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Effects of Storage Temperature on the Post-Mortem Changes of Wild and Cultured Olive Flounder Muscle

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The rigor-mortis progress of cultured olive flounder spiked at the brain started much faster than that of wild one. They attained full rigor state after 30 hrs at 0℃, 36 hrs at 5℃ and 50 hrs at 10℃ in the cultured flounder, while after 36 hrs at 0℃, 50 hrs at 5℃, and 60 hrs at 10℃ in the wild. ATP concentration in the muscle was around 5.9 μmol/g for wild and 6.2 μmol/g for cultured flounder. ATP breakdown progressed rapidly in 0℃ samples, followed by 5℃ and 10℃ samples. Mg^{2+}-ATPase activity of myofibrillar protein in the presence of 0.25 mM $CaCl_2$ was higher in cultured myofibrillar protein than in wild one. Mg^{2+}-ATPase activities of myofibrillar protein increased during storage in samples stored at 0℃ and 5℃ while decreased in samples stored at 10℃. The level of breaking strength of muscle immediately after death was higher in the wild muscle than in the cultured muscle. The breaking strength reached maximum level at 10 hrs after death in both samples.

Key words: olive flounder, wild, cultured, rigor-mortis, ATP, Mg^{2+}-ATPase, breaking strength.

Introduction

It is known that the taste and texture of cultured fish were deteriorated faster than those of wild fish. The protein content of wild fish was higher than that of cultured fish, but the lipid content was higher in the cultured fish than in the wild fish (Kim and Lee, 1986; Morishita et al., 1988; Nettlerton and Exler, 1992; Kim, 1994). The quality of raw fish flesh consumed as sliced is mainly evaluated by its texture. The toughness of raw fish flesh depends on fish species, degree of freshness of fish meat, content of collagen in the muscle, storage condition and killing methods (Noguchi and Yamamoto, 1955; Kim and Cho, 1992; Iwamoto et al., 1987; Ando et al., 1992; Kim et al., 1993). The improvement of muscle toughness after death was closely related to rigor-mortis progress. It is demonstrated that the ATP was the energy source of most of rigor-mortis progress and rigor-mortis occured by ATP depletion. The effect of storage temperature on rigor-mortis progress has been examined for tilapia (Poulter et al., 1981), horse mackerel (Bito et al., 1983), plaice (Iwamoto et al., 1987) and carp (Watabe et al., 1990; Hwang et al., 1991). Recently, the production of cultured fish was increased by the development of farming techniques, especially olive flounder.

The objective of this study was to investigate rigor-mortis progress of wild and cultured olive flounder during storage at 0℃, 5℃ and 10℃, and to elucidate the mechanism involved in change of physicochemical properties.

Materials and Methods

Materials

Live specimens of olive flounder, *Paralichthys olivaceus* (0.72~0.85 kg), were obtained from a local supplier. After being spiked at the brain, the samples were packed in plastic bags and stored in thermoregulated water baths adjusted to 0℃, 5℃, and 10℃.

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Rigor index

Rigor index was measured by the method described by Iwamoto et al. (1987) and used as a parameter of rigor tension. In this definition, rigor indices of 0% and 100% represent pre-rigor and full-rigor state, respectively.

Breaking strength

Breaking strength was measured using rheometer (Fudoh Kogyo Co.) according to the method of Ando et al. (1991). Muscle was sliced 40×40×10 mm with cutting knife. Elevation speed of the sample table was maintained 3 cm/min, and 8 mm cylindrical plunge was used. The results were represented means for 6 slices.

ATP determination

At the time when rigor index was measured, 2 g of the ordinary muscle was dissected from dorsal part of the carcass. The concentration of ATP was determined by Iwamoto et al. (1987) after being extracted with cold 10% perchloric acid and adjusted to neutral pH with potassium hydroxide solution.

