Non-destructive classification between unfrozen and frozen-thawed horse mackerel using visible/near-infrared spectroscopy

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Introduction

Food authenticity issues draw considerable attention with increasing consumers' concerns in Japan. Frozen and thawed fish usually have a lower market price than fresh, i.e. unfrozen fish; therefore, the substitution of frozen-thawed, for unfrozen fish is a significant authenticity issue. Fishery products that have been frozen-thawed must be labelled as "thawed" in accordance with the Quality Labelling Standard for Fishery Products[1] based on the Japanese Agricultural Standard (JAS) Law in Japan. Correct "thawed" labelling and the rapid and non-destructive method that can be used to check the "thawed" labelling are required to ensure fair trade and consumers' benefit. Although various methods based on changes in eye lens, erythrocyte, enzymatic activities and dielectric property have been proposed for identifying unfrozen and frozen-thawed fish,[2,3] they still have some difficulties in the practical use because these are destructive, time-consuming, technically demanding and/or depending on the type and quality of samples. This paper describes the results of exploring an alternative technique, utilising visible/near-infrared (NIR) spectroscopy.[4,5]

Experimental

Samples

A total of 139 live cultured horse mackerel *Trachurus japonicus* (body weight, 98–235 g; fork length, 19.5–24.5 cm) were used. Unfrozen samples were stored at 5°C while frozen-thawed samples were prepared by thawing at 5 °C after frozen storage below –40°C.

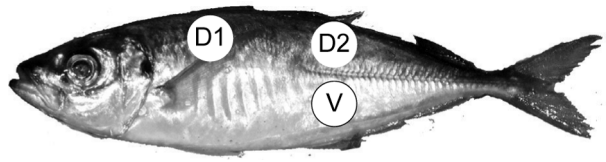

Figure 1. Spectral acquisition sites of horse mackerel.

Spectral acquisition

Diffuse reflectance (DR) spectra of the horse mackerel samples were measured in the 400–1100 nm region at a 2 nm interval using a NIR spectrophotometer (NIRSystems6500, Foss NIRSystems, Laurel, MD, USA) equipped with an interactance fiber optic probe. The visible NIR spectra were collected at two dorsal sites and a ventral site of each sample by placing the probe on the skin (Figure 1).

Data analysis

The samples were divided into a training set (54 unfrozen and 75 frozen-thawed samples) and a test set (27 unfrozen and 37 frozen-thawed samples). Spectral pretreatment[6] and principal component analysis (PCA) were performed using the Unscrambler (version 9.6, CAMO, Oslo, Norway). Based on the scores derived from PCA, misclassification rate was calculated by an in-house program coded in MATLAB (version 7.8, The MathWorks, Natick, MA, USA).[7]

Results and discussion

Figure 2 shows 193 DR visible/NIR spectra obtained at each spectral acquisition site of horse mackerel. Overall absorbance level of the original spectra obtained at dorsal sites was greater than that at the ventral site probably due to the difference in colour of the skin. The colour of the dorsal skin of horse mackerel is darker than that of ventral one, which is whitish/silver (Figure 1), leading to higher absorption of visible/NIR light. Since some peaks were strongly overlapped, and the spectra exhibited unwanted baseline shift probably due to the morphological variation among samples, second derivative was applied in order to enhance the peak resolution and reduce the baseline variations in the original spectra (Figure 2). The classification results using the second derivative spectra from each site are listed in Table 1. Sufficient classification models with low misclassification rate (less than 5%) were obtained for all the three sites. Figure 3 shows the score plots for the principal components (PC) used for misclassification rate calculation. It is noted that the thresholds to distinguish the unfrozen and frozen-thawed samples can be drawn along PC1 or PC2 direction in each case. The corresponding loading vectors are shown in Figure 4. The wavelength region around 630 nm was found to be highly important in classifying the unfrozen and frozen-thawed horse mackerel in all the models from the three different sites. Since it probably corresponds to the absorption related to metmyoglobin[8] and methemoglobin,[9] it suggests that the increase in their contents in dark and/or ordinary muscle under the skin can reflect the alteration during the freezing-thawing process.[10,11]

