Effects of Storage Temperatures on the Post-mortem Biochemical Changes in Scallop Adductor Muscle^{*1}

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Scallop adductor muscle was examined for changes in contents of ATP and its related compounds, arginine phosphate, octopine, arginine, polyamines, and lactic acids during storage at 5, 0, and -3°C .

The decrease in ATP and arginine phosphate and the increase in octopine were faster at -3 , 0, and 5°C , in that order. During storage, a low level of AMP accumulation, a rapid increase in inosine, and a gradual increase in hypoxanthine were observed. The K value increased fastest at -3°C , and slowest at 5°C , as opposed to the case of fish and prawn. However, results of sensory evaluation indicated that decomposition progressed fastest at 5°C . IMP as well as adenosine was detected during storage, suggesting AMP degraded through two pathways. L-Lactic acid was detected in a small amount, but D-lactic acid was detected in a fairly large amount and accumulated as decomposition progressed. Not only octopine but also D-lactic acid was thought to be the end-products of glycolysis in scallop adductor muscle. As for polyamines, agmatine, putrescine, cadaverine, and tryptamine were detected as decomposition progressed.

The consumption of scallop has been increasing considerably since its aquaculture was successfully applied on a commercial basis in the 1970's. Scallop can be utilized as a material in boiled, canned, dried, and smoked products. Furthermore, a large amount of scallop is consumed as a fresh material for a variety of dishes, including such popular dishes as *sashimi* and *sushi*. Consequently, retention of the freshness becomes a matter of serious concern, but rather limited information has been available for post-mortem biochemical changes in scallop adductor muscle. Hiltz and Dyer^{1,2} reported two major post-mortem changes occurring in scallop *Placopecten magellanicus* muscle: the accumulation of hypoxanthine (Hx), resulting from the degradation of nucleotides and the production of octopine as a principal end-product of the concomitant glycogen degradation. Diverse results were reported concerning ATP-degraded compounds of scallop adductor muscle: Arai³ reported that IMP and Hx were absent, whereas Nakamura *et al.*⁴ reported a high concentration of IMP at the early stage of storage, and a gradual increase in Hx during storage.

In this study, the changes in content of ATP and its related compounds, arginine phosphate, octopine, arginine, polyamines, and lactic acids were examined in scallop adductor muscle during storage at 5, 0, and -3°C .

Materials and Methods

Materials

Cultured live scallops *Patinopecten yessoensis* were purchased at the Tokyo Wholesale Market from July to October 1991. Each dissected adductor muscle (6 specimens, average 30 g in weight) was divided into three portions, then stored at 5, 0, and -3°C . At regular intervals, about 3 g of muscle was removed from each portion.

The muscle was homogenized in 10 ml ice-cold 6% perchloric acid and the suspension was centrifuged at 10,000 rpm for 10 min. The procedure was repeated, and the supernatants were combined. After neutralization with KOH, the supernatant was added to make 25 ml with distilled water. This solution was subjected to the following analysis.

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Determination of ATP and Its Related Compounds

ATP and its related compounds were determined with high performance liquid chromatograph (HPLC) as reported by Matsumoto and Yamanaka⁵⁾ with a slight modification: they were eluted with 200 mM NaH_2PO_4 (pH 2.9), absorbance at 260 nm.

Determination of Arginine Phosphate

Arginine phosphate was analyzed with HPLC equipped with a UV-VIS spectrophotometric detector (SPD-6AV, Shimadzu Co.) with absorbance at 205 nm on the Shim-pack CLC-NH₂ column (6 mm $\times$ 150 mm, Shimadzu Co.) as reported by Matsumoto and Yamanaka.⁶⁾

Determination of Octopine and Arginine

Octopine and arginine were determined with HPLC according to the method of Kai *et al.*⁷⁾ with a slight modification: octopine and arginine were converted to fluorescent derivatives with benzoin, and then analyzed with HPLC equipped with a fluorescence detector (FLC-6A, Shimadzu Co.) on the Kaseisorb LC ODS-300-5 column (4.6 mm $\times$ 250 mm, Tokyo Kasei Kogyo Co.).

Determination of Polyamines

Tyramine, putrescine, cadaverine, histamine, agmatine, tryptamine, and spermidine were determined with HPLC according to the method of Yamanaka.⁸⁾

Determination of Lactic Acid

L- and D-lactic acid were determined by using L-lactate dehydrogenase (E. C. 1.1.1.27, Boehringer Mannheim Co.) and D-lactate dehydrogenase (E. C. 1.1.1.28, Boehringer Mannheim Co.) according to the method of Noll.⁹⁾

Sensory Evaluation

The degree of freshness of scallop adductor muscle was evaluated by trained sensory panels and classified into three stages mainly based on its odor: acceptable stage (no smell); stage of initial decomposition (faintly putrid smell); stage of advanced decomposition (putrid smell).