Myofibrils

Myofibrils were prepared according to Perry and Grey's method (1956) with some modifications. All procedures were carried out at 0~4℃. The ordinary muscle was chopped and added 5 volumes of 39 mM borate buffer (pH 7.1) containing 25 mM KCl. The mixture was homogenized twice for 15 sec with Polytron PT3000 homogenizer and centrifuged at 1, 000×g for 15 min. After repeating the same procedure, the resulting precipitate was suspended in 4 volumes of 39 mM borate buffer (pH 7.1) containing 0.1 M KCl and centrifuged at 1,000×g for 15 min. Myofibrils obtained were suspended in a desired volume of 39 mM borate buffer (pH 7.1) containing 0.1 M KCl and used for ATPase assay.

ATPase assay

Myofibrillar Mg^{2+}-ATPase activity was assayed in a reaction mixture of 5 mM $MgCl_2$, 0.1 M KCl, 0.25 mM $CaCl_2$, 20 mM Tris-maleate buffer (pH 7.1), 2 mM ATP, and 0.5 mg myofibrils/mℓ. During the reaction, 2 mℓ aliquotes were taken at 1, 2, 4 and 6 min and stopped the reaction by adding 1 mℓ of 15% TCA. Liberated γ-inorganic phosphate was measured by the method of Fiske and Subbarow (1925).

Protein concentration

Protein concentration was determined by Biuret method using bovine serum albumin as a standard (Gornall et al., 1949).

Results

Changes of rigor index

The changes in rigor index of spiked wild and cultured olive flounder during storage at various temperatures were shown in Fig. 1 and, rigor onset, full rigor and ATP disappearance were summarized in Table 1. The onset time of rigor mortis in samples stored at 0℃ began at 3 hrs after death, and full rigor was attained after 36 hrs and 30 hrs in wild and cultured olive flounder, respectively. When stored at 5℃, the olive flounder began to become stiff after 3 hrs, attaining full rigor after 50 hrs and 36 hrs in the wild and cultured olive flounder, respectively. The samples at 10℃ exhibited the same onset time of rigor mortis with those at 0℃ and 5℃ samples, and the full rigor was attained after 60 hrs and 50 hrs in the wild and cultured sample, respectively. When the wild olive flounder was attained full rigor state, the rigor indices were observed among the following pairs: 0℃-96.8%, 5℃-90.2%, and 10-70.4%. The rigor indices of cultured samples were observed among the following pairs: 0℃-95%, 5℃-90%, and 10℃-72%. As shown in Fig. 1, the full rigor state in the wild samples was retained longer than that in the cultured ones.

Changes of ATP content

Fig. 2 shows time-courses of ATP degradation wild and cultured olive flounder muscle during storage at 0℃, 5℃ and 10℃. ATP concentration in the muscle were around 5.9 μmol/g in the wild samples and 6.2 μmol/g in the cultured. ATP breakdown progressed rapidly in 0℃ samples, followed by 5℃ and 10℃ samples. At 0℃, ATP concentration decreased rapidly to less than 1 μmol/g after 30 hrs and 25 hrs in both samples with the increase of rigor index. This relationship

Table 1. Rigor-mortis progress, changes of the compounds related to muscle energy and breaking strength of wild and cultured olive flounder during storage at 0℃, 5℃ and 10℃

Specimen	Temperature	Time (hrs) required for		
		Rigor onset and full rigor	ATP disappearance (<1 μmol/g)	Maxium breaking strength
Wild	0℃	3, 36	30	10
	5℃	3, 50	35	10
	10℃	3, 60	50	10
Cultured	0℃	3, 30	25	10
	5℃	3, 36	30	10
	10℃	3, 50	40	10

