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Improving the Effects of Cold Saturated Salt Solution Immersion on the Texture of Black Rockfish (*Sebastes schlegeli*) Muscle

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Abstract

In the present study we investigated the physicochemical changes in black rockfish (*Sebastes schlegeli*) muscle subjected to immersion in a cold saturated salt solution and determined the optimal immersion conditions. Fish were sacrificed instantly by a spike to the head and immersed in cold saturated salt solution under various conditions. The onset of rigor mortis was accelerated by a decrease in immersion temperature and an increase in immersion time. Additionally, the time to reach full rigor was significantly shorter. However, the rigor index of samples of fish immersed in the cold saturated salt solution was decreased more than in samples of fish immediately after death. The breaking strength of the samples immersed in the cold brine solution increased slowly but did not increase under every set of conditions. The breaking strength of all samples immersed in the cold saturated salt solution decreased significantly after reaching the maximum value following immersion at -12.5°C for 5 min. The ATP content was lowest under the aforementioned conditions (-12.5°C and 5 min) and then increased. The lactate levels also increased under the optimal conditions and then decreased, in contrast to the ATP content. Transmission electron microscopy showed that the sarcoplasmic reticulum used in the treatment of black rockfish had natural triad structures between the Z lines, which were disrupted in samples immersed in the cold saturated salt solution.

Keywords: Black rockfish, cold saturated salt solution, breaking strength, ATP, lactate, sarcoplasmic reticulum.

Introduction

Global per capita fish consumption increased from an average of 9.9 kg (live weight equivalent) in the 1960s to 18.4 kg in 2009 and preliminary estimates for 2010 point to a further increase to 18.6 kg (FAO, 2012). Of the 126 million tons available for human consumption in 2009, Asia accounted for two-thirds of the total, with 85.4 million tons (20.7 kg per capita), of which 42.8 million tons were consumed by Asian countries other than China (15.4 kg per capita). The annual seafood consumption in the Republic of Korea increased gradually to 59.3 kg per capita in 2011 (NOAA, 2012), of which fish represented 20.9 kg per capita (KREI, 2012). The per capita consumption of sliced raw fish was not reported, but has been estimated at 3.2 kg per person (Cho, 2008). In 2012, the total amount of cultured fish produced in Korea was 97,663 tons (MIFAFF, 2012), most of which was consumed as sliced raw fish.

Sliced raw fish, known as 'Saengseonhoe' in Korea or 'sashimi' in Japan, consists of a fresh raw fish fillet sliced into thin pieces after removal of the intestines, blood, skin and bones. It is enjoyed in several Asian countries, including Korea, Japan and China, but Koreans prefer fish containing a large amount of white muscle such as flounder, black rockfish, sea bream and sea bass. In Korea, a particularly important factor that determines the quality of raw sliced fish is texture. However, unlike the dietary custom in Japan, most people in Korea enjoy eating sliced raw fish immediately after the death of the fish (Cho *et al.*, 2003). The texture of the fish muscle is considered tough in the rigor state and is affected by various handling conditions (Ando *et al.*, 1999; Nakayama *et al.*, 1992; Abe and Okuma, 1991; Hwang *et al.*, 1991; Iwamoto *et al.*, 1987; Nazir *et al.*, 1963). However, delaying rigor development during storage by spinal cord destruction and air pressure treatment has been investigated in Japan (Nagai *et al.*, 2002; Nakayama *et al.*, 1996). Rigor mortis in fish occurs within a few hours after death as the first post-mortem change. However, its progress depends on the storage temperature, killing method,

washing and whether the fish is bled (Mishima *et al.*, 2005; Mochizuki and Sato, 1996; Abe and Okuma, 1991; Hwang *et al.*, 1991). Much research has focused on improving muscle toughness by changing the seawater temperature, the feeding of medicinal herbs, inducing focused movement by altering the fluid velocity and electrical simulation (Kim, 1998; Hwang *et al.*, 1991; Tachibana *et al.*, 1988).

