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＜研究ノート＞

Maintaining meat freshness through spinal cord destruction in rainbow trout *Oncorhynchus mykiss*

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Summary

Rainbow trout *Oncorhynchus mykiss* is very delicate and sensitive to the stress associated with capture. When we used the regular storage method, killing in iced water and chilling the fish with ice, the maximum stage of rigor mortis was observed only half an hour after death, and then the fish quality deteriorated rapidly. Since keeping the fish fresh without changing the killing procedure appeared difficult, we examined the effectiveness of inserting a spike into the medulla oblongata, followed by bleeding, and also by passing a wire through the neural canal to destroy the spinal cord to stop body struggling that induce ATP consumption toward earlier postmortem rigor. By using these methods, we managed to delay the maximum stage of rigor mortis and increasing *K*-value above 10 hours in icing storage.

Keywords：rainbow trout, *K*-value, rigor mortis, freshness, spinal cord

Introduction

Rainbow trout *Oncorhynchus mykiss* is consumed raw or grilled with salt, meuniere, and so on. As for freshness, it is well known that the *K*-value (an index of degradation in the freshness of meat) increases rapidly after death,[1), 2)] and the tenderness of the fish meat also increases as time goes by.[2), 3)] Since the changes are particularly rapid in fish and the duration of freshness is quite short, it's necessary to delay them.

In terms of the procedure for maintaining the freshness of fish, the effects of inserting a spike into the medulla oblongata,[4)-9)] and in addition to this process, destruction of the spinal cord using a wire[10)-12)] with or without bleeding process have been reported on many fish species. Among the latter reports, Nakayama *et al.*[10)] reported that the progress of rigor mortis in red sea-bream was delayed more when the spinal cord was destroyed than when a spike was inserted in the brain. Ando *et al.*[11)] also showed that in yellowtail and red sea bream, spinal cord destruction was effective in delaying ATP consumption and progress of rigor mortis, but this was not the case with plaice. On the other hand, referring to the report about the effect of bleeding,[13)] Mishima *et al.*[12)] suggested that the killing procedure most effective in delaying post-mortem changes was spinal cord destruction after bleeding, at least in the case of horse mackerel. Although there are some differences among fish species as to the effect of spinal cord destruction, the procedure might have possibilities for application to other species.

In this report, we examined the effect of killing procedures on rainbow trout, especially spinal cord destruction, and compared with killing in iced water, the regular procedure in fisheries industry.

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Materials and Methods

Fish samples

The fish used for this study was rainbow trout *Oncorhynchus mykiss* cultured in Fuji Trout Hatchery, Shizuoka Prefecture. The water temperature in the hatchery was kept at 11℃. Body length and body weight of the fish were 21.0-27.0 cm, and 180-280 g, respectively. Four kinds of fish samples were prepared by each killing procedure as follows.

(1) Stressed sample: The stressing condition was arranged by holding the fish out of the water until its death in the laboratory for 40 min. where the room temperature were 20℃ in common.

(2) Iced sample: The condition being killed in iced water, the commercially regular killing procedure in fisheries industry, was arranged by storing the fish in iced water for 40 min.

(3) Instant sample: The condition being killed instantly was set up by removing the fish gently from the water, killing immediately by insertion of a spike into the medulla oblongata, and letting the fish bleed for 1 min. in fresh water (11℃).

(4) Spinal-cord sample: The condition being killed by destruction of the spinal cord was set up by removing the fish gently from the water, killing immediately by insertion of a spike into the medulla oblongata, letting the fish bleed for 1 min in fresh water (11℃), and passing a wire (1.2 mm in diameter) through the neural canal to destroy the spinal cord three times after removing the fish from the water.

Then each fish sample was stored in crashed ice.

