Effects of Storage Temperature on Postmortem Changes of ATP and Its Related Compounds and Freshness Indices in Oyster Tissues*

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The effects of storage temperature on postmortem changes in the contents of ATP and its related compounds were investigated in the adductor muscle, mantle, gill, and body trunk of the oyster Crassostrea gigas in relation to freshness. The K, K′, and A.E.C. values taken for freshness indices were calculated from the contents of ATP and its related compounds.

In the adductor muscle, a marked decrease in ATP was observed together with the accumulation of AMP and IMP. The rates of ATP degradation during storage were on the order of 0 > 10 > 5°C storage. AdR was detectable only in the adductor muscle. In the other three tissues the level of AMP increased along with a slow decrease in the ATP level. The accumulation of IMP was not so marked as in the adductor muscle. The rates of ATP degradation during storage were on the order of 10 > 5 > 0°C storage in the body trunk, and 10 > 5 > 0°C storage in the mantle and gill. Although the K value remained low during the acceptable stage in all four tissues, the K′ value in the adductor muscle and the A.E.C. value in the other three tissues changed rapidly and continuously from the beginning of storage at all storage temperatures examined (0, 5, 10, 15, and 25°C). The change rates of K′ and A.E.C. values were higher at higher storage temperatures. The K′ value obtained on the adductor muscle and A.E.C. values obtained on the other three tissues appeared to be useful as freshness indices for oysters.

Key words: ATP and related compounds, postmortem changes, storage temperature, freshness index, oyster

We previously examined changes in the content of ATP and its related compounds in various tissues of the oyster during storage in ice, and reported the differences in contents and degradation patterns of those compounds among the tissues and the two pathways of AMP degradation (IMP and AdR pathways). We also proposed the K′ and adenylate energy charge (A.E.C.) values as potential freshness indices of oyster during ice storage. An interesting effect of storage temperature on ATP degradation, i.e. a faster ATP degradation at lower storage temperatures, has been observed in the muscle of fish, prawn, and shellfish. In those reports, ATP degradation was analyzed only in the muscle tissue of each species. The effects of temperature on ATP degradation in other tissues, such as the mantle, gills, and body trunk, remain unclear. In addition, Iwamoto et al. reported that the freshness index, K value, for the adductor muscle of itayagai scallop increased faster during storage at -3 and 0°C than at 5 and 10°C, while the muscle resulted in the worst organoleptic evaluation when stored at 10°C. Kawashima and Yamanaka also reported similar changes in K value at various storage temperatures in the adductor muscle of scallop. These results indicate that the K value is not suitable as a freshness index for the muscle of those shellfish. It is necessary to determine whether the K′ and/or A.E.C. values can be applied to the oyster as freshness indices at various temperature or not.

In this study, the effects of storage temperature on the changes of ATP and its related compounds and on the changes of freshness indices, K, K′, and A.E.C. values were examined in the adductor muscle, mantle, gill, and body trunk of the oyster Crassostrea gigas.

Materials and Methods

Materials
Live cultured oysters Crassostrea gigas were collected from a cultured farm in Matoya Bay, Mie Prefecture. They were artificially purified as reported previously. Each oyster was dissected into four tissue groups: adductor muscle, mantle, gill, and body trunk. The four tissue samples of each specimen were held separately in glass vial and stored at 0, 5, 10, 15, and 25°C. At each fixed time of storage, ten specimens of four tissues were used for the preparation of acid soluble fractions in the same manner as previously reported.

Determination of ATP and Its Related Compounds
ATP and its related compounds, i.e. ATP, ADP, AMP, IMP, AdR, HxR, Hx, and Ad, were determined by high performance liquid chromatography (HPLC) as reported previously.