We previously reported the physicochemical properties of black rockfish muscle immersed in a saturated salt solution (Cho *et al.*, 2003). In the present study, we investigated the physicochemical properties of black rockfish muscle immersed in a cold saturated salt solution and determined the optimal immersion temperature and time.

Materials and Methods

Device

A custom-made device consisting of a chamber and refrigerator was used to lower the temperature of the saturated salt solution to less than -20°C.

Fish Materials

Black rockfish (*Sebastes schlegeli*) cultured for 30 or 36 months in the South Sea of Korea were purchased alive form a local supplier (Cheongsu Fisheries Co., Busan, Korea). After recovery in 15°C seawater for approximately 6 h, the fish were immersed in a cold saturated salt solution at 0, -5, -10, -12.5, -15, or -20°C for 5 min and at -12.5°C for 2.5, 5, 7.5, 10, or 15 min. The control fish were killed by spiking the brains without immersion in a cold saturated salt solution.

Methods

Breaking Strength

Breaking strength was measured using a rheometer (Compac-100; Sun Scientific Co., Ltd., Tokyo, Japan) according to Ando *et al.* (1991). The muscle was sliced into cubes of 10 × 10 × 10 mm^3 using a cutting knife. The elevation speed of the sample table was maintained at 3 cm/min and a 10-mm cylindrical plunger was used. The results are presented as the mean value of 10 fish.

Rigor Index

The rigor index of the fish was measured as a parameter of rigor tension, essentially according to Bito *et al.* (1983). The body of each fish was placed on a horizontal table with half the body (tail portion) spread out from the edge of the table. Rigor index was measured by the equation:

$$\text{Rigor index (\%)} = Do - D/Do \times 100,$$

where *Do* and *D* represent the distances from the horizontal line to the base of the tail (or in a pre-rigor state) and that during storage, respectively.

Lactate Content

Lactate was measured as described by Barker and Summerson (1941).

ATP Content

The ATP content was measured as described by Iwamoto *et al.* (1987). Dorsal ordinary muscle (1 g) was dissected from the sample fish and added to 5 mL 10% ice-cold perchloric acid. The mixture was homogenized using a glass rod and centrifuged at 900 × *g* for 5 min. The pellet was extracted in the same manner. The combined supernatants were adjusted to pH 6.5–6.8 using KOH. The formed precipitate was removed by centrifugation at 900 × *g* for 5 min. The supernatant volume was increased to 10 mL using neutralized 10% ice-cold perchloric acid and analyzed for ATP and its related compounds using a Thermo high-performance liquid chromatograph equipped with an Agilent eclipse XDB-C18 column (4.6 × 250 mm, Surveyor Plus HPLC system; Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Light Microscopy

Fish muscle cubes (10 mm^3) immersed in cold saturated salt solution at 0, -5, -10, -12.5, -15, or -20°C for 5 min were excised immediately. Each muscle cube was compressed at 100 g/cm^2 for 10 s parallel to the orientation of the muscle fibers. Cross-sections (7.0 μm) were cut from the muscle fibers, which were prefixed, postfixed, dehydrated and embedded. Next, they were stained with hematoxylin and eosin and observed by light microscopy (Nikon type 104; Tokyo, Japan).

Transmission Electron Microscopy (TEM)

Samples (10 mm in length) of the anterior intestine and muscles from fish at 150 days post-hatching were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) containing 3.5% sucrose at 40°C. Following a total fixation time of approximately 3 h, the samples were rinsed in cacodylate buffer and post-fixed in 1% osmium tetroxide in cacodylate buffer, then dehydrated using a graded series of acetone, stained in bloc for 1 h in 1% uranyl acetate in 70% acetone and embedded in Araldite resin. Semi-thin sections (0.5–1 μm) were stained with toluidine blue. Ultra-thin sections were mounted on uncoated copper grids, stained with lead citrate and uranyl acetate and observed under a JEM 1200 EXII transmission electron microscope (JEOL Ltd., Tokyo, Japan) operated at 80 kV.

Statistical Analysis

All data were analyzed by analysis of variance using the general linear model procedure (SAS Institute, Cary, NC, USA). Duncan's multiple range test was applied to determine the significance of the differences between means.