Measurement of the Rigor Index

Post-mortem rigor was evaluated according to the method of Bito *et al.*[4] Briefly, fish were put on a horizontal table protruding the half of the body length from the edge of the table. The distance from the horizontal line to the base of the tail (L) was measured at selected time intervals after death, and the rigor index was calculated by applying these values to the following equation.

$$\text{Rigor index} = \frac{Lo - L}{Lo} \times 100$$

(Lo; the value immediately after death)

Chemicals

Perchloric acid (PCA), potassium hydroxide, sodium hydroxide, citric acid monohydrate, acetic acid, triethylamine, adenosine 5’-triphosphate (ATP), adenosine 5’-diphosphate (ADP), adenosine 5’-monophosphate (AMP), inosine 5’-monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Determination of ATP-related compounds by high performance liquid chromatograph (HPLC)

After each storage period (0-60 h.), the dorsal muscle fillets were excised from both sides of the backbone. ATP-related compounds in each fillet were then measured as follows.[14] Briefly, one gram portion of each sample was homogenized with 2 ml of iced 10% PCA, followed by centrifugation at 3,000 rpm for 3 minutes. The precipitate was washed with 2 ml of 5% PCA, followed by centrifugation at 3,000 rpm for 3 minutes, and the wash liquid was then added to the supernatant. This process was carried out twice, and the mixture of the supernatant and the wash liquid was neutralized to pH 6.5 with 10 M potassium hydroxide, and submitted as a test solution. The test solution was filtered through a 0.45 μm membrane filter (Nacalai Tesque, Kyoto, Japan) before injection. A 10 μl portion of test solution was injected into a Mightysil RP-18GP column (4.6×250 mm, Kanto Chemical, Tokyo, Japan) eluted with a mixture of 20 mM citric acid monophosphate, 20 mM acetic acid, and 40 mM triethylamine (pH 4.8). The flow rate of the elute was 1.0 ml/min and the column was at room temperature. The elute was monitored with UV absorption at 260 nm, and the ATP-related compounds were analyzed by comparing the retention times of HPLC peaks between samples and authentic compounds. The freshness of the muscle was judged from the *K*-value as defined by the following equation: $K\text{-value }(\%) = (HxR + Hx) / (ATP + ADP + AMP + IMP + HxR + Hx) \times 100$

Results and Discussion

Postmortem changes of Rigor Index

The rigor index of fish during postmortem ice storage is shown in Fig. 1. Immediately after death, the values for stressed fish (stressed sample) and iced fish (iced sample) began to increase, and reached 100% at 0.5 h. The rate of increase in the rigor index of this fish was much faster than reported values for horse mackerel,[6] chub mackerel,[7] round scab,[7] and red sea-bream.[10] In rainbow trout, killing in iced water was ineffective in delaying the development of rigor mortis, while in other species the procedure had more of a delay effect compared to that of stressed fish.[6, 7] This may be a significant issue in maintaining the quality of rainbow trout because killing in iced water was considered to be ineffective even though it is a regular procedure in fisheries industry. It can be proposed that a more appropriate regular killing procedure is needed for rainbow trout. Conversely, in fish killed instantly by inserting a spike into the medulla oblongata (instant sample), and samples with the added process of spinal cord destruction (spinal-cord sample), the values remained at 0% until 2 h. and then began to increase until the maximum level was reached at about 12 h. The rate of increase in the rigor index for the instant and spinal-cord samples was similar to those for horse mackerel,[6] chub mackerel[7] and round scab,[7] which were killed by inserting a spike into the medulla oblongata and stored in ice. After reaching the maximum level, the instant and spinal-cord samples remained above 50% at 38 h. while those of the values for the iced sample decreased and reached near 0%. The rate of decrease in the rigor index was the slowest in the spinal-cord samples. This effect is considered to be due to the destruction of the autonomic nerve to stop the fish body struggling that induces ATP consumption and leads to earlier postmortem rigor.[11] The shelf stability of spinal-cord sample was compared with that of the iced sample in terms of time-dependent change in the rigor index and ATP-related compounds as outlined below.

