Trials for keep the fish muscle quality during chilled storage

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SUMMARY: Destruction of spinal cord and bleeding at death were tried to keep the fish muscle freshness. In case of yellowtail and red sea bream, the sample whose spinal cord was destroyed (tested) showed the delay in onset of rigor mortis compared with the control sample. On the other hand, in case of plaice, the tested sample attained full-rigor faster than the control. Rate of ATP consumption was slower in the tested than the control in yellowtail and red sea bream, except for plaice. On the other hand, bleeding caused the delay of muscle softening in the pelagic fish, but not in the demersal fish. Transmission electron microscopy showed the delay of degradation of pericellular collagen fibrils in bled pelagic fish.

These results indicate that destruction of spinal cord and removal of blood have different effects for keeping the fish muscle quality.

KEYWORDS: post-mortem rigor, ATP, spinal cord, fish, collagen, bleeding, muscle, firmness

INTRODUCTION

Freshness is a very important factor for fish quality because they are easy to be decomposed. So, various procedures are utilized for keep of freshness. For the killing of a live cultured fish, punch at the brain or cut of the spinal cord is done generally in Japan. In some cases, a wire is pushed in to destroy the spinal cord completely in addition to the cut of the spinal cord. It is thought that the freshness of fish is maintained for longer period by this method, but there are few studies about the influence on the freshness by the method. Nakayama et al.\(^1\) has reported the delay effect on the post-mortem rigor and ATP consumption which was evaluated by ATP/IMP ratio by the destruction of spinal cord about red sea bream. ATP consumption has been reported as the cause of post-mortem rigor. In comparison of slaughter methods, death by struggling is the fastest in ATP exhaustion and onset of post-mortem rigor.\(^2\) According to these reports, the decrease of ATP content is the major factor of postmortem rigor progress.

Besides of rigor mortis, firmness of fish muscle is an important index of freshness as well. Fish muscle firmness is decreased rapidly after death during chilled storage.\(^3\)

It has been reported that the fish killing procedure affects the softening rate.\(^4,5\) Among several procedures, bleeding is supposed to be very important for keeping of the freshness. In the fish which are caught by net without bleeding, the muscle colors turns to unpleasant red and smells of blood. Therefore, those fish cannot be eaten raw such as sushi or sashimi.

Firmness of raw fish muscle has a direct relationship to content of collagen.\(^6\) In addition, post-mortem softening of fish muscle is reported to be caused by weakening of pericellular connective tissue which is constituted from collagen fibrils.\(^7-10\) Collagen is important in fish muscle firmness, but there are no reports about the effects of bleeding on fish muscle collagen during storage.

The purpose of the present study is to examine the effects of destruction of spinal cord on rigor mortis and of bleeding on muscle firmness and morphology of collagen fibrils.

MATERIALS AND METHODS

Samples
In the experiments for spinal cord destruction, yellowtail, red sea bream, and plaice were obtained alive and they were punched at the brain and cut their spinal cord (control sample). Tested samples were prepared following by the destruction of spinal cord by pushing a wire (2 mm in...
diameter) into the spinal cord (tested sample).

In the bleeding experiments, horse mackerel, striped jack, red sea bream, and rudder-fish were obtained alive. Bleeding was done by spiking the brain, and cutting one set of gill arches (bled sample). The control samples had only a spike at the brain without bleeding. After killing, the fish were stored at 5°C.

Rigor index
Post-mortem rigor was evaluated according to the method of Bito et al.11)

Determination of ATP content
ATP content was determined according to the method of Yokoyama et al.12) using HPLC.

Evaluation of muscle firmness
Change of muscle firmness was measured by a rheometer (RT-1002A, Fudoh, Tokyo, Japan).8)

Histological observations
Muscle structure was observed by a light microscope (LM) and transmission electron microscope (TEM) in the conventional methods.

