51st International Congress of Meat Science and Technology
August 7-12, 2005 – Baltimore, Maryland USA

NONDESTRUCTIVE NIR SPECTROSCOPY FOR DETECTING FRESH AND FROZEN-THAWED FISH MEATS

Musleh Uddin,* Emiko Okazaki and Yutaka Fukuda

Division of Food Technology and Biochemistry, National Research Institute of Fisheries Science, 2-12-4 Fukuura, Kanazawa-ku, Yokohama 236-8648, Japan

Key Words: NIR spectroscopy, fresh and frozen fish, PCA, SIMCA, LDA

Introduction

Given the perishable nature of fish or fillets, extension of its shelf-life is a requirement of normal trading. According to the Food and Agricultural Organization (FAO) and the Japan Agriculture Standard (JAS) regulations, labeling should state that the fish has been frozen and must not be refrozen (FAO 1982; JAS 2000). Fresh fish is, indeed, understood as being fish freshly caught or which has been chilled and stored for a short period at normal refrigeration temperature prior to purchase or use. For storage over longer periods freezing is normally utilized. The consumer perception of frozen fish is inferior to that of the fresh material and this is reflected in the price which it realises. In practice, a considerable number of frozen fish are thawed in fish shops, stored on ice and sold as unfrozen fish without being labeled as such. Control of labeling is only possible, if there any rapid and reliable methods exit, which allow food control authorities to distinguish between fresh and frozen-thawed fish or fillets.

To detect fresh and frozen-thawed fish, measurement of the electric properties of fish tissues, visual inspection of the eye lens, judgments of the integrity of red blood cells by microscopy, or estimation of the hematocrit value were proposed (Yoshioka and Kitamikado 1988; Rehbin 1992). In practice, proposed methods cannot be applied to those fish or fillets possessing no blood, eye lens or skin. Determination of enzyme activity (Rehbein and Cakli 2000) is also time-consuming or destructive. Near-infrared (NIR) spectroscopy is a physical and faster technique, requiring minimal or no sample preparation/reagent and its precision can be high. The method offers the possibility of measuring physical and chemical properties of fish or fillets. It has been widely used in the food industry, is based on the electromagnetic absorption of organic compounds (Uddin and others 2002; Blazquez and others 2004). No extractions are needed and no wastes are produced in visible/NIR spectroscopy using fibre optic probe, which would be an eco-friendly instrumental technique.

Objectives

To develop a nondestructive fast technique for identification of fresh and frozen-thawed fish meats.

Methodology

Live 108 each of red sea bream *Pagrus major* and horse mackerel *Trachurus japonicus,* were purchased from Kanazawa Prefecture, Japan. For fresh or unfrozen, 54 samples were used soon after killed while another 54 fish was kept at –40ºC. After 30 days of frozen stored samples were removed and thawed overnight at 5ºC then evaluated as frozen-thawed samples.

Samples were scanned using a NIRSystems 6500 spectrophotometer (Silver Spring, MD, USA) equipped with a surface interactance fibre optic accessory. Before spectra were measured on the fish, a reference spectrum was obtained by measuring the reflected radiation from a white ceramic plate. Spectra were recorded the wavelength range 400-1098 nm at 2 nm intervals. The spectra were stored in optical density units log (1/T), where T represents the percent of energy transmitted. Spectral data were analyzed with "The Unscrambler" software (Version 8.05, Camo, USA). The 108 samples in total, 54 of them fresh and 54 of them frozen-then-thawed, were divided into a modeling set and a prediction set. The modeling set contained 35 samples for the fresh and 35 for the frozen fish. Twenty-seven of those samples were picked as every odd numbered sample in the order of recording, and the remaining 8 samples were selected randomly. Thus, 19 samples for both fresh and frozen fish were allocated to the prediction set. Sample spectra for both sets were treated in exactly the same way with second derivative or multiplicative scatter correction (MSC), or no treatment was applied at all.

We used the classification method called Soft Independent Modeling of Class Analogy (SIMCA) and Linear Discriminant Analysis (LDA) using PCA (principal components analysis) scores. The former method is based on disjoint PCA models where for each group an independent PCA model is constructed which are then used to classify new, unknown samples. Later one uses the so-called scores values of PCA results as input variables to the LDA. By performing PCA first, we reduce the number of variables and make them independent and by doing so, only a small fraction of information is lost.