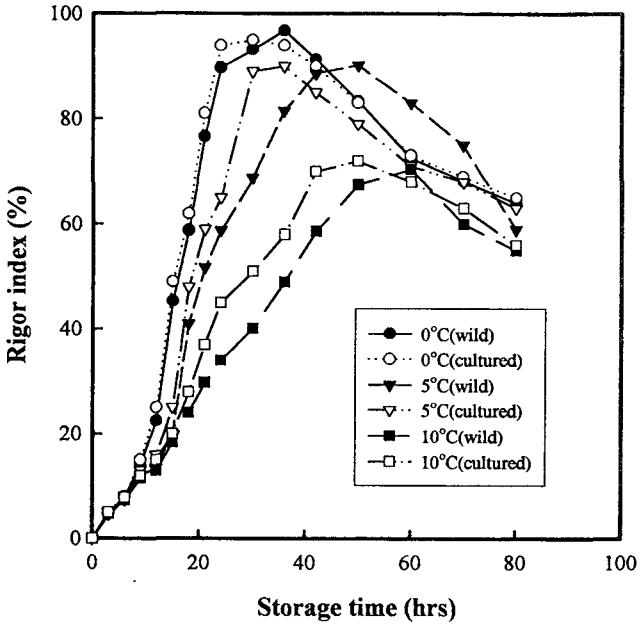

Fig. 1. Changes of rigor index in the muscle of wild and cultured olive flounder during storage at 0℃, 5℃ and 10℃.

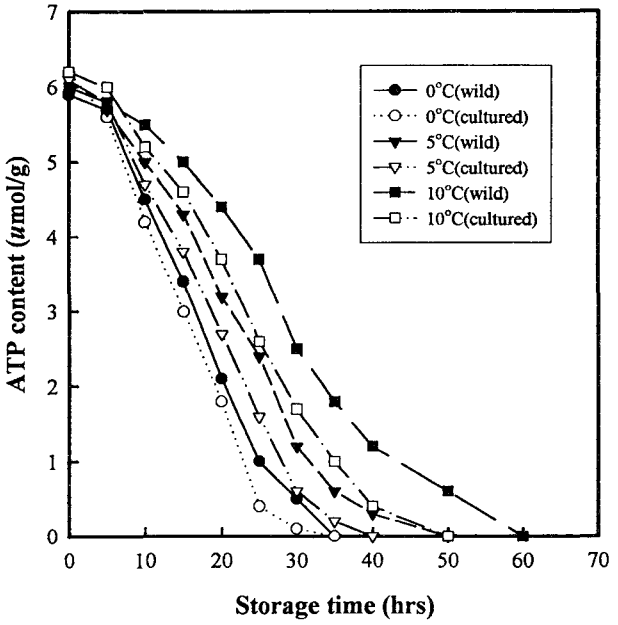

Fig. 2. Changes of ATP content in the muscle of wild and cultured olive flounder during storage at 0℃, 5℃ and 10℃.

was the same for the samples stored at 5℃ and 10℃. When the sample was stored at 5℃, ATP breakdown occurred to less than 1 μmol/g after 35 hrs and 30 hrs in both samples. At 10℃, ATP concentration decreased to less than 1 μmol/g after 50 hrs and 40 hrs in the wild and cultured sample. When the rigor index reached 50%, ATP concentration was about 3 μmol/g except in the case of 1.8 μmol/g at 10℃. The ATP breakdown in the wild samples was retarded than in the cultured ones.

Changes of myofibrillar ATPase activity

The myofibrillar ATPase activities were measured in the presence of 5 mM $MgCl_2$ and 0.25 mM $CaCl_2$, after preparation of myofibrils prepared from stored olive flounder muscle at various temperature. The changes of myofibrillar Mg^{2+}-ATPase activities of wild and cultured olive flounder muscle were shown in Fig. 3. The myofibrillar Mg^{2+}-ATPase activities were 0.777 ± 0.005 μmol Pi/mg min and 0.847 ± 0. 019 μmol Pi/mg min in the wild and cultured samples, respectively. The myofibrillar ATPase activities increased during storage in samples stored at 0℃ and 5℃ while decreased in samples stored at 10℃. After 20 hrs in samples kept at 0℃ and 5℃, the ATPase represented maximum activities, and then decreased slowly in both samples. However, in the case of sample kept at 10℃, the ATPase activity decreased readily during storage. As shown in Fig. 3, the myofibrillar Mg^{2+}-ATPase activities of the wild were lower than those of the cultured.

