) regression was applied to data analysis using JMP software (JMP pro. 12). The PLS model was built by setting wavelength pairs as predictors (X-variables) and the measured chemical data (NAD or NADH-content) as a response variable (Y-vector). The optimum number of latent factors of each PLS model was determined using ten-fold cross-validation method.

Results and discussion

Post-mortem changes in horse mackerel fillet over ice storage period

Immediately after death, a series of chemical and enzymatic changes occur in the fish body. NAD and NADH content were observed in the horse mackerel fillets over the ice storage periods which have a correlation with the post-mortem changes in fish muscle**.** At the beginning of ice storage, the NAD content was around 0.6 µmol/g, decreased gradually till 7 h and after 48 h declined around 0.2 µmol/g**.** On the other hand, the initial NADH content was less than 0.1 µmol/g, increased gradually till 12 h and at the end of ice storage reached around 0.8 µmol/g. The changes in NAD and NADH content of horse mackerel fillets were almost similar with the study of yellowtail and tuna muscle, respectively [8-9].

NAD decrease is highly associated with the development of rigor mortis and NADH level can influences the metmyoglobin formation in fish tissue [6]. The higher amount of NADH indicated the low amount of met-myoglobin formation. The color changes in fish muscle can be monitored by observing the NADH content incorporated with met-myoglobin reductase activity [9]. Therefore, the monitoring of NAD and NADH changes in fish muscle was revealed as very important parameters to know the state of rigor mortis as well as the post-mortem changes in fish muscle.

Prediction of post-mortem changes in frozen horse mackerel fillets by FFs

In the FF spectra of frozen horse mackerel fillets, the fluorescent molecules were distributed in the several wavelengths area. The standard NAD solution produced a fluorescent peak at wavelength combination of excitation (λ_{ex}) 290 nm, and emission (λ_{em}) 380 nm whereas the NADH solution showed a distinct fluorescence peak (λ_{ex} =340 nm and λ_{em} =460 nm) with high intensity. Therefore, the PLS regression models were developed for predicting the adenine nucleotides (NAD and NADH) in the frozen fish fillets**.** Firstly, in the PLS model of NAD content, the coefficient of determination (R^2) was 0.71 with root mean square error (RMSE) of 0.05 µmol/g by obtaining 8 latent factors (LF)**.** Then, the PLS model of NADH was observed with relatively higher R^2 value of 0.88 and RMSE of 0.07 µmol/g by producing the same number (8) of LF.

The relatively lower fitting of NAD content in fish fillets by PLS model might be the reasons of weaker fluorescence features of NAD molecule [10]. Moreover, the NAD peak of standard solution revealed that the intensity could be overlapped with ATP-related compounds [11]. The second model indicated a good correlation of FFs spectra with the NADH content in fish muscle. As the NADH is a highly fluorescent intrinsic component in fish body and produces a distinct peak, the prediction of this molecule by FF is much more conventional. Chang et al. [12] reported the fluorescence features of NADH content in fish muscle which is almost similar to the present study. Moreover, the NADH level in fish body can influences the met-myoglobin formation which is related to color changes [6]. Therefore, the nondestructive prediction of NADH content in fish body could be an efficient tool for monitoring the appearance of fish tissue at the post-mortem metabolic process.

Conclusions

From the results of destructive chemical analysis, it was confirmed that the NAD content decreased and NADH content increased gradually during the post-mortem changes. These fluorescent intrinsic compounds revealed a good correlation with the FFs data. Thus, FFs coupled with the chemometrics could be a rapid approach for monitoring the post-mortem changes in frozen fish muscle nondestructively.

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