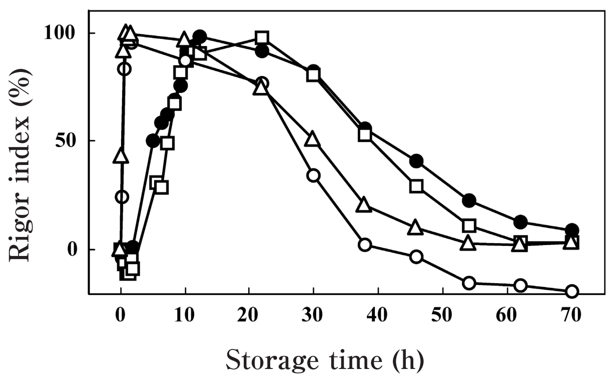

Fig. 1. The effect of four different killing procedures in rigor index of rainbow trout during ice storage at 0℃. Each point represents the mean value (n=5).
△: stressed sample, ○: iced sample, □: instant sample, ●: spinal-cord sample.

Postmortem changes of ATP-related compounds in the fish killed by the destruction of spinal cord and killed in iced water

Changes in the content of ATP-related compounds of spinal cord sample and iced sample during storage are shown in Fig. 2. For the iced sample (Fig. 2 (A)), the ratio of IMP content amounted to 84% of all ATP-related compounds at death. Content of the umami substance IMP decreased as time went by, while HxR content increased. Kumano *et al.*[3] and Mitsuhashi *et al.*[2] reported that the meat of rainbow trout began softening just after death with a simultaneous decrease in α-connectin content and breaking strength. From these results, the taste of rainbow trout killed in iced water was presumed to deteriorate as time went by. On the other hand, in case of the spinal-cord sample (Fig. 2 (B)), ATP remained when the fish died, and IMP content then gradually increased instead of a decrease in ATP until 17 h. This relationship between ATP and IMP content is shown in Fig. 3 with the time course of the rigor index. For the spinal-cord sample, the rigor index value increased as ATP content decreased, while IMP content increased

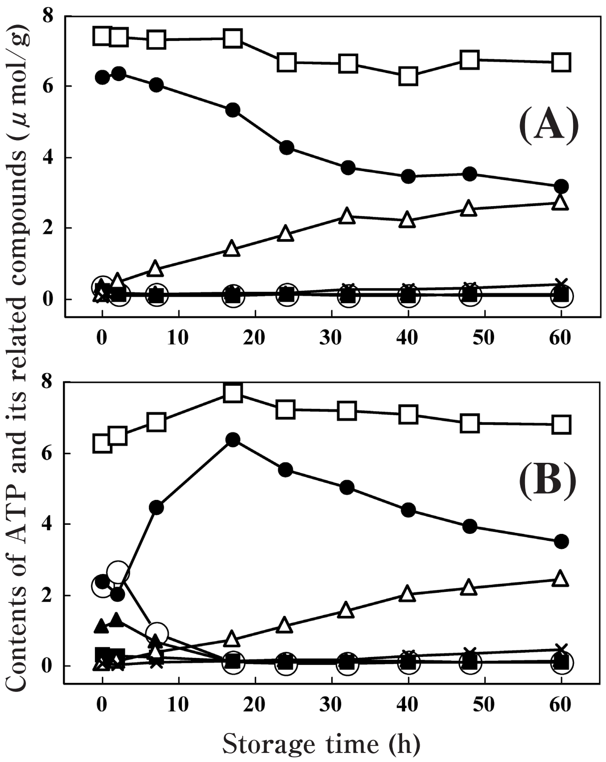

Fig. 2. Time-dependent changes of the contents of ATP-related compounds in iced sample (A) and spinal-cord sample (B) during ice storage at 0℃.
Each point represents the mean value (n=5).
○: ATP, ▲: ADP, ■: AMP, ●: IMP, △: HxR, ×: Hx,
□: total of ATP and its related compounds.

