Biological Metabolism of Frozen Whale Meat at Subzero Temperatures in Relation to Thaw Rigor

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Summary
In many cases, frozen whale meat in the Japanese market is prepared before rigor mortis (pre-rigor). A serious problem for frozen whale meat is the occurrence of thaw rigor, which is the strong development of rigor mortis during thawing. To prepare frozen whale meat without thaw rigor and maintain high meat pH, the temporal changes in adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD) contents of frozen meat stored at -2.5, -5.0, -7.5, and -10°C were investigated. The rate of decrease of ATP was higher than that of NAD at all storage temperatures. ATP nearly disappeared after holding the meat at -2.5°C for a few days; however, NAD existed yet, so pH decreased thereafter. ATP levels were maintained for a long period at a temperature of -5.0 to -10°C, resulting in the occurrence of thaw rigor. Compared to the muscles of fish such as tuna, the rates of decrease of ATP and NAD were extremely slow in whale meat.

Keywords: ATP, Thawing drip, Frozen meat, Mink whale, NAD, pH, subzero temperature

1. Introduction
The development of postmortem rigor in mammals, such as cows and pigs, is markedly slower than that in fish1-2). Therefore, the muscle tissue of domestic mammals intended for consumption is subjected to a comparatively long aging process for 2 weeks to 1 month. During the aging process, postmortem rigor solves, taste components gradually increase, and muscle becomes edible meat3). In contrast, because the whale is a marine mammal, its processing greatly differs from that of domestic mammals. Whale meat in the Japanese market is mainly a residual product from resource or biological surveys. The body is dissected and the meat block is frozen immediately after the survey. Therefore, frozen whale meat in the market is highly fresh and in a pre-rigor state. The frozen meat quickly undergoes thaw rigor, shrinking the meat, and dripping during thawing, greatly decreasing the meat quality1-4). This has also been reported for fresh frozen fish, such as skipjack5), sardine6), and bigeye tuna7). The membrane structure of the endomysium and sarcoplasmic reticulum is destroyed as ice crystals are generated upon freezing8). Thaw rigor is caused by leakage of Ca2+ from the sarcoplasmic reticulum, rapid consumption of adenosine triphosphate (ATP), and contraction of the muscle during thawing9-10).

The thaw rigor occurs likely because it is possible to quickly freeze various biological samples to maintain freshness because of recent developments in refrigerating equipment9). ATP, the energy source for muscle contraction, inhibits the thermal denaturation of proteins11-12). Furthermore, ATP inhibits the denaturation of myofibrillar protein and autoxidation of oxymyoglobin to metmyoglobin during frozen storage13-14). Therefore, fresh frozen fish meat containing highly concentrated ATP shows high freezing tolerance, but requires careful treatment to prevent thaw rigor during thawing.

We studied freezing characteristics focusing on highly fresh frozen bigeye tuna15). We found that nicotinamide adenine dinucleotide (NAD), a glycolytic coenzyme, decreased during storage at -10°C. Additionally, the anaerobic glycolytic pathway could be inhibited during and after thawing. Interestingly, NAD in bigeye tuna meat decreased during storage at -10°C, while ATP showed a minimal decrease at the same temperature. Thus, it was possible to prepare thawed meat at neutral pH by decreasing NAD at -10°C and then gradually thawing to 0°C. Fish meat at neutral pH shows high water holding property and sensory evaluation in comparison to that at low pH15).

In this study, we focused on highly fresh frozen whale meat. Whale meat contains high protein and low lipid contents as well as free amino acids, such as balene, and thus is an important marine food resource16). However, frozen whale meat exhibits high thawing drip containing taste components, as
described above. Therefore, changes in the biological characteristics of whale meat at subzero temperatures were investigated. The results showed that storage at subzero temperature not only decreases ATP, but also NAD, as has been observed in frozen bigeye tuna meat.

2. Experimental

2.1 Material
The ordinary muscle tissue of the minke whale *Balaenoptera acutorostrata* caught in the North Atlantic Ocean was used in this study. The frozen mass of 15 kg of highly fresh whale meat was provided by Kyodo Senpaku Co., Ltd. (Tokyo, Japan). The frozen mass was divided into blocks of approximately 150 g (25 x 50 x 125 mm) by Marukou-shouji Inc. (Yamaguchi, Japan). Meat blocks were stored at -50°C in the lab until use. At the beginning of the experiment, the blocks were transferred to a freezer at -2.5, -5.0, -7.5, and -10°C.