Results & Discussion

For a classification to be successful two things are needed. Firstly, samples belonging to the same group should be as similar as possible and secondly, the groups should be as far away from each other as possible. In our case, defining groups was easy, since the absorbance spectra of the fresh and frozen-thawed fish samples are very much different as seen in Figure 1. The second derivative spectra of fresh and frozen samples shown with the water band featuring strongly around 966 nm as a negative peak and similar observations were also noted in horse mackerel (data not shown). The major effect of freeze-thawing treatment involves a gross change in total absorbance after freezing and thawing; this arises from changes in light scatter presumably arising from alterations in the physical structure of at least the surface layer of fish. The existence of these differences suggests that it may, indeed, be possible to detect freeze-thaw treatment by means of this spectroscopic procedure.

Water absorbs strongly in specific wavelengths which is expected and usually exhibits a broad band because of H-bonding interactions with itself and with other components in the meat (Figure 1). In visible-NIR spectroscopy, the regions from 740–

760 nm and 960–980 nm are related to O–H bond of the water in the sample (Buning-pfaue 2003). In this figure, only the 900-1098 nm is displayed as we cut off the visible region and excluded it from any calculations. We have tested other spectral intervals also, but the distance of the two groups was the biggest and the spread of data points within each group the smallest when the 900-1098 nm interval with original absorbance spectra was used. The PCA score plot clearly shows us that the fresh and the frozen-thawed samples are well separated (Figure 2). Similar separation was also observed in DESIR analysis of fresh and frozen-thawed fish was performed on the meat juices (Uddin and Okazaki 2004). Using the results of this exploratory stage for all spectral treatments applied, two independent PCA models were generated with the modeling sets and then they were used to build SIMCA models. SIMCA models were applied to the prediction set and results of the prediction can be best visualized by plotting the sample-to-model distances for all samples as shown in Figure 3 where the two groups are well defined and separated. All prediction samples are much closer to the group that they should belong to. However, not every sample is within membership limits for both the modeling and the prediction samples. As can be seen some samples are located in the upper right quadrant, indicating that they belong to none of the defined models. No sample is in the lower left quadrant, meaning that no sample was classified to both groups simultaneously. The upper left and lower right quadrants define samples which belong to one group. However when the sample spectra were subjected to MSC transformation, modeling and classification seem much more uncertain (data not shown). The two groups are very close; in fact, they almost overlap even at the modeling stage. This means that the MSC transformation removed information, i.e. scattering, on which the previous model is based, therefore models are not that far apart.

As regards LDA, the results are much more clear-cut as seen in Table 1. To perform modeling and classification the same wavelength range, spectral transformation and prediction samples were used for LDA analysis as well. It is clear from the table that the model using original absorbance spectra achieved much better (100%) classification accuracy for the prediction samples. The same figures for MSC treated spectra are considerably worse, indicating again that scattering is the information that makes classification work. We think that for fresh fish, the cellular structure is intact. When light enters the fresh fish, cells not only absorb the light but also change its direction until the light reaches the next cell. This multiple changes in the direction of light is called scattering, which increases the distance the light travels from the entry point to the exit point of the sample. This increase results in increased absorbance as seen in Figure 1a. On the other hand, when freezing and thawing is done, the cell membranes get damaged leaking the intracellular contents into the extracellular space. Thus, there is much smaller number of cells that can scatter light as it travels trough the sample, reducing the distance the light has to cover. As a result, light interacts with a smaller number of molecules, which in turn results in a decrease of absorbance (Figure 1). Nevertheless, results are promising that a fast measurement method can be developed to detect fraud such as when frozen-thawed fish are sold as fresh.

Conclusions

The applicability of visible/NIR technique has been successfully demonstrated to differentiate between fresh and frozen-thawed fish. The technique uses the fact that fish muscle absorbs and reflects light in different ways during storage and thawing. Spectroscopically measuring raw materials using known characteristics, models can be developed that can again be used to estimate the characteristics for unknown specimen. Once have the models, differentiation between fresh and frozen thawed fish could be a matter of seconds.

References

Blazquez C, Downey G, O'Donnell C, O'Callaghan D, Howard V. 2004. Prediction of moisture, fat and inorganic salts in processed cheese by near infrared reflectance spectroscopy and multivariate data analysis. J Near Infrared Spectrosc 12: 149–158.

Buning-pfaue H. 2003. Analysis of water in food by near infrared spectroscopy. Food Chem 82: 107–115.

FAO, 1982. Reference manual to codes of practice for fish and fishery products. FAO Fish, Circ. 750, 217.