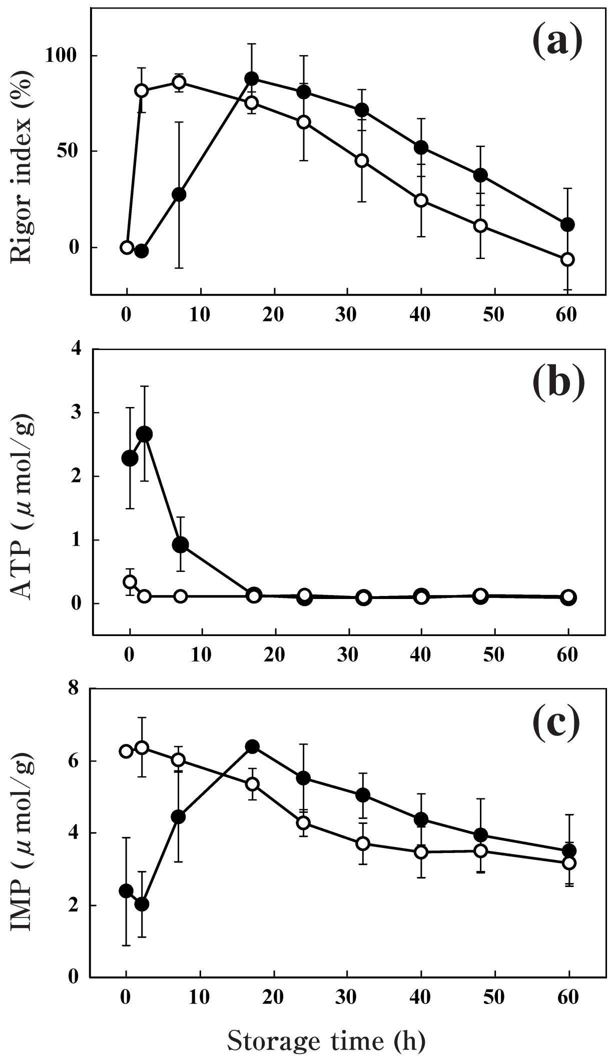

Fig. 3. Postmortem changes of rigor index (a), ATP content (b), and IMP content (c) in iced sample (○) and spinal-cord sample (●) during ice storage at 0℃. Data are mean ± standard deviation (n=5).

until 17 h. After the ATP was consumed, both the rigor index value and IMP content decreased gradually from their maximum levels as time went by. A similar tendency to that seen between the rigor index and IMP content was also observed in the iced sample in which most of the ATP had been consumed when the fish died. From these results, it was inferred that rigor mortis and ATP consumption could be delayed by spinal cord destruction, and that IMP content could be estimated by evaluating the rigor index in rainbow trout chilled with ice. Figure. 3 (a) shows a rigor mortis graph with a sharp up-and-down curve. This type of curve characterizes the return to an initial rigor index of 0% from the maximum value near 100%. Such curves were not seen for most of the fish in the study and there was a tendency of no return to 0%, while only round herring,[4] ground shark[4] and cherry salmon[9] were similar in type. It can therefore be considered to be typically possible in rainbow trout chilled with ice to estimate IMP content from the rigor index because their time course curves have a similar tendency (Figs. 3 (a), (c)).

The time-dependent change of the *K*-value in spinal-cord sample and an iced sample are shown in Fig. 4. At the 20% point, which can be seen as a standard value for fish to be eaten raw, the storage period for the spinal-cord sample was 25 h., while that of the iced sample was 15 h. and was considered to be delayed by 10 h. in the procedure. In rainbow trout, IMP content decreases rapidly after reaching its maximum level and the peak rigor index value. The *K*-value increases in accordance with the decrease in IMP content.

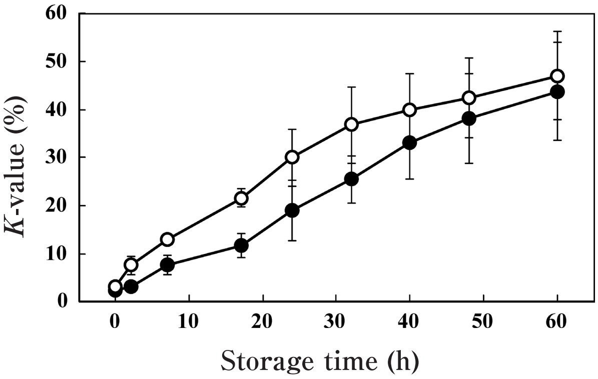

Fig. 4. Time-dependent changes of *K*-value in iced sample (○) and spinal-cord sample (●) during ice storage at 0℃.
Data are mean ± standard deviation (n=5).

