Changes of ATP and ATP-related compounds contents and K value in Sebaste sthompsoni in different storage temperatures

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Abstract. The changesofAdenosine Triphosphate (ATP) and its related compounds were investigated inSebastes thompsoni muscle stored at 0℃, 10℃ and alternate temperatures. The investigation was performed by HPLC system. Different extents of ATP-related compounds were found in different temperatures but the tendency is similar. K value was calculated as the chemical indices to show freshness. The results showed that: The changes of ATP and its degradation products were regular and 0℃ is an appropriate temperature to storage Sebastes. It's the species that shows slow increase in K-value and belong to the Hypoxanthine (Hx) type.

Introduction

Sebastes thompsoni is a common species mainly distributed in the North Pacific, living in coastal waters. The raw fish is delicious and rich in nutrition[1]. Iced storage is an effective preservation method to inhibit the growth and reproduction of a variety of microorganisms and restrain the enzyme activity of body itself [2]. However, there is still a series of changes in fish body when storage in low temperature result in change of quality and flavor, such as the formation of amines (volatile and biogenic) and hypoxanthine[3-4]. ATP and its decomposing products are the major component of aquatic animal muscle nucleotides. It is well known that ATP in the fish decomposed as this way: ATP→ADP→AMP→IMP→HxR→Hx[5]. The proportion of (HxR + Hx) content in total ATP-related compounds is defined as K value. [6].

Foreign researchers[7-9] have studied onscad, rainbow trout, yellow-fin tuna and indicated that K value can be evaluated as the important chemical indices to assess fish freshness. Li Sha[10] researched on quality change of tilapia fillets during chilling storage and declared that K value was well correlated with TVB-N, sensory evaluation and Ca^{2+}-ATPase activity. However, there is little study on K value of Sebastes thompsoni and comparing the tendency of ATP and ATP-related compounds storage in different temperatures to find its change rule.In this paper, we focused on the changes of ATP-related compounds and K value of wild cultured Sebastes thompsoni and compared the tendency when stored in different temperatures. It aims to provide theoretical guidance for preservation of Sebastes thompsoni.

Materials and Methods

Materials. Live cultured Sebastes thompsoniwere purchased in a fresh food market at Hakodate, Hokkaido, Japan. Sebastes were transferred to laboratory in refrigerated container soon after harvesting. After killing, cut the fish into two pieces along its spine. Then removed skin, toke back muscle and removed red meat and fat. White meat was held in polyethylene plastic bag, numbered andfrozen ,respectively at 0℃, 10℃, and these two temperatures alternate day by day.All chemicals used were of analytical grade.

Extraction of ATP and ATP-related Compounds from Flounder Muscle.1g sample was mixed with 10mL of 5% PCA. The mixture was combined and neutralized with 1M KOH solution to adjust pH to 2.0-2.5 then filled up to 20mL with distilled water. All the operation was on ice at

about 0℃.The mixture was centrifuged at 3000rmp for 15min at 4℃ and the supernatant was stored at 4℃.

Determination of ATP and ATP-related Compounds by HPLC. The PCA extraction was filtered through membrane (0.45μm) and the filtrate was mixed with phosphate buffer(pH7.5). A 20μL portion of the mixture was injected onto GS-320HQ column(Shodex, Asahipak) eluted by 1mol L^{-1} NaH_2PO_4 and Na_2HPO_4 mixed solution(pH2.7). The flow rate was 0.8mL min^{-1} and column temperature was held at 25℃. The elution was monitored by UV absorption at 256nm. Standard sample, ATP, ADP, AMP, IMP, HxR and Hx mixed solution was also determined by HPLC in the same method. ATP and ATP-related compounds contents were calculated by comparing retention time of HPLC peaks between samples and standard.