Results

Breaking Strength

The changes in breaking strength of black rockfish muscle immersed in a cold saturated salt solution at 0, -5, -10, -12.5, -15, or -20°C for 5 min are shown in Figure 1. The breaking strength values of black rockfish muscle immediately after killing and after immersion in cold saturated salt solution at 0, -5, -10, -12.5, -15 and -20°C for 5 min were 1.57, 1.69, 1.72, 1.86, 1.97, 1.74 and 1.69 kg/cm^3, respectively. The highest breaking strength was observed at -12.5°C. The breaking strength of the muscle decreased at temperatures higher or lower than -12.5°C.

The changes in breaking strength of black rockfish muscle immersed in cold saturated salt solution at -12.5°C for 2.5, 5, 7.5, 10, or 15 min are shown in Figure 2. The breaking strength values for black rockfish muscle immediately after killing and after immersion in cold saturated salt solution at -12.5°C for 2.5, 5, 7.5, 10 and 15 min were 1.57, 1.75, 1.97, 1.85, 1.71 and 1.65 kg/cm^3, respectively. The highest breaking strength was observed at -12.5°C for 5 min.

The changes in breaking strength of black rockfish muscle immersed in cold saturated salt

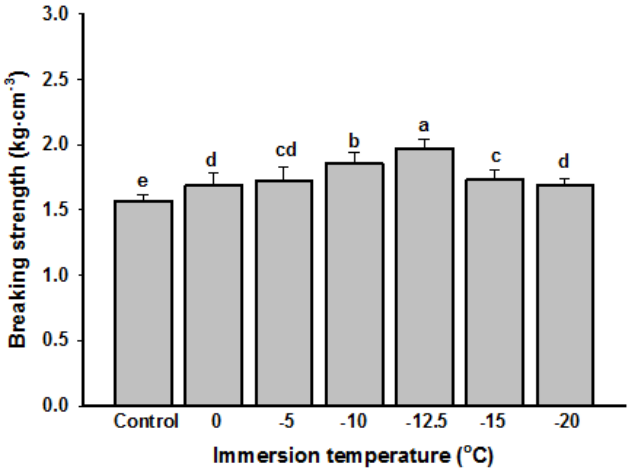

Figure 1. Effect of temperature on the breaking strength of black rockfish following immersion for 5 min in a cold saturated salt solution. The control fish were killed instantly by a spike to the head. Means represented by different superscripts above the bar were significantly different according to Duncan's multiple range test (P<0.05).

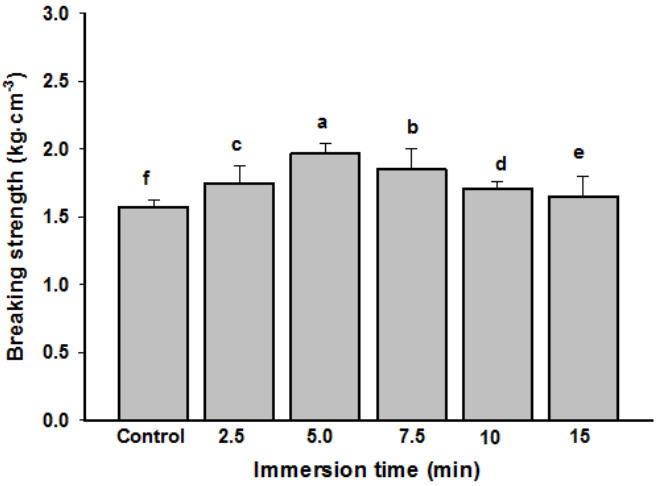

Figure 2. Effect of immersion time on the breaking strength of black rockfish exposed to cold saturated salt solution at -12.5°C. Means represented by different superscripts above the bar were significantly different according to Duncan's multiple range test (P<0.05).

solution at -12.5°C for 5 min were compared among fish weighing 300500 g and 500-700 g (Figure 3). The ratio of the breaking strength of muscle immersed in cold saturated salt solution to that of muscle immediately after sacrifice was 28.3% in the 300-500 g group and 25.4% in the 500-700 g group. The decreased breaking strength in the heavier fish was likely mediated by the enhanced transfer of heat from the tissues to the saturated salt solution.