Changes of breaking strength

Fig. 4 shows the changes of breaking strength of olive flounder muscle during storage at various temperatures. The levels of breaking strength in

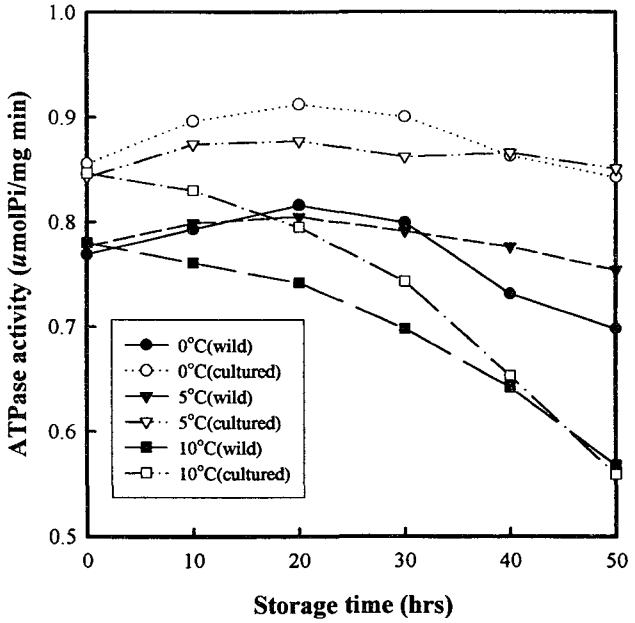

Fig. 3. Changes of myofibrillar Mg^{2+}-ATPase activity in the presence of 0.25 mM $CaCl_2$ in wild and cultured olive flounder during storage at 0℃, 5℃ and 10℃.

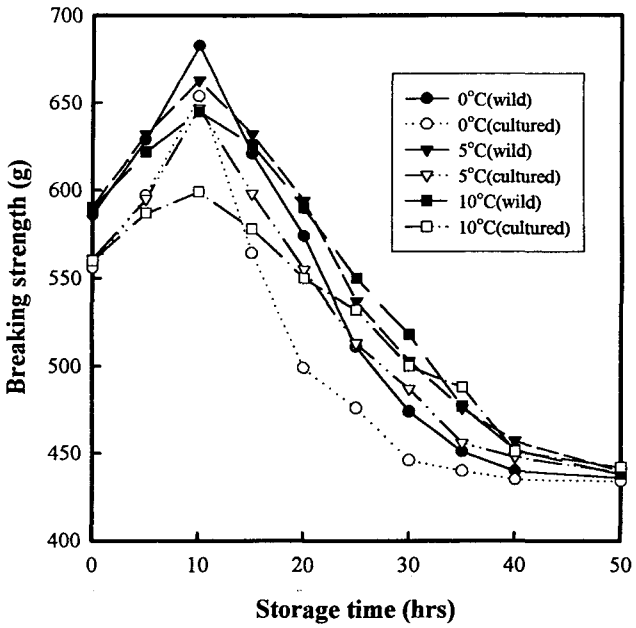

Fig. 4. Changes of breaking strength in the muscle of wild and cultured olive flounder during storage at 0℃, 5℃ and 10℃.

muscle immediately after death were 589 g in the wild samples and 559 g in the cultured. The breaking strength reached the maximum level after 10 hrs in both samples. The maximum values of breaking strength stored at 0℃ were 629 g and 597 g in the wild and cultured samples, respectively. At 5 ℃, the maximum value were 663 g and 647 g in the wild and cultured samples, while at 10℃, they were 645 g and 599g in both samples, respectively. The values of breaking strength increased rapidly at 0℃ sample, followed by 5℃ and 10℃. The breaking strength of samples stored at 0℃ and 5℃ decreased remarkably after 10 hrs, while the breaking strength of samples stored at 10℃ decreased slowly. Meanwhile, the breaking strength of the cultured sample exhibited about 95% level against that of the wild sample immediately after death. The breaking strength of the cultured samples reached 95.7% at 0℃, 97.5% at 5℃ and 92.8% at 10℃, compared to that of the wild samples at each storage temperature after 10 hrs.