Calculation of Chemical Freshness Indices
The K, K′, and A.E.C. values were calculated from the contents of ATP and its related compounds from the following equations as reported previously:

K (%) = (HxR + Hx)/(ATP + ADP + AMP + IMP + HxR + Hx) x 100

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The following abbreviations are used: AdR, adenosine; HxR, inosine; Hx, hypoxanthine; Hx, xanthine; Ad, adenine; A.E.C. value, adenylate energy charge value.
K' (%) = \frac{(\text{IMP} + \text{HxR} + \text{Hx})}{(\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{HxR} + \text{Hx})} \times 100

A.E.C. (%) = \frac{1}{2}(2\text{ATP} + \text{ADP})^{1/2} \times 100

Organoleptic Test

The sensory ratings of the four tissues were evaluated using the organoleptic test as reported by Matsumoto and Yamanaka.8)

Results

Changes in Content of ATP and Its Related Compounds of Oyster Tissues during Storage at Various Temperatures

Figure 1 shows the changes in average content of ATP and its related compounds in the adductor muscle (n = 10) stored at 0, 5, 10, 15, and 25°C. During storage at 0°C, the ATP level decreased rapidly from 3.57 ± 0.38 μmol/g (mean ± S.D.) at time 0 to 0.45 ± 0.17 μmol/g after eight hours of storage. On the other hand, ATP levels decreased slowly during storage at 5°C, and became 1.44 ± 0.21 μmol/g after eight hours of storage. The rate of decrease in the ATP level during the first eight hours of storage at 0°C was significantly higher than that at 5°C (p < 0.05, Student's t-test). During storage at 10°C, the rate of decrease in ATP level was also lower than that at 0°C. The rate of ATP degradation during the first eight hours of storage was on the order of 25 > 15 > 0 > 10 > 5°C storage. The ADP level at time 0 was 2.53 ± 0.31 μmol/g. Although the ADP level decreased gradually during storage at all temperatures, the levels after eight hours storage at 0°C, 1.47 ± 0.19 μmol/g, was significantly lower than those at 5 and 10°C, 2.46 ± 0.28 and 2.17 ± 0.27 μmol/g, respectively (p < 0.05). With the decrease in ATP level, the levels of AMP and IMP increased markedly during storage at 0, 5, and 10°C. Additionally, the levels of AMP and IMP after eight hours of storage at 0°C, 5.22 ± 0.63 and 0.76 ± 0.24 μmol/g, respectively, were significantly higher than those at 5°C, 3.38 ± 0.27 and 0.11 ± 0.04 μmol/g, respectively (p < 0.05). The levels of IMP after 1 and 2 day storage at 0°C were also higher than those at 5°C (p < 0.05). Although the level of AMP after eight hours of storage at 10°C, 3.16 ± 0.45 μmol/g, was lower than that at 0°C (p < 0.05), the IMP level after eight hours of storage at 10°C did not show any significant difference compared with that at 0°C. ADP was detected in small amounts during storage at all temperatures. The levels of HxR, Hx, and Xt increased rapidly during storage at higher temperatures. The total contents of ATP and its related compounds tended to decrease during storage at 0°C.

In the mantle, the level of ATP decreased during storage (Fig. 2). The rate of decrease in ATP level in the mantle was very low in contrast to that in the adductor muscle. The levels of ATP after two days of storage at 0, 5, 10, and 15°C were 1.03 ± 0.11, 1.06 ± 0.13, 0.87 ± 0.17, and 0.56 ± 0.12 μmol/g, respectively. There was not a significant difference among those values at 0, 5, and 10°C during two days of storage (p > 0.05), unlike those in the adductor muscle. However, the ATP level at 10°C tended to decrease faster than those at 0 and 5°C (p < 0.1). The rate of ATP degradation in the mantle during storage seemed to be on the order of 25 > 15 > 10 > 5 > 0°C storage. The level of ADP increased at first, and then remained constant, after which it decreased during storage at all temperatures. The AMP level in the mantle increased to about 2 μmol/g during storage. The levels of IMP, HxR, Hx, and Xt in the mantle were not as high as those in the adductor muscle. AdR was undetectable during storage at any temperature.

In the gill, the changes in ATP and ADP levels were similar to those in the mantle, and the rate of ATP degradation in the gill after the first day of storage was on
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Fig. 2. Changes in average content of ATP and its related compounds in the mantle of oysters during storage at various temperatures (n = 10).

Fig. 3. Changes in average content of ATP and its related compounds in the gills of oysters during storage at various temperatures (n = 10).