Rigor Mortis

The effects of immersion in saturated salt solution at 0, -5, -10, -12.5, -15, or -20°C for 5 min on rigor mortis in black rockfish muscle are shown in Figure 4. We found that 90% of the maximum muscle stiffness was reached after storage for 29 h.

The times required for black rockfish muscle immersed in cold saturated salt solution to reach maximum stiffness were 23 h for the 0°C group (87%), 23 h for the -5°C group (86%), 20 h for the -10°C group (82.4%), 18.5 h for the -12.5°C group (78.8%), 16.5 h for the -15.0°C group (74.4%) and 14.5 h for the -20°C group (70.8%).

The time required for black rockfish muscle immersed in saturated salt solution at -12.5°C to reach maximum stiffness was 24 h for the immediately following sacrifice group (89.5%), 22 h for the 2.5 min group (84.8%), 18.5 h for the 5 min group (78.8%), 17 h for the 7.5 min group (74.8%), 13 h for

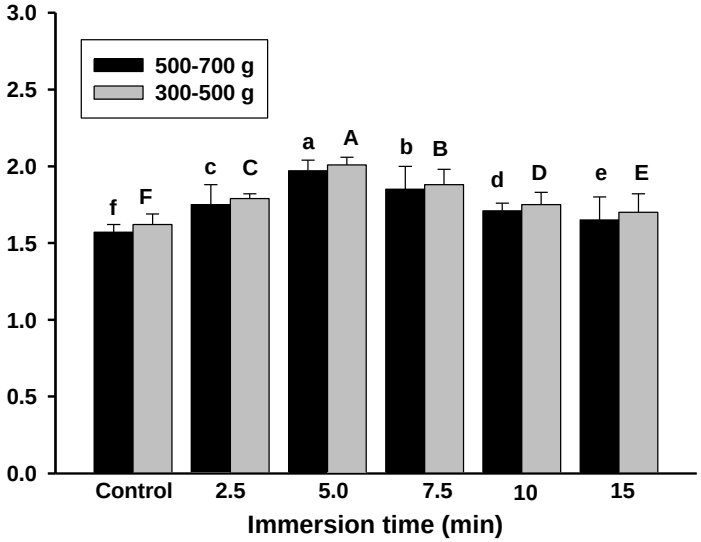

Figure 3. Effect of fish weight on the breaking strength of black rockfish following immersion for 5 min at -12.5°C in cold saturated salt solution. Means represented by different superscripts above the bar were significantly different according to Duncan's multiple range test ($P<0.05$).

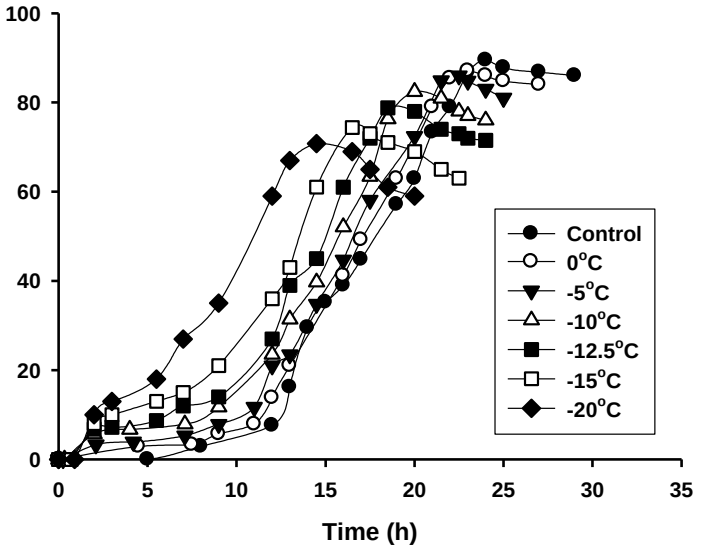

Figure 4. Changes in rigor mortis in black rockfish during storage at 5°C after immersion in cold saturated salt solution at various temperatures.

the 10 min group (68.7%) and 7.5 h for the 15 min group (59.8%) (Figure 5).