2.2 ATP content
According to the method described in our previous report\(^1\), ATP contents of frozen meats at subzero temperatures were analyzed. Frozen meat samples about 5 g were quickly removed from meat block in the freezer room at -5.0°C. The meat sample was also quickly homogenized in 15 mL of cold 10% perchloric acid, and then centrifuged at 1,500 x g for 3 min. The supernatant was filtrated through filter paper of No. 2 and the pH was adjusted to neutral. The ATP content was determined by high-performance liquid chromatography.

2.3 NAD content
NAD content was measured from the same extract obtained as described in Section 2.2 according to the method of Ehira *et al.*\(^2\).\(^3\)

2.4 pH measurement
The pH of whale meat at subzero temperatures and immediately after thawing was measured according to the method of Bito *et al.*\(^4\). Frozen meat samples about 5 g were quickly removed from meat block in the freezer room at -5.0 °C. Thawed meat samples about 5 g were also removed immediately after thawing up to 5.0 °C. And then, meat sample was also quickly homogenized in 25 mL of cold 20 mM iodoacetic acid. pH was measured with pH meter (Horiba, F-52).

2.5 Thawing drip
Frozen block meat about 150 g was packed into a polyethylene bag without air under normal pressure and thawed under running water at approximately 12°C until the central temperature reached 5.0°C. It took about 10 minutes from -2.5 °C and 25 minutes from -10 °C for thawing up to 5°C. Thawing drip (%) was calculated as the difference in weight of meat before and after thawing.

3. Results

3.1 Change in ATP content
Frozen whale meat blocks of approximately 150 g stored at -50°C were transferred to a freezer at -2.5, -5.0, -7.5 and -10°C and preservation experiments were conducted. The changes in ATP content during storage at four subzero temperatures are shown in Fig. 1. The initial amount of ATP was 11.9-15.5 μmol/g. After storage at -2.5°C, ATP content rapidly decreased to 0.47 μmol/g after 2 days and nearly disappeared to 0.11 μmol/g after 5 days. In contrast, ATP content after storage at -5.0°C remained at 4.86 μmol/g even after 21 days. Following storage at -7.5 and -10°C, ATP contents were 1.61 and 1.92 μmol/g after 108 and 133 days, the final day of storage, respectively.

![Fig. 1 Changes in the amount of ATP in frozen minke whale meat at storage temperatures of -2.5, -5.0, -7.5, and -10°C.](image)

3.2 Change in NAD content
The changes in NAD content during storage at four subzero temperatures are shown in Fig.2. The initial amount of NAD was 0.59 μmol/g. The rate of decrease of NAD was greatest under storage of -2.5°C, but NAD content was 0.34 μmol/g 5 days later. NAD content was 0.35 μmol/g at -5.0°C after 21 days, 0.25 μmol/g at -7.5°C after 108 days, and 0.27 μmol/g at -10°C after 133 days. The temperature dependency was similar to that of ATP, although the rate of decrease was slower than that of ATP at the same temperatures.
3.3 Change in pH
The changes in pH before and after thawing during storage at different temperatures are shown in Fig. 3. The initial pH of the frozen whale meat was 6.7–6.9, which is nearly neutral. The pH of the frozen meat after storage at -2.5°C rapidly decreased to below 6.0 after 2 days, even at subzero temperatures. Furthermore, the pH decreased to 5.7 after 5 days, and pH after thawing was nearly the same. After storage at -5.0°C, the pH decreased more gradually than at -2.5°C, and was around 6.0 after 21 days. At that time, the pH did not change before and after thawing. Similarly, pH gradually decreased during storage at -7.5 and -10°C, but did not decrease to below 6.0 after 108 and 133 days, respectively. At these two storage temperatures, the changes in pH before and after thawing were very large. ATP and NAD were also present at the end of storage days as shown in Fig. 1 and 2, and thus the pH under these conditions would decrease over additional storage time.

3.4 Changes in thawing drip
The changes in thawing drips at the four storage temperatures are shown in Fig. 4. Initially, a large amount of ATP and NAD remained and thawing drip was approximately 30%. After storage at -2.5°C, thawing drip decreased to approximately 3% and thaw rigor was not observed. At other storage temperatures, a large amount of thawing drip (approximately 20%) and thaw rigor were observed after the final storage—21 days at -5°C storage, 108 days at -7.5°C, and 133 days at -10°C. These results suggest that frozen whale meat containing 1.5–2.0 µmol/g ATP exhibits thaw rigor and high drip during thawing.
Next, changes in thawing drip for 30 days of storage at -2.5°C were evaluated (Fig. 5). As shown in Fig. 4, thawing drip rapidly decreased to below 5% after 3 days. During storage, thawing drip gradually increased and reached approximately 10% after 30 days.