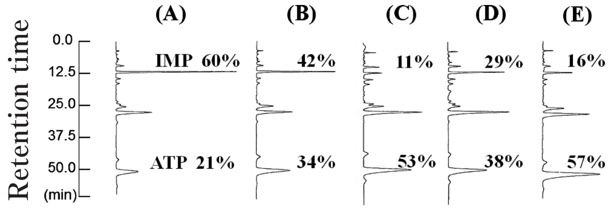

Fig. 5. HPLC chromatograms of ATP-related compounds of each spinal-cord sample at death ((A)-(E)).
The percentage of ATP and IMP are in the total content (μmol/g) of ATP-related compounds.

For this reason, the effective method to delay the period within small *K*-value is also considered to leave ATP in fish as much as possible when they die to delay the formation of IMP. In other words, it's considered that having fish in an unstressed condition at death and making them stop consuming ATP after their death are significantly important to maintain the freshness of rainbow trout.

Evaluation of the spinal cord destruction for rainbow trout

As discussed above, spinal cord destruction after killing instantly followed by bleeding was proposed as the most effective of four procedures to keep rainbow trout fresh. However, there may be related issues in dealing with fish. Figure 5 shows chromatograms of ATP-related compounds for each of five spinal-cord samples ((A)-(E)). The chromatograms represent the condition of each test fish treated with the same procedure, but there were large differences in ATP and IMP content among the five fish. The quantity of remaining ATP reflects how stress-free the fish were. The figure also indicates that these fish were subjected to some kind of unknown stress during the procedure. From these results, it was inferred that rainbow trout may be easily influenced by various stress factors, such as being cought, removed from the water or fixed tightly. That is why, in terms of rainbow trout, the killing procedure with spinal cord destruction may stress fish more than killing them instantly, and may be appropriate to end the procedure by insertion of a spike into the medulla oblongata and letting the fish bleed. However, significant effects of spinal cord destruction have also been demonstrated in red sea-bream[10] and yellowtail.[11]

In this experiment, the effect of spinal cord destruction in rainbow trout was demonstrated. However, the procedure is considered to be more effective if stressors for rainbow trout throughout the process are removed.

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<研究ノート>

ニジマスの鮮度保持に及ぼす脊髄破壊の効果

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要　約

ニジマスはデリケートであり取扱いや環境によるストレスを受けやすい。ニジマスに水産業界で一般的に行われている水氷締めを施し氷蔵したところ、魚体の硬直の度合は死後30分後に最大に達し、その後、軟化を含めた品質の劣化が速やかに進んだ。この劣化の速さは苦悶死魚にも匹敵するものであり、手法の変更が望まれた。そこで、広く海水魚に適用されている延髄切断、それに引き続いての脱血と針金による脊髄破壊を施し、その効果を先の水氷締めの場合と、魚体の死後硬直と魚肉中ATP関連物質量の経時変化から比較した。その結果、脊髄破壊の手法によりニジマスの死後変化が10時間以上遅延することが判明した。これは死後すぐに脊髄の自律神経を破壊することで死後硬直を早める要因となる筋肉中のATP消費を抑えることができたためと考えられるが、脊髄破壊魚は、単に延髄切断を施した魚よりも解硬が緩やかであることも認められた。また氷蔵ニジマスにおいては、硬直指数とうまみ物質IMP含量の増減パターンが非常に類似しており、延髄破壊魚、水氷締め魚ともに魚体の最大硬直時にはIMP含量が最大値に達した。

延髄切断、脱血、そして脊髄破壊という一連のプロセスによりニジマスの鮮度は保持されることが判ったが、ストレスを与えない安定的な処置が難しいこともATP残存量における個体差の大きさから推察された。実用にあたっては、さらにストレスを除く方向性とともに、延髄切断と脱血にとどめ，不用なストレスをかけない方向性も提案される。

キーワード：ニジマス，K値，死後硬直，鮮度，脊髄