Additionally, the time to reach full rigor in fish muscle immersed in cold saturated salt solution was significantly shorter than that in fish muscle immediately after sacrifice. However, the rigor index of fish muscle immersed in the cold saturated salt solution decreased more than that of the muscle of fish that were sacrificed immediately. The rigor index was the highest in the muscle of fish that were sacrificed immediately, followed by fish immersed in saturated salt solution at 0, -5, -10, -12.5, -15 and -20°C and for 2.5, 5, 7.5, 10 and 15 min, in that order.

Lactate and ATP contents

The lactate and ATP contents of black rockfish muscle immersed in saturated salt solution at 0, -5, -10, -12.5, -15 and -20°C for 5 min while stored at 5°C are shown in Figure 6.

The lactate contents of black rockfish muscle immersed in saturated salt solution at 0, -5, -10, -12.5, -15, or -20°C for 5 min were 7.2±0.1, 7.7±0.2, 8.2±0.6, 8.7±0.2, 6.6±0.4, or 6.2±0.5 μmol/g, respectively. The ATP contents were 6.6±0.2, 6.2±0.2, 6.1±0.1, 5.9±0.2, 6.1±0.2 and 6.4±0.1 μmol/g, respectively, under identical conditions.

The lactate contents in muscle after immersion for 2.5, 5, 7.5, 10 and 15 min in cold saturated salt solution were 7.4±0.3, 7.7±0.2, 7.1±0.5, 6.6±0.3 and 6.3±0.5 μmol/g, respectively. The ATP contents were 6.5±0.3, 5.9±0.1, 6.3±0.1, 6.8±0.2 and 6.5±0.3 μmol/g, respectively, under identical conditions (Figure 7).

The muscle of fish immersed in salt water at -12.5°C for 5 min showed the greatest lactic acid content and the lowest ATP content.

Microscopy

Cross-sections of ordinary muscle samples from black rockfish showed clear disruption of the intercellular portion in the immersed samples as compared with the control samples (Figure 8). Immediately after death and at -5 and -10°C, the detachment of muscle fibers by compression was very slight. At -12.5°C, the interfibrillar space began to increase and at temperatures below -15°C, the space was enlarged.

The microstructures of fish muscle excised immediately after death and of muscle immersed at -5, -10, -12.5, -15 and -20°C for 5 min are shown in Figure 9. Immediately after death, I bands were observed but were narrow (*i.e.*, the muscle was beginning to contract). At temperatures below -12.5°C, the I bands disappeared and the M and Z lines became dense (Figure 9).

Discussion

The toughness of raw fish is directly related to the collagen content (Sato *et al.*, 1986; Hatae *et al.*, 1986). Some fish species have similar collagen contents and the toughness of muscle is a result of the effect of the actomyosin complex on the binding of myosin to F-actin. Muscle toughness is also affected by the procedure used to kill the fish (Cho *et al.*, 2003; Mochizuki and Sato, 1996; Ando *et al.*, 1991, 1993; Oka *et al.*, 1990). Immersion in a cold saturated salt solution after killing results in the greatest muscle firmness.

The accelerated rigor mortis and increased breaking strength of black rockfish muscle after immersion in a cold saturated salt solution were due to the strong muscle contraction induced by cold