The order of 25 > 15 > 10 > 5 > 0°C storage (Fig. 3). IMP increased slowly during storage at all temperatures. AdR was undetectable, as in the mantle. The levels of HxR, Hx, Xt, and Ad in the gills increased slowly at a rate slightly higher than that in the mantle.

In the body trunk, the changes of ATP and its related compounds during storage were similar to those observed in the gills (Fig. 4), but the rate of ATP degradation in the body trunk during storage was on the order of 25 > 15 > 10 > 5 > 0°C storage. IMP was detected in low levels, but AdR was undetectable.
Fig. 4. Changes in average content of ATP and its related compounds in the body trunk of oysters during storage at various temperatures (n=10).

Fig. 5. Changes in average K, K', and A.E.C. values in the adductor muscle of oyster during storage at 0 (○), 5 (●), 10 (▲), 15 (▲), and 25°C (▼) (n=10).

Arrow (▼) indicates the stage of initial decomposition, thereafter the decomposition progressed.

Chemical Freshness Indices

Figure 5 shows the changes in K, K', and A.E.C. values with sensory ratings obtained for the adductor muscle during storage at various temperatures. The adductor muscle gave off a faintly putrid smell on the 2nd, 4th, 7th, or 10th day of storage at 15, 10, 5, or 0°C, respectively, and this stage was recognized as the stage of initial decomposition. The K value increased very slowly during the acceptable stage at all storage temperatures, and then increased rapidly as decomposition progressed. On the contrary, the K' value increased from 2% to about 60-80% by the stage of initial decomposition, and then remained constant. Although the K' values on the 1st and 2nd day of storage at 0°C were significantly higher than those at 5°C (p<0.05) because of the higher levels of IMP at 0°C than those at 5°C (Fig. 1), the K' value at 5°C was higher than that at 0°C on the 4th day of storage and thereafter. The A.E.C. value fell from 60.3% at time 0 to about 10-20% on the 1st day. Thereafter, the A.E.C. value tended to increase because of the very low levels of ATP and the differences in rates between ADP and AMP decrease, but the values were low compared with that at time 0.
In the mantle, the K value was very low during the acceptable stage (Fig. 6). The K values was only from 2.7 to 13.9% at the initial decomposition stage. During storage at 10°C, the K value was 8.4% on the 4th day and then decreased as decomposition progressed. HxR and Hx increased until the 4th day, and then decreased. By way of compensation, Xl was detected for the first time on the 4th day, and then increased. As a result, the K value decreased as the decomposition progressed at 10°C. On the other hand, the K' value increased from 3% at time 0 to about 20–30% at the initial decomposition stage during storage at 0, 5, or 10°C. The K' value increased during 15°C and 25°C storage, but the K' values at the initial decomposition stage were only 10.7 and 13.4%, respectively. The A.E.C. value decreased rapidly and continuously from 80.3% at time 0 during storage. The A.E.C. values at the initial decomposition stage were slightly high, 36.2% and 27.7%, during storage at 15 and 25°C, respectively.

The K value obtained on the gills was low at the acceptable stage (Fig. 7), and then rose rapidly as decomposition progressed at 0 and 5°C. The K' value increased linearly and rapidly compared with the K value during storage at 0, 5, and 10°C. However, at 15 and 25°C, the K' value did not show a marked increase. The A.E.C. value in the gills decreased rapidly and continuously during storage as observed in the mantle.

In the body trunk, the changes in K and K' values during storage were similar to those in the gills, but a marked increase of K' value was observed during the decomposition stage at 15°C (Fig. 8). The A.E.C. value decreased rapidly and continuously from 80.3% at time 0 during storage. The A.E.C. values at the initial decomposition stage were slightly high, 36.2% and 27.7%, during storage at 15 and 25°C, respectively.
rapidly from 71% at time 0 to about 20–35% at the initial decomposition stages at various storage temperatures.

