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Development of Raman Technique for Detection of Formalin and Biochemical Changes in Japanese Catfish (*Silurus asotus*)

Graduate School of Science and Technology, Kwansei Gakuin University
Department of Bioscience, Hidetoshi Sato Laboratory, Erwan Yudiar Darussalam

【Introduction】 The purpose of this study is to develop a new method to determine formalin contamination as well as biochemical changes in fish meat using Raman spectroscopy, which is quick, low cost and nondestructive. Recently, the International Agency for Research on Cancer (IARC) has classified formaldehyde as one of the poisonous chemical food contaminations, which is carcinogenic to humans. It is illegal to dip fish in formalin solution as well as to use it for any preservation to increase the shelf life of fish meat. Many methods have been proposed to determine formaldehyde in food. The Chinese official method of analysis formaldehyde based on spectrophotometer shows simple and convenient methods, but has the drawback of poor selectivity. In contrast, chromatographic analyses, such as high performance liquid chromatography (HPLC) and gas chromatography (GC), have become popular for quantitative analysis of formaldehyde in food and in environment as well. However, have a drawback time consuming and destructive in preprocessing the sample. Hence, a new practically useful method is expected for the detection of the formalin in the food science.

【Experimental】 A catfish was sacrificed with the hypothermia method, and was cut on abdomen side with 1 cm thickness. The blood was removed by washed with distillate water. The fresh meat was immersed for 12 hours with different concentrations of formalin (20, 10, 2, 0.2 and 0.02%). A control sample was prepared by immersing in distillate water. After the first measurement, the samples were kept in a freezer (-21°C) for 24 hours, then the second measurement was carried out. The Raman instruments was equipped with a ball lens installed hollow optical fiber Raman probe (BHRP) and the measurement was made in contact with the samples. The spectra were analyzed with principal component analysis (PCA) and partial least squares regression (PLSR). Frozen storage experiment was carried out by comparing the result between fresh fish and that frozen in freezer -21°C, for 2 weeks, 1 month, and 2 months. Orange G staining was used for observation of tissue structure.

【Results and Discussion】 Present result shows that two formalin bands (911 and 1492 cm^{-1}) and one methanol band 1042 cm^{-1} appears in the Raman spectra. The concentration limit of formalin solution applied to the fish meat, that can be detected by Raman spectroscopy was 2%. Bands at 855, 940 and 1248 cm^{-1} observed in loading plots of the partial least square regression (PLSR) analysis of frozen fish was assignable to collagen, which I assumed that biochemical changes had occurred in the fish meat. PLSR discrimination analysis was applied to improve the detection potential to the data sets for 2% formalin soaked samples. The correlation co-efficiency (R^2) was 0.9490434 for the validation result of one-leave-out-cross validation. Another experiments have been made to confirm the appearance of collagen bands in the previous result, by formalin-immersed pure collagen experiment and different time of frozen storage experiment. The results showed that bands due to collagen observed in the previous result were not because of the formalin treatment but due to frozen storage treatment. Microstructure analysis shows ice crystallization on the fish tissue after frozen storage, which shows good agreement with the previous study.

【Conclusion】 Raman spectroscopy is a good technique for detection of formalin contamination. Furthermore, Raman spectroscopy could distinguish between fresh and frozen fish.