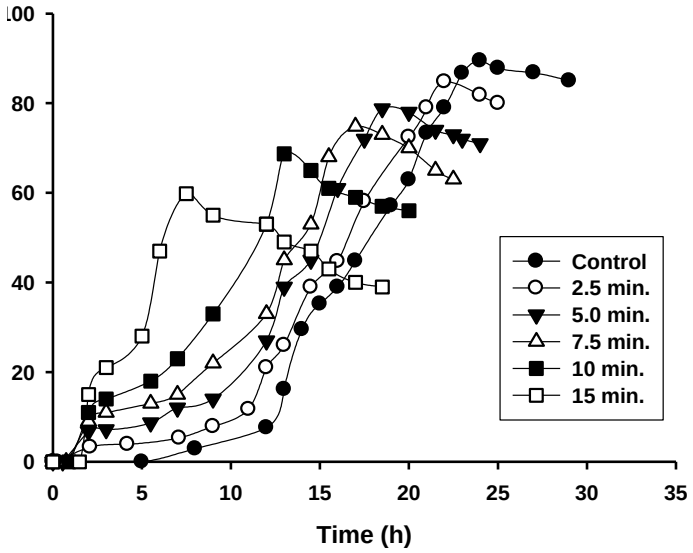

Figure 5. Changes in rigor mortis in black rockfish during storage at 5°C after immersion in cold saturated salt solution for various immersion times.

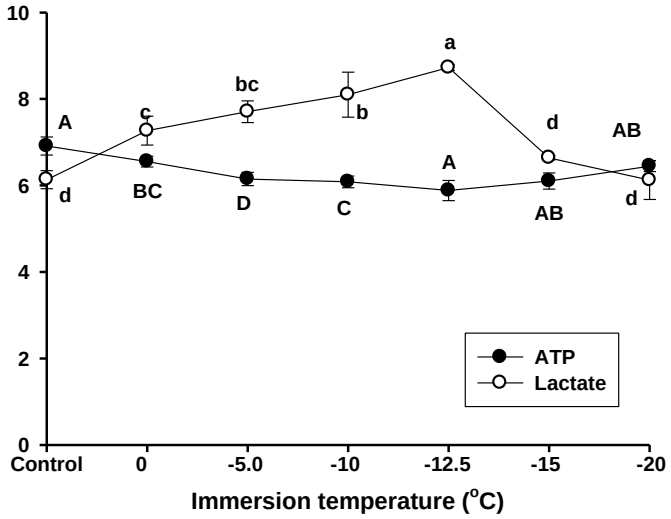

Figure 6. Effect of temperature on the ATP and lactate contents in black rockfish immersed for 5 min in cold saturated salt solution. Means represented by different superscripts above the symbol were significantly different according to Duncan's multiple range test (P<0.05).

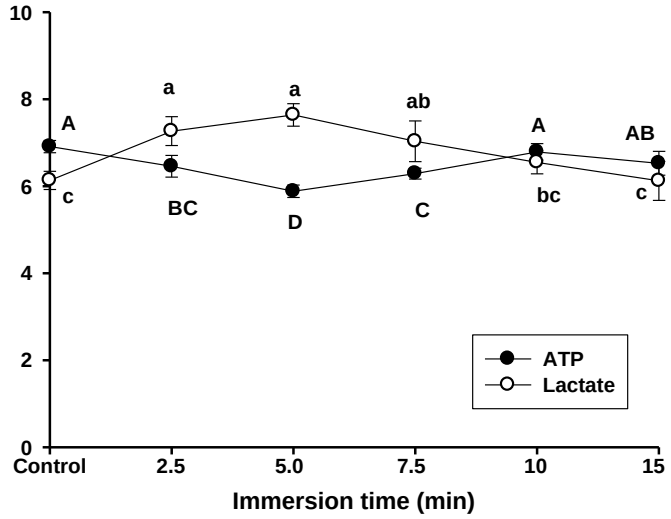

Figure 7. Effect of immersion time on the ATP and lactate contents in black rockfish immersed in cold saturated salt solution at -12.5°C. Means represented by different superscripts above the symbol were significantly different according to Duncan's multiple range test (P<0.05).

stress. The breaking strength of black rockfish muscle was highest under the most stressful condition (*i.e.*, immersion in cold saturated salt solution for 15 min) (Cho *et al.*, 2003). However, the fish were under stress not only from the salinity but also the low temperature; this could also induce strong muscle contraction (Cho *et al.*, 2003; Nakayama *et al.*, 1996). Under stress, fish muscle generates strong contractile tension and enters the rigor state (Nakayama *et al.*, 1996). When a fish dies while struggling, rigor mortis, ATP breakdown and lactate accumulation occur rapidly in the muscle (Roth *et al.*, 2002; Iwamoto *et al.*, 1990).