JAS (Japan Agricultural Standard), 2000. Quality labeling standard for perishable foods. Notification No. 514.

Rehbein H. 1992. Physical and biochemical methods for the differentiation between fresh and frozen-thawed fish or fillets. Ital J Food Sci 2: 75–86.

Rehbein H, Cakli S. 2000. The lysosomal enzyme activities of fresh, cooled, frozen and smoked salmon fish (Onchorhyncus keta and Salmo salar). Turk J Vet Anim Sci 24: 103–108.

Uddin M, Ishizaki S, Okazaki E, Tanaka M. 2002. Near-infrared reflectance spectroscopy for determining end-point temperature of heated fish and shellfish meats. J Sci Food Agric 82: 286–292.

Uddin M, Okazaki E. 2004. Classification of fresh and frozen-thawed fish by near-infrared spectroscopy. J Food Sci 69: C665–668.

Yoshioka K, Kitamikado M. 1988. Differentiation of freeze-thawed fish fillet from fresh fish fillet by the examination of erythrocyte. Nippon Suisan Gakkaishi 54: 1221–1225.

Tables and Figures

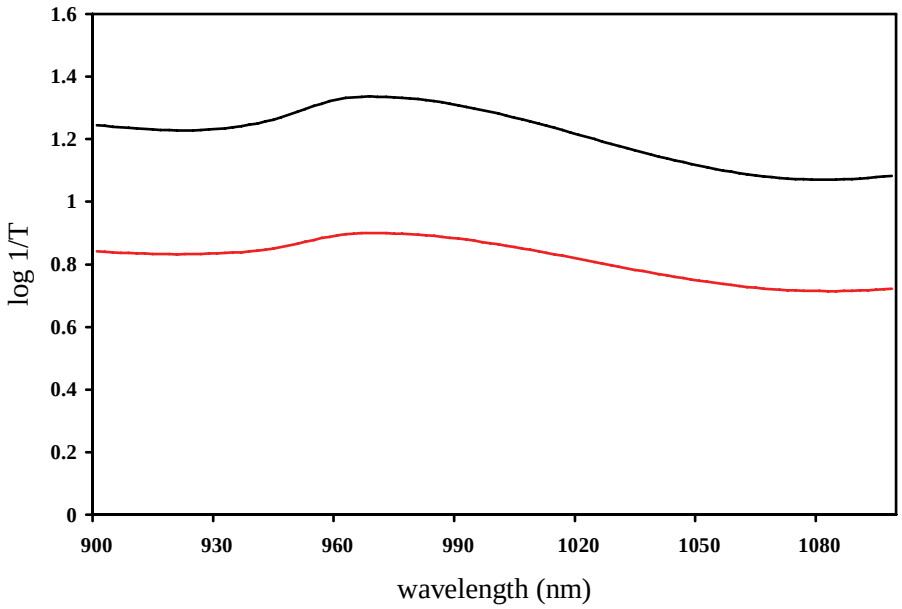

Figure 1. Average original absorbance spectra of fresh (upper line) and frozen (lower line) red sea bream sample spectra in the 900-1098 nm wavelength range

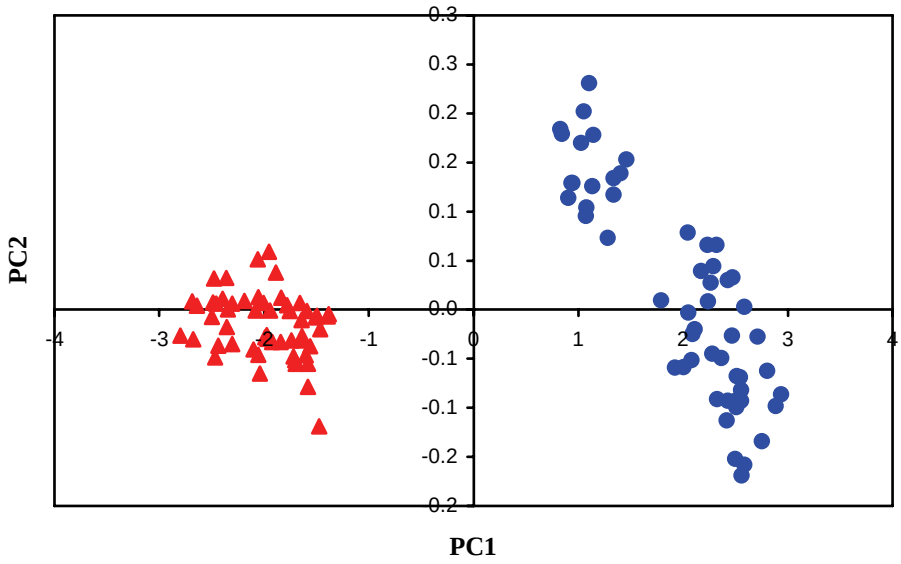

Figure 2-Two dimensional PCA score plot of all 108 red sea bream samples. Samples on the left side of the ordinate axis are frozen samples (triangles), while those on the right are fresh samples (circles).

