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Smart technique for accurate monitoring of ATP content in frozen fish fillets using fluorescence fingerprint

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ABSTRACT

The aim of the present study was to develop a fast and nondestructive method based on fluorescence fingerprints (FFs) to predict the ATP content in frozen fish meat frozen at early stages after death using fillets of horse mackerel (*Trachurus japonicus*) as a model. Fifty-six fish were sacrificed instantly, stored in ice for different periods (0–48 h), and then filleted and frozen. The fluorescence fingerprints of the frozen fillet samples were acquired using fluorescence spectrophotometer with fiber probe installed inside a freezer. Subsequently, the ATP-related compounds of the same samples were determined using HPLC. Finally, four different models based on partial least squares (PLS) were developed to predict ATP contents from HPLC and the FFs data. The best PLS model with a correlation coefficient (R^2) of 0.88 and root mean square error estimated by cross validation (RMSECV) of 0.97 μmol/g was obtained when the most important combinations of excitation-emission wavelengths were used for prediction. This methodology offers a simple and rapid approach to detect the ATP contents in frozen fish nondestructively without thawing the sample during the assessment that could be applied during any stage of fish marketing, facilitating quality control activities and the determination of fishery market price.

1. Introduction

In the fish muscle, a series of chemical and autolytic changes that involve enzymes occur during post-mortem metabolism, for instance, adenosine 5′-triphosphate (ATP) is sequentially hydrolyzed to breakdown products and phosphate (Hong, Regenstein & Luo, 2017). Because the degradation of ATP indicates changes in the fish meat in terms of freshness and quality, deep freezing (≤ −30 °C) is a method of long-term preservation, which is useful to the fishing industry, for keeping the original quality as well as the ATP content unchanged (Burgaard & Jørgensen, 2010). Frozen fish with high ATP content exhibit a high tolerance to protein denaturation and muscle discoloration during frozen storage (Inohara, Kimura, & Yuan, 2013). Moreover, fish (such as Atlantic salmon, cod, and mackerel) that are frozen in the pre-rigor stage retain good quality with high ATP content, and better color and meat texture than the fish, which are frozen in the post-rigor stage (Skjervold et al., 2001; Einen, Guerin, Svein Olav Fjæra, & Skjervold, 2002; Cappeln & Jessen, 2001; Fukuda, Tarakita, & Arai, 1984).

In commercial tuna fishing, different types of fish (dead and alive) are often hauled onto the deck after day long fishing using long lines, and most fish are immediately frozen at ultra-low temperatures (−60 °C) in the ship (Inohara et al., 2013). Some species are stored in ice for several hours/days on a fishing vessel and then frozen, upon arrival at the landing center. This makes it difficult to distinguish dead catch and alive catch in the frozen state, with respect to quality, by the naked eye. In the fish markets of Japan, the price of frozen tuna is empirically determined based on the color and degree of shrinkage of the caudal region after rapid thawing, since high shrinkage (thaw-rigor) indicates the presence of high ATP levels, and it appears that these fish are frozen in the pre-rigor stage, which increases the market prices (Okazaki, 2009). Furthermore, the rapid thawing of ATP-replete frozen fish may cause muscle contraction, which reduces the quality of the fish (Peters et al., 1968; Einen et al., 2002). Consideration on the treatment methods such as thawing conditions suitable to the fish materials will be paid depending on their ATP content. Thus, real-time measurement of ATP levels is strongly desired from the viewpoint of fishery-related

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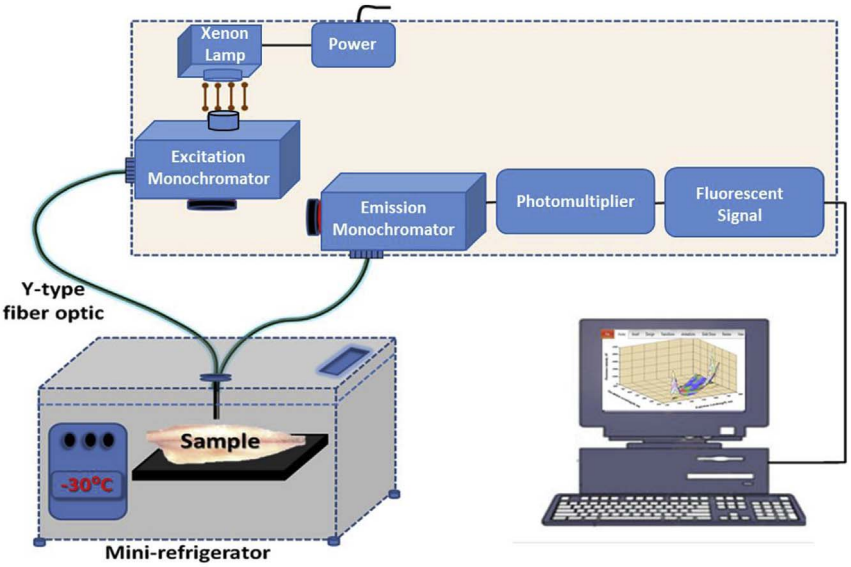

Fig. 1. Measurement system of fluorescence fingerprint of frozen fish samples inside a mini-refrigerator with a Y-type fiber optic probe.

industries and food distribution.