Discussion

We previously reported that the total content of ATP and its related compounds differed with oyster tissues, such as adductor muscle, mantle, gills, and body trunk. \(^1\) Watanabe et al. also reported similar results in which the content of ATP and its related compounds were different among disk abalone tissues including adductor muscle, foot muscle, mantle, and viscera. \(^1\) In this study, we confirmed the previous findings that the total content of ATP and its related compounds in the adductor muscle was much higher than those in the mantle, gills, and body trunk of oyster (Figs. 1, 2, 3, and 4). The total contents of ATP and its related compounds in oyster tissues tended to decrease during storage at 0°C. Although the exact reasons for this phenomenon are unclear, one of the possible reasons is the higher efflux of body fluid from the tissues due to the lower temperature and longer period of storage. The degradation pattern of ATP and its related compounds differed among the four tissues. The rate of ATP degradation in the adductor muscle was on the order of 0 > 10 > 5°C storage. Faster ATP degradation at a lower storage temperature has also been observed in the muscle of red sea bream, \(^3\) plaice, \(^5\) kuruma prawn, \(^8\) itayagai scallop, \(^9\) disk abalone, \(^10\) and scallop. \(^1\) This phenomenon was reported in fish muscle by Watake et al. \(^1\) and the same mechanism proposed by them should be applicable to the adductor muscle of the oyster. On the other hand, the rate of ATP degradation in the body trunk was on the order of 10 > 5 > 0°C storage. Although the temperature effects on the ATP degradation in the body trunk was different from those in the adductor muscle, the exact reason of this disagreement is not clear. The temperature effects on the rates in ATP degradation during storage in the gills and mantle were intermediate between those in the adductor muscle and body trunk. The rates of changes in ATP, ADP, and AMP in the mantle, gills, and body trunk were generally much slower than those in the adductor muscle at each storage temperature of 0, 5, 10, 15, and 25°C. The enzyme systems responsible for the degradation of ATP, ADP, and AMP, that is ATPase, myokinase, and AMP deaminase, respectively, seemed to be highly active in the adductor muscle compared with those in the other three tissues as reported previously. \(^1\) The presence of AdR and IMP was confirmed with enzymatic analysis by Kawashima and Yamanaka. \(^11\) in scallop muscle. In this study, IMP and AdR were detected in the adductor muscle at all storage temperatures. Obviously, AMP was degraded in the adductor muscle of oysters through two pathways as reported previously. \(^1\) In the other three tissues at various storage temperatures, however, IMP but not AdR was detected. The IMP pathway of AMP degradation seemed to be predominant during storage in these three tissues.

For the possible freshness indices of oyster, K, K', and A.E.C. values were calculated from the levels of ATP and its related compounds (Figs. 5, 6, 7, and 8). Iwamoto et al. \(^9\) reported a faster degradation of ATP and faster increase in K value at lower storage temperatures in itayagai scallop. Similar changes in K value were also reported in scallop muscle by Kawashima and Yamanaka. \(^11\) They concluded that the K value could not be applied as a freshness index to scallop. On the contrary, the faster increase in K value at lower storage temperatures was not detected in the oyster adductor muscle, since AMP and IMP were accumulated rapidly and further degradation to HxR and Hx was not observed at the beginning of storage in the oyster muscle, unlike in the scallop muscles. \(^8\) \(^1\) Although the K value increased with storage time, its increasing rate during the acceptable stage was slow in the adductor muscle. The K value in the mantle, gills, and body trunk showed little increase and/or fluctuated with increasing storage temperatures. The K value was not suitable for oysters as a freshness index. On the other hand, the K' value in the adductor muscle increased rapidly from the first day of storage, reaching 70–80% at the initial decomposition stage, thereafter remaining constant during storage at 0, 5, and 10°C. At higher storage temperatures of 15 and 25°C, the K' value reached approximately 60% at the initial decomposition stage. The index for freshness must give information on freshness before the onset of decomposition as described by Watanabe et al. \(^10\) on disk abalone muscle. Therefore, the K' value obtained on the adductor muscle appeared to be useful as a freshness indicator of oyster muscle because of its rapid and continuous increase during the acceptable stage. From the same point of view, the A.E.C. values in the mantle, gills, and body trunk that showed a rapid and continuous decrease during storage at all temperatures appeared to be useful as a freshness indicator for oysters.

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