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Non-destructive prediction of initial freshness condition in frozen fish fillets using excitation-emission matrix**Md. Mizanur Rahman, Mario Shibata, Naho Nakazawa, Tomoaki Hagiwara, Kazufumi Osako and Emiko Okazaki**
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Fish freshness is very important to the international fish markets and especially for Japanese people who are accustomed to consuming the raw fish as 'Sushi' and 'Sashimi'. However, once the raw fish with a variety of freshness are getting frozen, it's very difficult to distinguish them instantly by naked eyes. To know the freshness status of frozen fish fillets, it is necessary to let them thaw first and subsequent assessment of freshness using conventional chemical methods, which are destructive and time-consuming. The non-destructive freshness assessment of frozen fish fillets without thawing is a big challenge. A few previous studies have proved the potentiality of fluorescence spectroscopy for the freshness assessment of fish based on some freshness indices (e.g. K-value). Therefore, the present study was aimed to propose a non-destructive method using the excitation-emission matrix (EEM) of fluorescence spectroscopy based on the most important intrinsic fluorophores for determining the freshness of frozen fish fillets at early stages after death. Right fillets of fifty-six horse mackerel fish (*Trachurus japonicus*) were vacuum packed with a plastic bag, kept in ice for 14 different periods (0-48 hour) to prepare samples with different freshness conditions (n=4) and finally stored at -30°C. EEM spectra of the samples were then acquired directly from the frozen fillets using fluorescence spectrophotometer (F-7000) and an external fiber optic probe installed inside the freezer. Subsequently, the ATP-related compounds and nicotinamide adenine dinucleotides (NADH) content of the same samples were determined using relevant biochemical methods. Partial least square (PLS) regression models were developed under a 10-fold cross-validation method by comparing the chemical data with the masked EEM spectra (1054 variables). The PLS model of adenylate energy charge (AEC) values (relative ratio of ATP, ADP, and AMP) showed the coefficient of determination (R^2) of 0.94 and root mean square error (RMSE) of 5.48% with 9 latent factors (LF). Furthermore, the PLS model of NADH content was observed with R^2 of 0.88, RMSE of 0.07 $\mu\text{mol/g}$ and LF of 8. As a freshness index, the AEC values and NADH content indicated the energy status and color changes in fish fillets, respectively which could be predicted by PLS model. Thus, the EEM spectra coupled with chemometrics offers a simple and rapid approach to predict the freshness status at the early stage in post-mortem fish muscle non-destructively keeping the sample in a frozen state during the assessment.

Biography

Md. Mizanur Rahman is a Japanese Government Scholarship student (MS leading doctorate program) of Tokyo University of Marine Science and Technology under the supervision of Professor Dr. Emoko Okazaki who has her expertise in the non-destructive prediction of fish freshness using NIR and fluorescence spectroscopy. He is now trying to establish a new smart technique for non-destructive prediction of frozen fish freshness using fluorescence fingerprint, which is also called excitation-emission matrix (EEM). He has a diversified knowledge on fisheries technology, as he is a teaching staff in Bangladesh

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