Non-invasive sensing of seafood quality using fluorescence fingerprints coupled with chemometrics（ケモメトリックスと組み合わせた蛍光指紋法による水産物品質の非破壊計測）

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博士学位論文内容要約
Summary

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論文題目 Title	Non-invasive sensing of seafood quality using fluorescence fingerprints coupled with chemometrics （ケモメトリックスと組み合わせた蛍光指紋法による水産物品質の非破壊計測）		

Seafood consumed as ‘Sushi’ and ‘Sashimi’ are often distributed as raw and frozen state which require high quality due to hygienic, good properties of texture, color, flavor, and taste. However, it’s ticklish to know the exact post-mortem quality of frozen meat instantly, without thawing. So, nondestructive monitoring of post-mortem quality changes in frozen seafood is a big challenge for the seafood industry. Conventional chemical methods (e.g. HPLC) for quality evaluation of seafood are tedious, destructive, and time-consuming. Additionally, seafood consumers are facing a variety of problems related to authenticity such as mislabeling or misclassification and adulteration of fishery products. Therefore, a fast, noninvasive and smart protocol for quality authentication of seafood is strongly anticipated from the industry. Three-dimensional fluorescence fingerprints (3D-FFs) is also called excitation-emission matrix (EEM). 3D-FFs has been introduced for the quality measurement of different food materials but very limited for seafood. Thus, the present study has been attempted to address several issues of seafood quality and authenticity using FFs as well as to develop a new technique to visualize the seafood quality.

In the chapter 1, the study was conducted to characterize fluorophores in the fish body using 3D-FFs and to utilize these 3D-FFs obtained from frozen horse mackerel (*Trachurus japonicus*) fillets to predict early post-mortem changes. Alive fish were sacrificed instantly, preserved in ice until 2 days, and then filleted, vacuum packed, and frozen. Subsequently, 3D-FFs of the frozen fillets were acquired using a fluorescence spectrophotometer (F-7000) aided with a fiber probe. Post-mortem freshness changes were tracked by measuring adenylate energy charge (AEC) values and nicotinamide adenine dinucleotide (NAD and NADH) content. Partial least squares regression (PLSR) models for predicting AEC values and NADH content in frozen fish meat showed good fittings, with R^2 of 0.90 and 0.85, by utilizing eight and five excitation wavelengths, respectively, based on their fluorescence features acquired from standard fluorophores.

In the chapter 2, a new approach was aimed to monitor the pH changes in frozen fish meat using FFs. Frozen fish fillet with different post-mortem ice storage condition (0-48 h and 0-40 h for horse mackerel and spotted mackerel fish, respectively) were used for FFs and subsequently pH of frozen meat was measured (n=63). PLSR analysis of FFs spectra was performed to predict pH changes. Standard solutions of ATP-metabolites, NAD, and NADH with different pH (5.0-8.0) were also scanned by F-7000. PLS models (validation) of pH prediction in frozen horse mackerel and spotted mackerel muscles showed the coefficient of

determination (R^2) of 0.71 and 0.90, respectively. The fluorescence intensities of pure ATP-metabolites and NAD solutions were highest at low pH (5.0) and showed a decreasing trend with increasing pH. As the significant effects of pH on the FFs of different fluorophores were observed, PLS models of FFs spectra revealed a good prediction of pH changes in frozen fish meats.

In the chapter 3, FFs was applied as a rapid and non-invasive quality authentication method to differentiate between ordinary and dark meat of frozen fish. Spotted mackerel fillet *(Scomber australasicu*s) with different freshness condition (0-40 h ice stored) were yielded and then frozen. FFs of the frozen fillets (containing dark and ordinary meat) were acquired using FFs. Then, pH and ATP-metabolites were determined and, principal component analysis (PCA) and PLSR chemometrics were performed. Higher K-values observed in dark muscle might be due to a faster decomposition rate of IMP rather than ordinary muscle. Chemometric treatment i.e. PCA of FFs spectra demonstrated clear discrimination between dark and ordinary meat of frozen fish muscle. Further, validation models of PLSR revealed that the freshness changes of ordinary fish meat could be predicted at frozen state with high accuracy than that of dark meat.

In the chapter 4, a study was attempted to classify seafood species by fluorescence EEM nondestructively even at their frozen state. Post-mortem freshness variation of five seafood species (horse and spotted mackerel, cod, flounder and shrimp) was stimulated by ice storage (0-48 h) and then frozen. EEM of frozen samples was acquired to discriminate among them nondestructively. PCA of EEM demonstrated clear discrimination among the seafood category and fish fillets with different muscle properties which was also revealed from the freshness data of chemical analysis.

In the chapter 5, the study investigated the potentiality of FFs to differentiate between fresh and frozen-thawed spotted mackerel fillets. Fresh and frozen-thawed fillets were yielded with different post-mortem freshness variation (ice storage for 0-40 h), and then FFs acquisition and chemical analysis were performed. PCA of FFs spectra showed clear discrimination (PC1+PC2 = 94.65%) between fresh and frozen-thawed fish meat, contributed by freshness variations. Further validation models of PLSR revealed that the nondestructive freshness prediction by FFs was also influenced by thawing process and the prediction accuracy was lower in frozen-thawed fillet ($R^2 = 0.59$) rather than fresh fillet ($R^2 = 0.84$).

In the chapter 6, as the FFs can only estimate quality at one point, the freshness condition of other parts of seafood body could not be tracked. In this study, we focus on a novel fluorescence imaging method, in which 3D-FFs was combined with 4D-imaging technique, to visualize the distribution of shrimp freshness such as K-value. Visualization was successfully performed to reveal the spatial-temporal changes of frozen shrimp freshness with an accuracy of $R^2 = 0.80$ using only one excitation light. The proposed method offers a more practical way for large-scale commercial purposes.

Non-invasive assessment of potential freshness indices by 3D-FFs is possible to predict the post-mortem quality of seafood at frozen state. 3D-FFs has proved to show the potentiality for authenticating seafood quality to prevent unfair trade. 4D-imaging proposed a promising method to visualize the spatial-temporal changes of frozen seafood freshness by overcoming the limitation of point measurement of 3D-FFs. Thus, Multidimensional fluorescence imaging would be one of the potential candidates as state-of-the-art authentication methods of frozen seafood quality for the industries.