RESULTS AND DISCUSSION

Process of postmortem rigor
In case of yellowtail and red sea bream (Fig. 1A, B), the control sample body started to be stiff within 3 h storage, and was in maximum rigor in 6-9 h after death. On the other hand, tested sample showed slower process of rigor than the control. By the destruction of the spinal cord, the fish body stopped struggling promptly. This effect would be due to the destruction of the autonomic nerve. In case of the control sample, autonomic nerve would function even after the death of fish for a short time, so the muscle moves at random till autonomic nerve stops their function.

In case of plaice (Fig. 1C), however, the onset of rigor mortis was earlier for 3 h in the tested than the control. As the case of plaice (Fig. 2C), ATP content was almost the same during 0-15 h storage, but it was higher in the control than the tested in 18-24 h. These differences in ATP contents would influence on the onset of as pelagic fishes. Then, instead of stopping the function of autonomic nervous system, some stimulation would be given to plaice muscle by the destruction of the spinal cord, and the post-mortem rigor progressed earlier.

Change of ATP contents
In the cases of yellowtail (Fig. 2A) and red sea bream (Fig. 2B), decrease of ATP content was slower in the tested samples than the control. As the case of plaice (Fig. 2C), ATP content was almost the same during 0-15 h storage, but it was higher in the control than the tested in 18-24 h. These differences in ATP contents would influence on the onset of
rigor mortis.

Muscle cell is connected with neural end and it accepts neural pulse at the cell membrane. Ca\textsuperscript{2+} is released from sarcoplasmic reticulum by the pulse, and muscle contracts by the elevation of Ca\textsuperscript{2+} concentration. The central nervous system exists in the spinal cord and it links with peripheral nerves, and the pulse from the central nervous system causes the muscle contraction. As the case of the present study, destruction of the central nervous system might stop the pulse to the muscle fiber, and stop the release of Ca\textsuperscript{2+} from sarcoplasmic reticulum, and resultantly, muscle contraction was also stopped completely.

**Effect of bleeding on muscle firmness**

In the cases of yellowtail (Fig. 3A), horse mackerel (Fig. 3B), and striped jack (C), the pelagic fishes, the bled muscle firmness decreased slower than that of the control. On the other hand, in the cases of red sea bream (Fig. 4A), flatfish (Fig. 4B), and rudder-fish (Fig. 4C), the demersal fish, those values were not significantly different. These results show that bleeding have an effect on muscle softening specific for pelagic fish.

**Histological observations**

The number of blood cells was counted according to light microscopy (Table 1). The number in bled sample was about half that of the control. Especially in the case of flatfish, the cell number of the bled was about one-fifth of that of the control. These results suggest that over half volume of blood in muscle was drained by bleeding. However, except for the blood cell number, any structural differences were not observed by LM.

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<tr>
<th>Table 1. Numbers of blood cells in muscle (cells/mm\textsuperscript{2})</th>
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<td>Bled (A)</td>
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<td>Striped jack</td>
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<td>Yellowtail</td>
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<td>Horse mackerel</td>
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Ando et al., J. Food Sci., 64, 423-428

Muscle fine structure of yellowtail was observed by TEM (Fig. 5). Fibril structure of collagen was clearly observed in the fresh muscle. In the case of bled samples, collagen fibrils maintained their structure at 6h (Fig. 5C). In the control sample at that time, however, the collagen fibrils were disintegrated almost (Fig. 5D). Almost the same results were obtained in horse mackerel and striped jack (data not
shown). On the other hand, there were no structural differences between the two groups in bottom fishes (data not shown).

These results show that collagen fibril disintegration was delayed by bleeding, and this may be the reason why bled muscle softened relatively slow.

Bleeding is done in the live stock industry also, and is very important for preservation and improvement of meat taste. However, influence by bleeding on muscle texture was not been resolved. Present study is directed to work improvement of fish muscle texture, but if the mechanism which influences fish muscle texture by bleeding is clarified, it may become important for the meat industry as well.

Fig. 5 Change of fine structure of pericellular collagen fibrils in yellowtail muscle during storage at 5°C. A, C; bled, 0 and 6 h, B, D; control, 0 and 6 h (Ando et al., J Food Sci., 64, 423-428).

REFERENCES