Calculation of K value.ATP in the fish degraded soon after death and ATP decomposed as this way: ATP→ADP→AMP→IMP→HxR→Hx. So the K value【6】 was defined as following,

$$K = \frac{(\mathrm{HxR} + \mathrm{Hx})}{(\mathrm{ATP} + \mathrm{ADP} + \mathrm{AMP} + \mathrm{IMP} + \mathrm{HxR} + \mathrm{Hx})} \times 100\%$$

Result and Discussion

Changes of ATP and ATP-related compounds composition in Sebastes muscle stored at 0℃. Sebastes was stored at 0℃ for 20 days. All of the six ATP-related compounds were detected in Sebastes muscle and were well separated. The degradation products of main ATP-related compounds were showed in fig.1 and some of them changed evidently during the storage. When stored at 0℃,ATP degraded rapidly in 1d then almost disappeared after 2d. The sharp drop of ATP content is related with the high activity of ATP decomposing enzyme [11]. ATP took off 1 molecular phosphoric acid degraded to ADP then degraded to AMP. ADP and AMP slightly decreased in 1-2d and then remained at a low level indicating a quick conversion of ATP to IMP through ADP and AMP.

At first IMP increased rapidly in 1d then decreased slowly until 15d and almost disappeared at 19d. IMP is a kind of tasty agent as the main flavoring material contributes to the flavor of umami in animal muscle [12]. It would be better to eat fish when IMP accumulated high during the early storage for its delicacy. Yang Wenge [13] declared that ATP and ADP degrade quickly and IMP increased rapidly in 1-2d then accumulated in fish for some days. However, AMP did not accumulate during the whole storage. This conclusion is identified with our result.

HxR is the degradation product from IMP. HxR content rose rapidly during 0-2d then continued to rise to and then declined until 20d. With time of storage, HxR degraded into Hx and Hx content increased slowly until 15d rose to 0.73mmol/Kg. From 16d Hx suddenly increased high as the result of sharp decline of HxR. Hx contributes to the flavor of bitter and lead to corruption. According to the quantity of HxR and Hx accumulated in the muscle, fish can be divided into three types [14]: 1) HxR type. 2) Hx type. 3) HxR and Hx type. As Hx was the main accumulated product during the latter storage, Sebastes belongs to the Hx type.

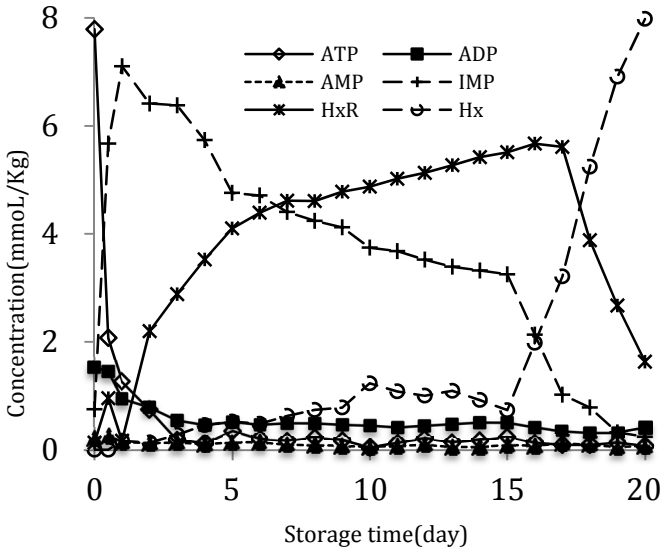

Fig.1 Changes of ATP and ATP-related compounds composition in Sebastesmuscle at 0℃.

Changes of ATP and ATP-related compounds composition in Sebastes muscle stored at 10℃ and alternate temperature(0℃, 10℃). Sebasteswas respectively stored at 10℃ for 8 days(fig.2), and these two temperatures alternate day by day(fig.3). When stored at 10℃, The main tendency of ATP-related compounds in Sebastes stored at 10℃ was same with that stored at 0℃. But the whole process of ATP's degradation was much faster indicating that the metabolic enzymes were more active at 10℃. Another evident different is that the highest content of IMP at 10℃ is lower than that at 0℃. We supposed that IMP decomposing enzyme was active enough to conversed IMP to HxR quickly result in little time for IMP to accumulate high.

When stored at alternate temperatures(0℃, 10℃),the main tendency of ATP-related compounds in Sebastes stored at was same with that stored at 10℃. But the whole process of ATP's degradation was a little slower than that stored at 10℃ but much faster than 0℃ showing that the metabolic enzymes were also active at 0℃ and 10℃ alternately. But the highest content of IMP is higher than that stored at 10℃indicating that IMP decomposing enzyme was more active at 10℃.