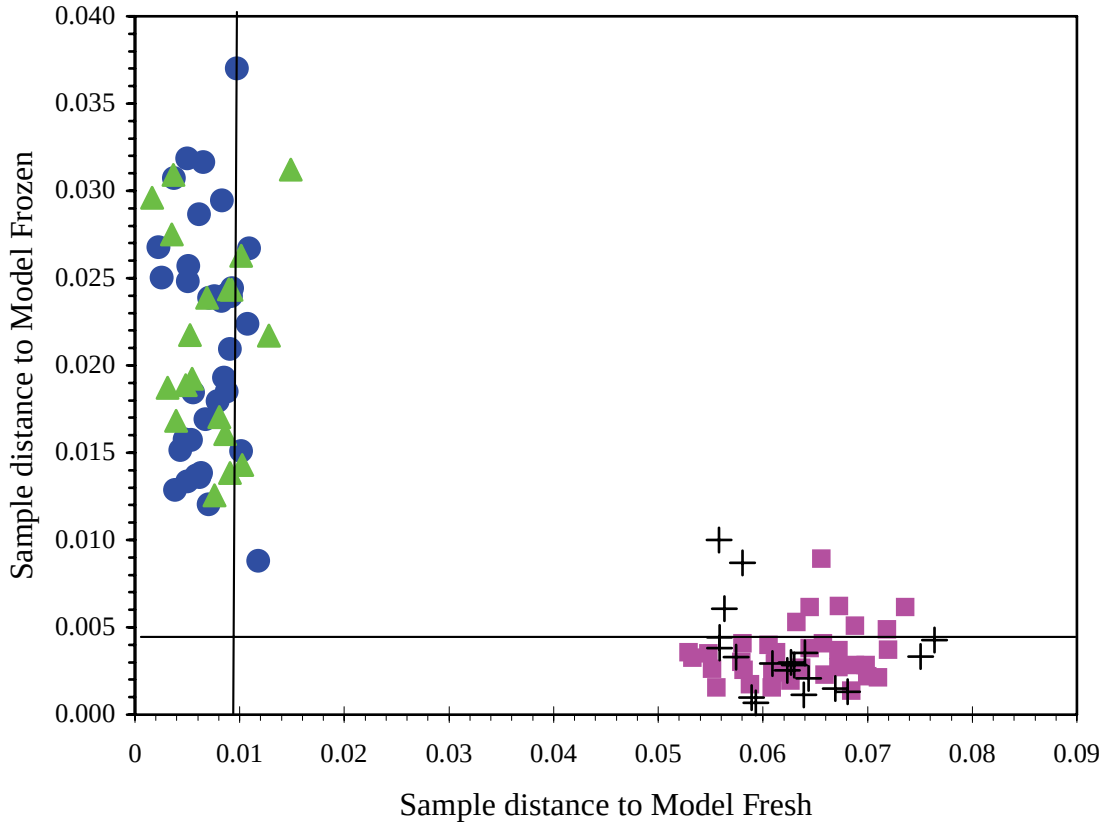

Figure 3-Cooman's plot for discrimination between fresh and frozen-thawed red sea bream. Spectra were submitted to SIMCA without any treatment. Circle Rs are fresh modeling samples, square Fs are frozen-thawed modeling samples, and triangle Rs and plus Fs are fresh and frozen-thawed prediction samples, respectively. The horizontal and vertical gray lines are class memberships limits calculated at a 5% confidence limit.

Table-1. Discrimination results between fresh and frozen-thawed prediction red sea bream using LDA with PCA scores as input variables

Spectral transformation	N correct in Groups[a]		Group proportion correct		Overall proportion correct
	Type of fish		Type of fish		
	Fresh	Frozen	Fresh	Frozen	
None	19	19	100%	100%	100%
MSC	15	16	79%	84%	81%

[a]The number of correctly classified samples out of 19 prediction samples for the fresh and frozen-thawed red sea bream groups, respectively