Thesis abstract

Of

Fluorescence Spectroscopy Prediction of Fish Freshness
(蛍光分光による魚の鮮度予測)

By

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Freshness is one of the most important attributes for assessing fish quality, sale ability, and consumption. To date, numerous of traditional as well as newer techniques have been used for fish freshness assessment. However, most of these assessments are limited to the variety of fish products they can be applied to because of being time consuming, high cost, chemical reagent required or expert personnel required. Therefore, due to the fast changes seen in fish freshness, a simple, rapid and convenient freshness assessment is desired.

In the current study, the aim was to explore the potential use of fluorescence spectroscopy techniques with none or little damage to assess fish freshness. Time series fluorescence characteristics of fish eye fluid and scales were observed for fast and simple prediction of fish freshness. In these studies, Japanese dace (*Tribolodon hakonensis*) and red sea bream (*Pagrus major*) were chosen as representative fish from freshwater and seawater environments, respectively. These two kinds of experimental fish were stored at 20 ºC and 80% of humidity for 36 hours and sampled every 3 h from the start of the storage. Meat from the dorsal part of experimental fish was sampled at different storage times and used to measure the standard freshness indicator, *K* value (which was calculated from adenosine triphosphate (ATP) and its decomposition compounds), by the paper electrophoresis method. While at the same time, eye fluid samples from Japanese dace and red sea bream, as well as dorsal part scales from red sea bream were collected for fluorescence spectra acquisition and biochemical analysis.

As for the results, the *K* value (standard fish freshness indicator) of Japanese dace and red sea bream increased with storage time. The classification used for the Japanese dace and red sea bream was for raw consumption up until 6 h and 9 h in storage, respectively; both fish remained fresh up until 18 hours, and then deteriorated quickly thereafter when stored at 20 ºC and 80% of humidity. While on the other hand, time series of fluorescence characteristics of eye fluid and scales were observed. Firstly, the main fluorescent compounds in Japanese dace fish eye fluid were distinguished as aromatic proteins, uric acid and dityrosine. Aromatic proteins showed a complicated change with storage time and dityrosine, which was found to be occurred at very late storage time, made them of limited value to assess fish freshness. While uric acid regularly increased with storage time indicating that the change of uric acid in the fish eye fluid during storage could be employed as a meaningfully predictor of fish freshness. Next, fluorescence characteristics of uric acid in the eye fluid as a potential rapid and simple assessment for fish freshness continued to be explored for the red sea bream. The results of uric acid, which were measured by High Performance Liquid Chromatography (HPLC), showed that uric acid also

existed in red sea bream eye fluid and increased with storage time. The fluorescence intensities of uric acid in red sea bream eye fluid were observed with storage time and showed a similar tendency to increase as observed in Japanese dace eye fluid. The fluorescence intensities of uric acid were then plotted against with the standard fish freshness indicator, *K* value, and showed a good exponential relationship (R^2 = 0.94) between these two parameters. A higher linear relationship (R^2 of 0.94 and RMSEP of 6.37%) between the predicted *K* value, which was calculated from the uric acid fluorescence intensities, and the measured *K* value detected by paper electrophoresis method indicated that measuring the changes of uric acid fluorescence characteristics in fish eye fluid presented a high potential for fast and simply assessment of fish freshness. Finally, the fluorescence characteristics of red sea bream scales were also identified as a potential rapid and non-destructive means for assessing fish freshness. Two kinds of fluorescent compounds, tyrosine and collagen, were identified as the main fluorescent compounds in red sea bream scales. Time dependent changes on fluorescence intensities of either tyrosine or collagen fluctuated with storage time due to various biochemical reactions or moisture losses from the scales, which limited their use to assess fish freshness by themselves. However, subsequent analysis showed that the fluorescence intensities ratio of collagen to tyrosine increased linearly during storage (R^2 = 0.95) and were proposed as a potential non-destructive index of fish freshness.

The conclusion from these studies showed that: firstly, fish freshness decline is related to the fluorescence compound changes in eye fluid. Uric acid as the one of the main fluorescent compounds in Japanese dace eye fluid can provide valuable insights into fish freshness decline. Secondly, fluorescence characteristics of uric acid in the red sea bream eye fluid accurately predicted the standard fish freshness indicator, *K* value, indicating that it has the potential to be employed as a new, fast and simple fish freshness indicator. Finally, the fluorescence intensities ratio of collagen to tyrosine (which were the main fluorescent compounds in red sea bream scales) linearly increased with storage time, indicating that fish scale fluorescence also can be used for fast, simple and nondestructive assessment of fish freshness status. By combining the above fundamental research, the overall conclusion of this study is that a rapid and simply sensing technique based on fluorescence spectroscopy of the fish eye fluid or scales could be developed to provide a measurement solution for fish freshness determination. However, fluorescence characteristic of fish eye fluid and scales typically could use as an indicator of fish freshness, but given their variability depends on several factors, e.g. the wide diversity in the patterns of fish deterioration metabolism from one species to another, postmortem time and temperature, storage conditions, handling conditions or method of kill. Therefore, numerous modifications of the fluorescence characteristics in eye fluid and scales for fish freshness assessment versus fish species, storage condition and specific handling must be examined before it could be used as a practical application. In the future, more advanced research and application systems, such as fluorescence imaging or a fluorescence multiplex sensor, also could be developed based on the findings of this research.