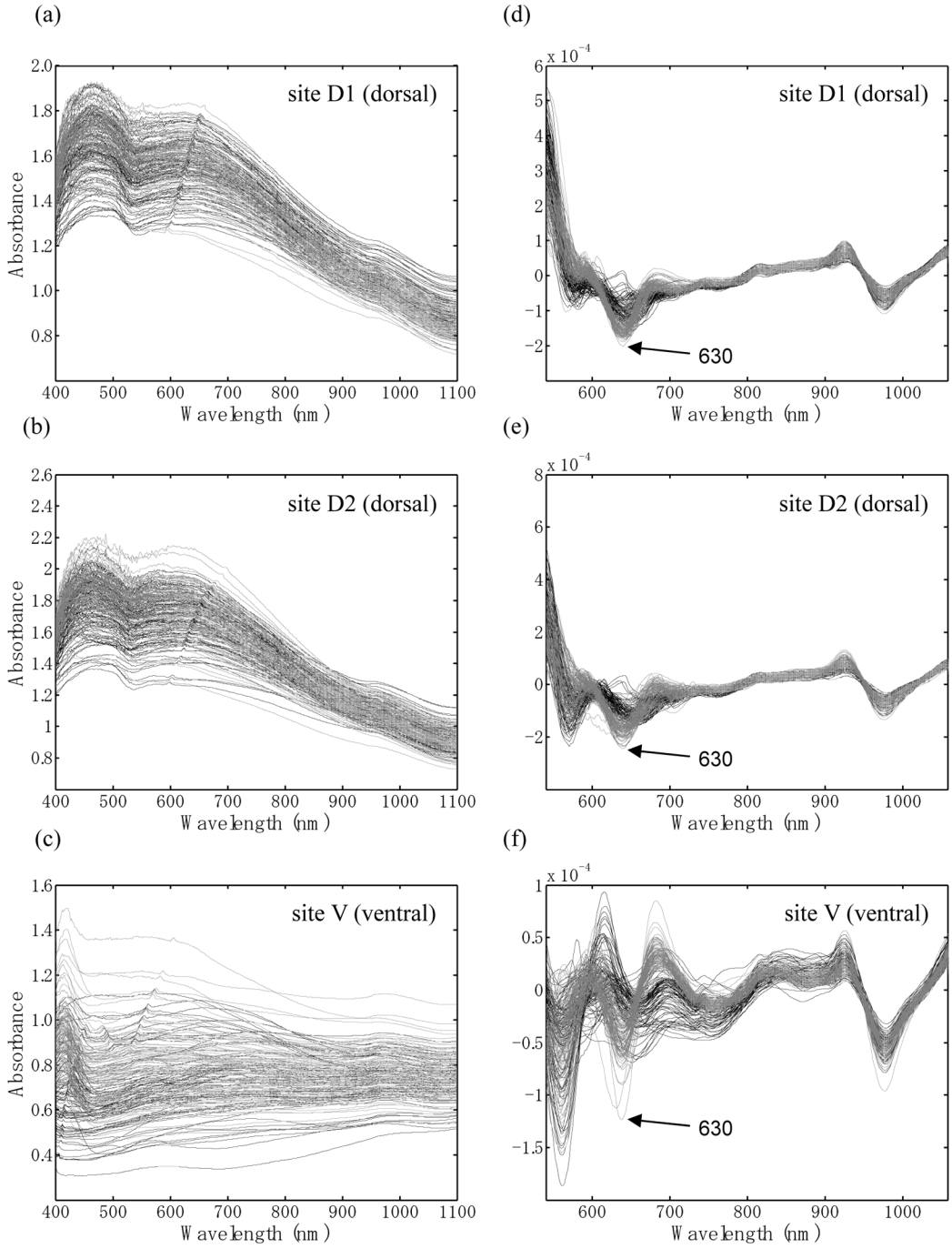

Figure 2. Visible/NIR spectra of unfrozen (black) and frozen-thawed (gray) horse mackerel. (a) site D1 (dorsal), original spectra. (b) site D2 (dorsal), original spectra. (c) site V (ventral), original spectra. (d) site D1, second derivative spectra. (e) site D2, second derivative spectra. (f) site V, second derivative spectra.