Results

ATP and Its Related Compounds

The changes in contents of ATP and its related compounds in scallop adductor muscle stored at 5, 0, and -3°C are shown in Figs. 1, 2, and 3.

Fig. 1. Changes in percentage distribution of ATP and its related compounds during storage at 5°C .

Fig. 2. Changes in percentage distribution of ATP and its related compounds during storage at 0°C .

Fig. 3. Changes in percentage distribution of ATP and its related compounds during storage at -3°C .

ATP contents immediately after death ranged from 4.2 to 8.8 $\mu\text{mol/g}$ among the 6 specimens, and consisted of a molar ratio of around 70% of total ATP-related compounds. The average nucleotide profile at the start was 67% ATP, 23% ADP, 8% AMP, traces of IMP, adenosine (AdR), inosine (HxR), and Hx.

During storage at 5°C , ATP slowly decreased and its concentration reached a 5% molar ratio of the total ATP-related compounds on the 6th day of storage. On the contrary, ATP

Table 1. Changes in contents of polyamines in scallop adductor muscle during storage at 5, 0, and -3°C

(mg/100 g)							
Temperature	Days	TYM	PUT	CAD	HM	AGM	TPM
5°C	0	—	—	—	—	—	—
	3	—	tr	0.22	—	0.92	0.13
	6	—	2.23	1.77	—	2.78	0.45
0°C	3	—	—	—	—	—	—
	6	—	—	—	—	0.42	—
	9	—	tr	tr	—	0.79	—
	14	—	0.37	0.45	—	2.05	—
-3°C	3	—	—	—	—	—	—
	6	—	—	—	—	—	—
	9	—	—	—	—	—	—
	14	—	—	—	—	tr	—

decreased rapidly when stored at -3°C , and remained as only a 11% molar ratio of the total ATP-related compounds even on the 2nd day of storage. The change in ATP content during storage at 0°C was intermediate between those at 5 and -3°C . With the decrease in ATP, AMP slowly accumulated to around 20% of the total nucleotide concentration at all the storage temperatures, whereas HxR increased considerably at -3°C , moderately at 0°C , and slowly at 5°C . Hx also increased but at a slower speed than that of HxR. IMP and AdR, which are not shown in Figs. 1, 2, or 3, slowly increased during storage, and the highest concentrations were 2 and 1% molar ratios of the total ATP-related compounds, respectively. The occurrence of IMP and AdR were confirmed by enzymatic analyses.

Arginine Phosphate

Figure 4 shows the changes in content of arginine phosphate in scallop adductor muscle during storage. Immediately after death, the content of arginine phosphate was $5\text{ }\mu\text{mol/g}$. Arginine phosphate decreased noticeably to al-

Fig. 4. Changes in content of arginine phosphate during storage.

most $1\text{ }\mu\text{mol/g}$ on the 3rd day of storage at -3°C , then maintained this level until the 14th day of storage. When stored at 5 or 0°C , it increased to little more than $6\text{ }\mu\text{mol/g}$ on the 2nd day of storage, then rapidly decreased to $1\text{ }\mu\text{mol/g}$ on the 6th day of storage.

Octopine and Arginine

The changes in contents of octopine and arginine in scallop adductor muscle during storage are shown in Figs. 5 and 6. Immediately

Fig. 5. Changes in content of octopine during storage. **Fig. 6.** Changes in content of arginine during storage.

Fig. 7. Changes in total amounts of D-lactic acid and L-lactic acid during storage.

after death, the contents of octopine and arginine were 1 $\mu\text{mol/g}$ and 10 $\mu\text{mol/g}$, respectively. Octopine increased during storage, whereas arginine decreased. The rate of octopine increase and of arginine decrease was largest at storage at -3°C , and smallest at storage at 5°C . The contents of octopine and arginine reached 12 and 3 $\mu\text{mol/g}$ respectively regardless of the storage temperatures.

Polyamines

Table 1 shows the changes in contents of polyamines in scallop adductor muscle during storage. Initially, polyamines were absent. Putrescine (PUT), cadaverine (CAD), agmatine (AGM), and tryptamine (TPM) were detected in small amounts after 3 days of storage at 5°C , then they gradually increased. When stored at 0°C , AGM was detected on the 6th day of storage, and on the 9th day, PUT and CAD were also detected. Only a trace of AGM was detected on the 14th day of storage at -3°C .

Lactic Acid

The changes in the total amount of D- and L-lactic acid in scallop adductor muscle during storage are shown in Fig. 7. The total amount of D- and L-lactic acid was 40 $\mu\text{g/g}$ at the beginning, and D-lactic acid consisted of around 80% of the total. The D-lactic acid content increased noticeably after 2 days of storage at 5°C , and increased moderately after 9 days of storage at 0°C , whereas the L-lactic acid content showed almost no change. On the other hand, both D- and L-lactic acid contents remained at the same level when stored at -3°C .