The black rockfish muscle exhibited increased tension at temperatures less than -12.5°C for a short period due to actomyosin; moreover, the breaking strength was decreased upon removal of the cold stress, which blocked the signaling pathway, after 5 min. In addition, the muscle breaking strength after immersion at 0, -5 and -10°C was lower than that after immersion at -12.5°C due to limited ATP degradation. At -15 and -20°C, actomyosin toughness increased briefly; however, the lower temperature weakened the collagen matrix and so reduced the background toughness. The relative weakness of the pericellular connective tissue of black rockfish immediately after death may be responsible for the softer texture of the muscle.

Light microscopic observation of black rockfish revealed muscle fiber detachment by compression following immersion for longer time periods and at lower temperatures (Figure 8). This suggests that the

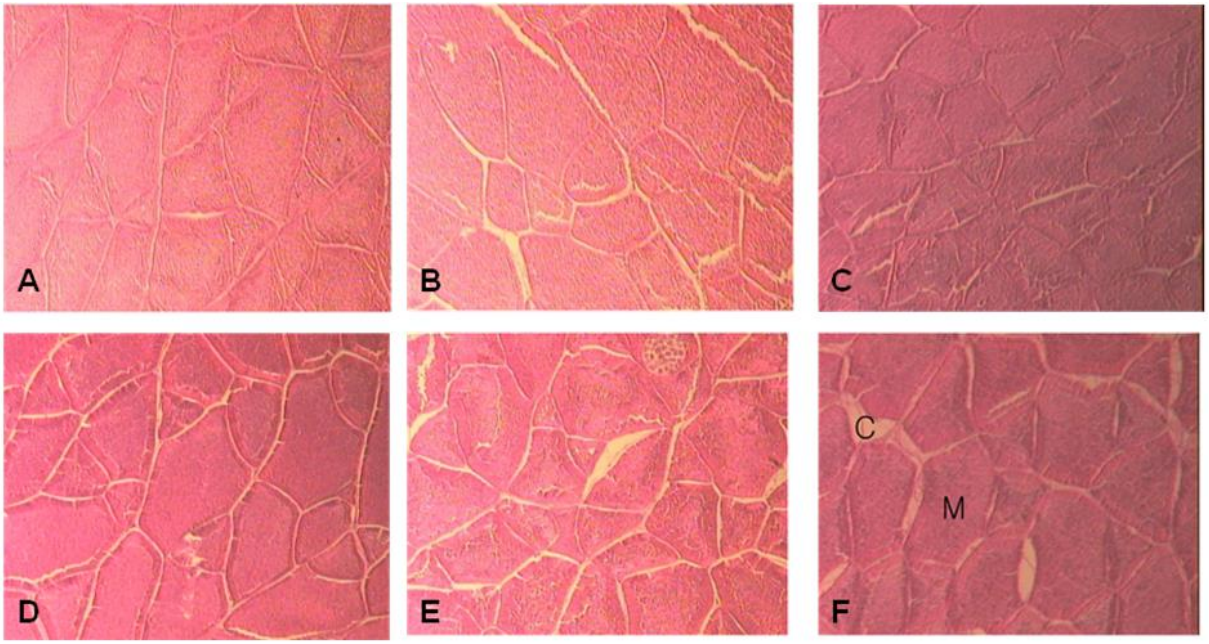

Figure 8. Light microscopic observation of black rockfish muscle compressed at 100 g/cm^2 after immersion for 5 min in cold saturated salt solution at various temperatures. A, Control (immediately after death); B: -5°C; C, -10°C; D, -12.5°C; E, -15°C; and F, -20°C. C: Structure of the extracellular matrix. M, muscle fiber.