However, conventional chemical methods for determining ATP concentration or changes in ATP levels are more complex because of space and time limitations and the destructive nature of the technique. Thus, the development of a simple, rapid, reliable, non-invasive, and selective method for the detection of ATP without thawing the sample is challenging. Although there are some fast and destructive methods utilizing paper strips and luminescence for the detection of ATP (Drew & Leeuwenburgh, 2003; Hattula & Wallin, 1996), these methods are not suitable for the accurate monitoring of ATP levels in frozen fish meat because ATP breakdown begins immediately after thawing.

Techniques based on fluorescence spectroscopy appear to fulfill the requirements imposed by the fisheries sector for providing critical information on quality during the different stages of food production and in regulatory affairs.The method of the fluorescence fingerprints (FFs), which is also called excitation-emission matrix (EEM), is based on repeated records of emission signals for multiple numbers of excitation wavelengths. The technique has been demonstrated in specific pieces of work for the prediction of the freshness of frozen fish (ElMasry et al., 2015; ElMasry, Nakazawa, Okazaki, & Nakauchi, 2016), detection of microbial spoilage (Oto et al., 2013; Yoshimura et al., 2014), and estimation of the levels of various constituents (Dufour, Frencia, & Kane, 2003; Engelen, Møller, & Hubert, 2007).

A few previous studies using fluorescence spectroscopy have examined the freshness of frozen horse mackerel samples, which were previously stored in a refrigerator at 4 °C until 12 days based on several indices of freshness (*K*, K_1, *P*, *G*, *H-values*) (ElMasry et al., 2015; ElMasry et al., 2016). However, the implicated fluorescent compounds (such as ATP-related compounds), which were related to the indices of freshness, could not be specified and the fish bodies in which ATP was almost depleted, were treated. Therefore, the present study was aimed at specifically examining the ATP content in horse mackerel fillet (as a model) to know the early post-mortem quality of frozen fish by using a fast and nondestructive method of detection based on fluorescence spectroscopy and multivariate analyses. This is the first study to investigate the use of a fluorescence spectroscopy technique for detecting ATP contents in frozen fish at a very early post-mortem stage.

2. Materials and methods

2.1. Samples

Assuming the frozen fish meat with different storage period after death and different ATP content, the samples of horse mackerel (*Trachurus japonicus*) (20.07 ± 0.53 cm and 132.60 ± 9.81 g of average body length and weight, respectively) were used in this study as a model sample. Alive fish were transported to the laboratory from the fish store (Tokyo, Japan). Fifty-six fish were sacrificed instantly by spinal cord destruction and stored in ice for different periods (0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 12, 24, and 48 h) to prepare different ATP content among the samples. After chilled storage, the fish were beheaded, gutted, filleted and packed individually in vacuum packs. Afterward, the fillet samples were quickly frozen in an air blast freezer and kept at −30 °C until the FF measurements. FF measurements were then performed directly for the frozen fillet samples. Once the fluorescence spectra were acquired, the samples were repacked and kept frozen at −60 °C to maintain original quality until the determination of ATP-related compounds. Four samples were prepared for each storage condition ($n = 4$).

2.2. Instrumental configuration for acquiring fluorescence spectra

To alleviate the effect of environmental conditions during measurements, fluorescence fingerprints (FFs) of the frozen fillet sample were acquired inside the freezer (SC-DF25; Twinbird Corp., Niigata, Japan) at −30 °C without thawing the samples. All the measurements were performed in a dark room using a fluorescence spectrophotometer (F-7000; Hitachi High-Tech Science Corp., Japan) equipped with an Y-type fiber optic probe as shown in Fig. 1. Each frozen sample was positioned in the middle of the freezer. The optical fiber probe was held 2 mm above the sample to capture its fluorescence spectrum. The fluorescence intensity was measured by scanning the excitation wavelengths from 250 mm to 800 nm at 10 nm steps, and detecting the emission intensities at 10 nm intervals between 250 and 800 nm. For both excitation and emissions, the slit width was adjusted at 20 nm, with scan speed at 30,000 nm/min. The resulting FF spectra were three-dimensional matrix: excitation wavelength λ_{Ex}, emission wavelength λ_{Em} and fluorescence intensity *F* that contains information about the concentrations of ATP and the other intrinsic fluorescence compounds.

2.3. Extraction of ATP from frozen meat

After the acquisition of FFs of all the frozen fillet samples, their corresponding ATP contents were determined according to the method described by Ehira and Uchiyama (1986). First, cylindrical subsamples (1 cm in diameter) were cut using a rotary saw from the same locations