Sensory Evaluation

Results of sensory evaluation are summarized in Fig. 8, together with the changes in the K value.

Fig. 8. Changes in K value and sensory rating during storage. Symbols used are: ☆, evaluated as initial decomposition; ★, evaluated as advanced decomposition.

After 4 days' storage at 5°C , scallop adductor muscle was evaluated to be at the stage of initial decomposition, conversely no evidence of decomposition was detected during the storage at 0 and -3°C for 14 days.

Discussion

ATP degradation, along with the decrease in arginine phosphate and the increase in octopine, was fastest at -3°C , followed in order by those at 0°C and at 5°C . Faster ATP degradation at a lower storage temperature has also been observed for plaice,^{10,11} red sea bream,¹²⁻¹⁴ and kuruma prawn.⁵ Watabe *et al.*¹⁵ concluded that this phenomenon occurs because Ca^{2+} uptake ability of sarcoplasmic reticulum decreases at a lower storage temperature, resulting in an increase in Ca^{2+} concentration inside myofibrils. Then, the increased Ca^{2+} ion activates myofibrillar Mg^{2+} -ATPase and thus accelerates ATP consumption. The same mechanism would be applicable to scallop adductor muscle.

As a result of the ATP degradation, IMP generally accumulates in fish and AMP in molluscs. Further degradation to HxR or to Hx is very slow, but after reaching the stage of initial decomposition, a marked increase in Hx occurs because of the microbial growth.¹⁶ Accordingly the K value,¹⁷ a freshness index defined as the molar ratio of HxR plus Hx to the total of ATP-related compounds, increases faster during storage at higher temperature, which allows active growth of microbes. On the contrary, in the case of scallop adductor muscle, AMP accumulation was fairly low, and further degradation to HxR and Hx was observed from the beginning. Therefore, the K value increased most rapidly when stored at

−3°C, where ATP degradation was fastest. Similar changes in the K value were reported for itayagai scallop *Pecten albicans*,¹⁸⁾ where the K value increased faster at −3 and 0°C than at 5 and 10°C.

On the 4th day of storage at 5°C, scallop adductor muscle was evaluated to be at the stage of initial decomposition when the K value was 42%. On the other hand, scallop adductor muscle stored at −3°C was evaluated to be acceptable even on the 14th day of storage, but the K value was already 45% on the 2nd day of storage. Thus, it is considered that the K value as a freshness index could not be applicable to scallop adductor muscle.

Arai¹⁹⁾ has reported the presence of adenosine deaminase with high activity and the absence of AMP deaminase, which resulted in absence of IMP in scallop adductor muscle. In contrast, Nakamura *et al.*,⁴⁾ Sakamoto *et al.*,²⁰⁾ and Nagamine *et al.*²¹⁾ have detected IMP. In this study, though at low levels, both AdR and IMP were detected during storage. The presence of AdR and IMP were independently confirmed by enzymatic analyses. From this result, AMP was considered to degrade to HxR through two pathways.

Arginine phosphate in molluscs, like creatine phosphate in fish, is used to generate ATP from ADP. Arginine phosphate in scallop adductor muscle decreased with ATP decrease, whereas arginine phosphate in prawn or creatine phosphate in fish^{11,14)} decreased to certain levels prior to the beginning of ATP decrease.

A fairly large amount of D-lactic acid and a small amount of L-lactic acid were detected at the beginning, then D-lactic acid accumulated as decomposition progressed. L-lactic acid is an end-product of glycolysis in vertebrates, but in molluscs, octopine^{2,22)} has been said to accumulate instead of L-lactic acid. The present study confirmed that both octopine and D-lactic acid accumulated. Therefore, the end-products of glycolysis in scallop adductor muscle were supposed to be both D-lactic acid and octopine. When Grieshaber and Gäde²³⁾ argued the absence of lactic acid in scallop *Pecten Jacobaeus* adductor muscle under the anaerobic condition, they determined lactic acid by using lactate dehydrogenase (E.C. 1.1.1.27), which is L-lactate dehydrogenase. That was why they could not detect D-lactic acid. Baldwin *et al.*²⁴⁾ have reported that anaerobic stress led to accumulation of D-lactic acid and tauropine in the foot and

adductor muscle of New Zealand abalone *Haliotis iris*. The mechanism of D-lactic acid formation in shellfish post mortem is of great interest.

Polyamine formation, as well as D-lactic acid accumulation, occurred as decomposition progressed, and accordingly showed a reverse temperature dependency to ATP degradation.

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