![Graph showing changes in thawing drip over 30 days](image)

**Fig. 5** Changes in thawing drip (%) of quickly thawed minke whale meat after storage at -2.5°C for 30 days (n = 3).

4. Discussions

In this study, changes in biochemical properties at subzero temperatures effective for inhibiting thaw rigor in frozen whale meat were investigated. The rate of decrease in ATP content in frozen whale meat was clearly slower than that in fish, such as bigeye tuna meat. In the case of frozen bigeye tuna meat, ATP nearly disappeared at -5.0°C within 10 hours. In contrast, ATP content of frozen whale meat at -5.0°C remained about initial half after 2 weeks (Fig. 1). ATP in the muscle is thought to be resolved by various enzymes, such as myofibrillar ATPase. At the same time, ATP is reproduced through the anaerobic glycolytic pathway, and so on. Therefore, the result in Fig. 1 showed that the decreasing rate of ATP exceeded the reproducing rate.

Similarly, changes in NAD during the storage at four subzero temperatures were investigated (Fig. 2). The initial concentration of NAD was 0.6 µmol/g, which was lower than that of bigeye tuna at 0.9 µmol/g. Additionally, the rate of decrease in NAD differed. In bigeye tuna, NAD content decreased to 0.2 µmol/g at -10°C after 2 days and 0.1 µmol/g after 5 days. In contrast, the NAD content of frozen whale meat decreased to 0.42 µmol/g at -2.5°C after 2 days and 0.34 µmol/g after 5 days. If NAD content in bigeye tuna meat is below 0.2 µmol/g, the anaerobic glycolytic pathway does not function during thawing and the pH of meat would no longer decrease. In frozen whale meat in this study, ATP nearly disappeared after 5 days at -2.5°C. At that time, the NAD content remained at 0.34 µmol/g, so it was possibly not to stop the anaerobic glycolytic pathway.

Normally, lactic acid may have been generated with the resolution and reproduction of ATP, decreasing pH. Based on the changes in pH, lactic acid may have been produced during storage even at subzero temperatures. The rate of decrease of ATP was clearly greater than that of NAD in frozen whale meat, in contrast to in frozen fish meat. However, it was recently reported that the rate of decrease of ATP in chub mackerel *Scomber japonicus* frozen meat was greater than that of NAD. Thus, the relationship between the rates of decrease of ATP and NAD is not the same in all fish species. Interestingly, the pH of thawed meat after the storage period at -5.0, -7.5 and -10°C did not decrease to the minimum, 5.7 as same as that at -2.5°C (Fig. 3). These meats occurred thaw rigor, so it was possible that various enzymes and substrates were expressed as thawing drip and the pH of meat did not decrease so much.

The changes in thawing drip (Fig. 4) were well-correlated with ATP content, and thaw rigor occurred in the presence of ATP. ATP contents were 1.5–2.0 µmol/g after the storage period at -7.5°C and -10°C, but both samples showed thaw rigor and expressed more than 20% thawing drip. Therefore, it is important to reduce ATP content to below 1.0 µmol/g to inhibit thaw rigor. Changes in thawing drip after storage at -2.5°C were observed during the 30 days (Fig. 5). The thawing drip decreased to below 5% after 3 days, and then gradually increased to 10% by the final storage day. This may be because protein was no longer preserved by ATP, resulting in protein denaturation during freezing to increase drip. In addition, low pH would relate the denaturation and increase of thawing drip.

In this study, the ATP content of highly fresh frozen whale meat decreased during storage at -2.5°C and thus, thaw rigor was inhibited. In contrast, the rates of decrease of NAD were slower than those of ATP at four storage temperatures, -2.5, -5.0, -7.5, and -10°C. Unfortunately, it is difficult to maintain a neutral pH in whale meat because NAD content does not decrease more rapidly than ATP content, as in bigeye tuna. On the other hand, it has been reconfirmed that thaw rigor is inhibited by the storage at -2.5°C for a few days. However, it was noted that the decrease of pH at -2.5°C is fast, resulting in protein denaturation and the increase of thawing drip in the later storage.

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