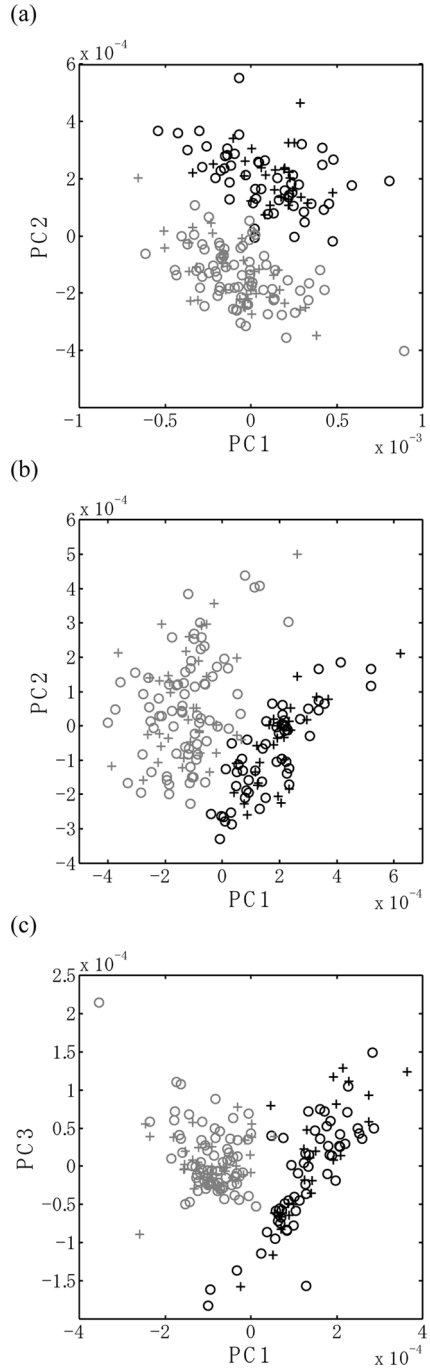

Figure 3. PCA scores for (a) site D1 (dorsal), (b) site D2 (dorsal) and (c) site V (ventral). Training set (o), test set (+); unfrozen (black), frozen-thawed (gray).

Table 1. Representative classification models between unfrozen and frozen-thawed horse mackerel based on PCA.

Measurement site	Wavelength region(nm)	Principal component (PC)	Misclassification rate (%)	
			Training set (129 samples)	Test set (64 samples)
D1 (Dorsal)	540–1058	1–2	2.3	0.0
D2 (Dorsal)	600–1058	1–2	1.6	4.7
V (Ventral)	600–1058	1–3	2.3	3.1

Conclusion

A high potential of visible/NIR spectroscopy for non-destructive classification between unfrozen and frozen-thawed horse mackerel has been demonstrated. Importantly this technique based on monitoring the increase in the metmyoglobin/methemoglobin content in dark and/or ordinary muscle under the skin may be applied to detection of frozen-thawed fish in other fish species because fish generally has dark muscle containing myoglobin and hemoglobin under the skin.

Acknowledgments

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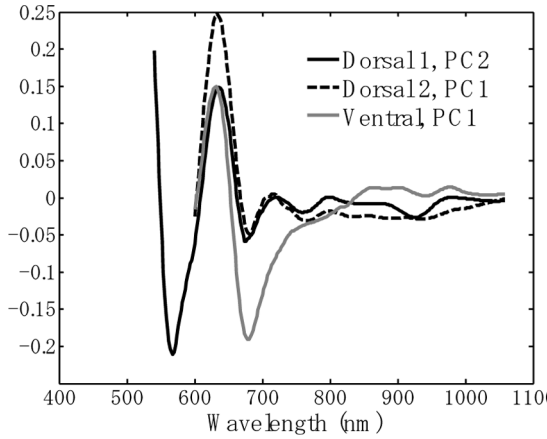

Figure 4. Loading vectors corresponding to the PCA scores in Figure 3.

References

1. Ministry of Agriculture, Forestry and Fisheries (Japan), Notification No. 516 (2000).
2. H. Rehbein, *Ital. J. Food Sci.* **2,** 75 (1992).
3. G. Duflos, B. Le Fur, V. Mulak, P. Becel and P. Malle, *J. Sci. Food Agric.* **82**, 1341 (2002).
4. M. Uddin and E. Okazaki, *J. Food Sci.* **69,** C665 (2004).
5. M. Uddin, E. Okazaki, S. Turza, Y. Yamashita, M. Tanaka and Y. Fukuda, *J. Food Sci.* **70,** C506 (2005).
6. A. Savitzky and M.J.E. Golay, *Anal. Chem.* **36,** 1627 (1964).
7. H. Shinzawa, S. Morita, Y. Ozaki and R. Tsenkova, *Appl. Spectrosc.* **60,** 884 (2006).
8. W.D. Brown, M. Martinez, M. Johnstone and H.S. Olcott, *J. Biol. Chem.* **237,** 81 (1962).
9. F. Matsuura and K. Hashimoto, *Bull. Japan. Soc. Sci. Fish.* **21,** 1158 (1956).
10. C.J. Chow, Y. Ochiai and K. Hashimoto, *Bull. Japan. Soc. Sci. Fish.* **51,** 2073 (1985).
11. C.J. Chow, Y. Ochiai, S. Watabe and K. Hashimoto, *Nippon Suisan Gakkaishi*, **54,** 473 (1988).