Discussions

The rigor-mortis progress and changes in ATP concentration of plaice and red sea bream muscle during storage at 0℃ and 10℃ were reported (Iwamoto et al., 1985; 1987; Iwamoto and Yamanaka, 1986). The progress of rigor-mortis in spiked olive flounder muscle was the fastest at 0℃ among tested temperatures, confirming the results from fish muscle stored at various temperatures ranging from 0℃ to 25℃ (Iwamoto et al., 1987). The rigor-mortis in the cultured fish happened faster than in the wild one. These results suggest that ATP breakdown causing rigor-mortis was faster in the cultured olive flounder muscle than in the wild counterpart, and the fastest in sample stored at 0℃ of tested temperature, confirming the present experiments (Fig. 2, Table 1). Iwamoto and Yamanaka (1986) also reported that rigor-mortis progress and ATP breakdown in the cultured were faster than in the wild red sea bream. To elucidate the mechanism of ATP breakdown in two types of samples and at various temperatures, we tested the changes of myofibrillar ATPase activities during storage.

Iwamoto et al. (1988) reported the influence of reaction temperature on plaice myofibrillar Mg^{2+}-ATPase activity, creatine kinase activity and sarcoplasmic ATPase activity, and they said that these factors showed high relationship. In present experiments, myofibrillar Mg^{2+}-ATPase activity in the presence of 0.25 mM $CaCl_2$ in the cultured samples was higher than in the wild ones. During storage of olive flounder muscle, the myofibrillar Mg^{2+}-ATPase activity was the highest of samples kept at 0℃, followed by samples kept at 5℃ and 10 ℃. These results explained that ATP breakdown in the wild olive flounder muscle was faster than that in the cultured sample, and ATP breakdown in samples stored at 0℃ was faster than that in samples stored at 5℃ and 10℃. Since myofibrillar Mg^{2+}-ATPase activity in contracted muscle is much higher than in the relaxed state, relatively high Mg^{2+}-ATPase activity in the cultured myofibrils result in higher rigor index, suggesting that the contraction in the cultured muscle occur strongly than in the wild muscle. We confirmed the fact from results of rigor-mortis progress, the changes of ATP concentration and the myofibrillar ATPase activity in the cultured olive flounder muscle. The increase of breaking strength after 10 hrs storage suggest that muscle contraction takes place strongly in both samples. Especially muscle contraction in the samples kept at 0℃ was stronger than in the samples at 5℃ and 10℃ (Fig. 1). It is due to increased actomyosin toughness by myosin-actin complex formation. In spite of strong contraction in the cultured olive flounder, the lower breaking

strength value in the cultured samples was due to the histological difference between wild and cultured muscles: the difference of toughness by the distribution and content of collagen in the muscle of wild and cultured fish so called background toughness (Kim, 1998).

It is suggested that the increase of breaking strength at early after death take place by strong contraction by ATP breakdown resulted from the activation of myobrillar Mg^{2+}-ATPase. It is known that fish myofibrils have Ca^{2+}-dependent Mg^{2+}-ATPase activity and Mg^{2+}-ATPase activity in the absence of Ca^{2+} is extremely low (Konno et al., 1977). Ca^{2+} might easily be released from sarcoplasmic reticulum at around 0℃, resulting in the increase of ATP consumption by activation of myofibrillar Mg^{2+}-ATPase in the presence of Ca^{2+} (Watabe et al., 1989). And the decrease of Ca^{2+}-uptake at around 0℃ has already been demonstrated in muscle (Whiting, 1980; Iwamoto et al., 1989; Hwang et al., 1991). In previous paper (Kim et al., 1998a; 1998b), it was demonstrated that the strong contraction was occurred by the rapid release of calcium from sarcoplasmic reticulum.

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