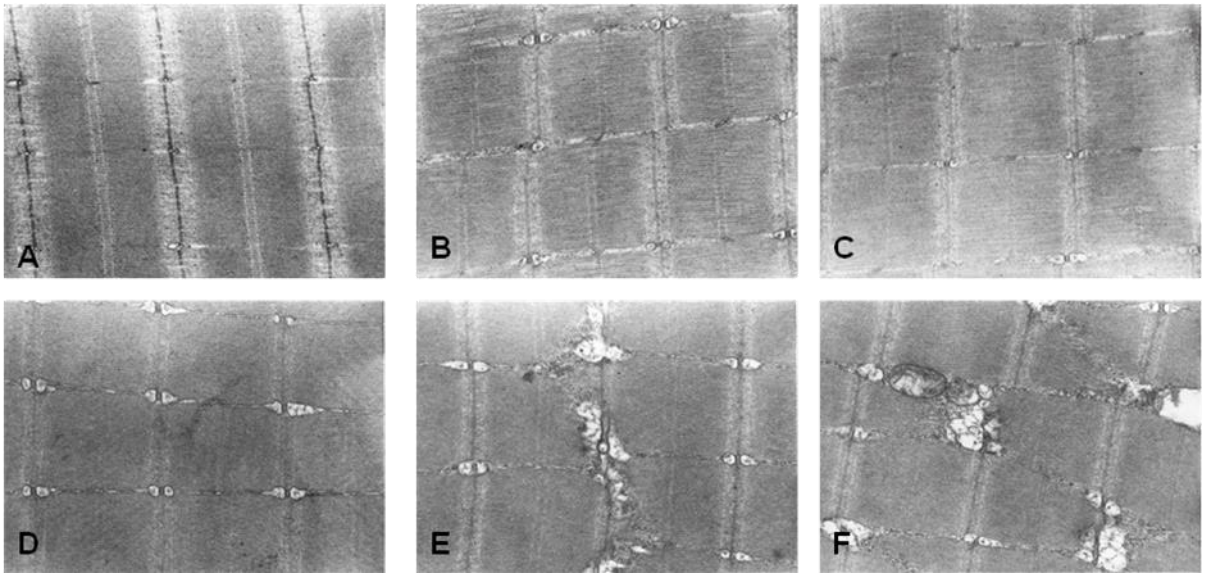

Figure 9. Changes in the structure of black rockfish after immersion for 5 min in cold saturated salt solution at various temperatures as observed by transmission electron microscopy. A, Control (immediately after death); B, -5°C; C, -10°C; D, -12.5°C; E, -15°C; and F, -20°C.

breaking strength of the pericellular connective tissue among the muscle fibers was weakened during immersion under these conditions.

To elucidate the mechanisms underlying the effects of immersion in a cold saturated salt solution, changes in the A band, H zone and Z lines of the sarcomere were visualized by TEM. In myofibrils prepared from the dorsal muscle of fish immediately following sacrifice, the A band, H zone and Z lines of the sarcomeres were clearly distinguishable (Figure 9). In myofibrils prepared from the dorsal muscle of fish immersed in cold saturated salt solution, the H zone, I band and Z lines of the sarcomeres were almost indistinguishable, indicating that the duration of immersion should be increased.

TEM showed that the sarcoplasmic reticulum (SR) contained natural triad structures between the Z lines, which were disrupted in the samples subjected to immersion in cold saturated salt solution. Muscle contraction occurs across the surface of the SR, utilizing the energy from ATP and causing a reduction in the Ca^{2+} ion concentration (Lee *et al.*,

2000; Cho *et al.*, 1999). This in turn causes an increase in the intracellular Ca^{2+} concentration. We suggest that treatment with the cold saturated salt solution caused changes in the SR (*e.g.*, Ca^{2+} release and Ca^{2+} uptake).

Therefore, the toughness of fish muscle immersed in cold saturated salt solution increased rapidly due to the combined effects of myosin and actin and accelerated ATP degradation. The background toughness of the fish was reduced due to collagen matrix weakness, which accelerated the development of rigor mortis in fish muscle immersed in cold saturated salt solution.

Acknowledgments

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