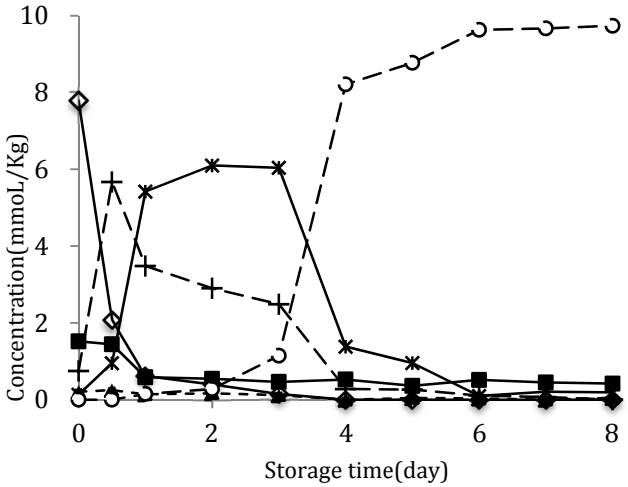

Fig.2 Changes of ATP and ATP-related compounds composition in Sebastesmuscle at 10℃.

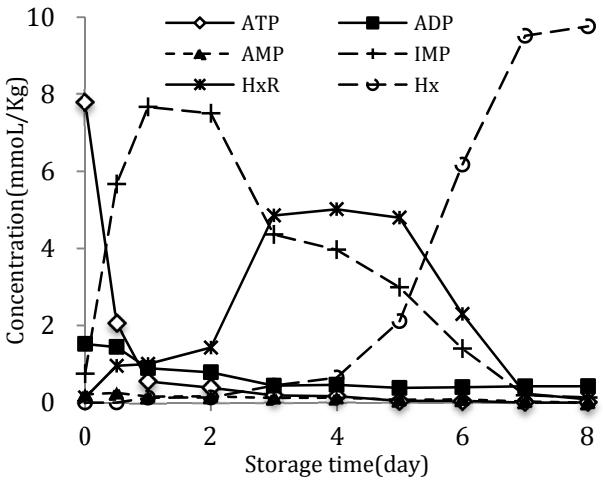

Fig. 3 Changes of ATP and ATP-related compounds composition in Sebastesmuscle at 0℃ and 10℃alternately.

Changes of K value in Sebastes muscle stored at different temperatures. K value is an important freshness index widely used for indication of nucleotide degradation and assessment of chemical spoilage[15]. The chemical freshness index K value was calculated from the contents of ATP-related compounds as 2.3.

When stored at 0℃, the tendency of K value can be divided into three parts as table1,2 shows. Firstly with the loss of freshness K value increased to 44.43% quickly in 0-5d and then continued to rise gently after 10 days. At last K value increased sharply from 15d and reach as high as 92.4% at 20d. It is generally believed that K value of instantaneous dead fish is lower than 10% and K value of fresh fish should be lower than 20%. With the loss of freshness K value rise to 60%, it is judged as primeval corruption. Sebastes thompsoni is the species that shows slow increase in K-value.

At 10℃, K value rose to 53.59% within only 1d showing its fast corruption compared with 0℃. However, K value did not show quick increase at the alternate temperature(0℃, 10℃) and its tendency almost same with that at 0℃at early storage. At 2d K value rose to 15% indicating the fish is still fresh and then increased fast but still slower than 10℃. It is suggested that low temperature conduces to retain freshness and 0℃ is an appropriate temperature. If taking both freshness and sensory into account, it is better to eat Sebastes1-2d after its death because of its low K value and high IMP content(fig.1)which contributesto flavor of umami.

Table.1 Changes of K value in Sebastes muscle at 0℃(0-10d)

	Storage time (day)										
	0	1	2	3	4	5	6	7	8	9	10
K value（%）	1.25	3.16	22.37	30.36	38.25	44.43	46.83	50.38	51.40	53.46	58.68

Table.1 Changes of K value in Sebastes muscle at 0℃(11-20d)

	Storage time (day)									
	11	12	13	14	15	16	17	18	19	20
K value（%）	58.65	58.94	61.65	60.96	60.02	73.56	84.81	87.69	92.12	92.40

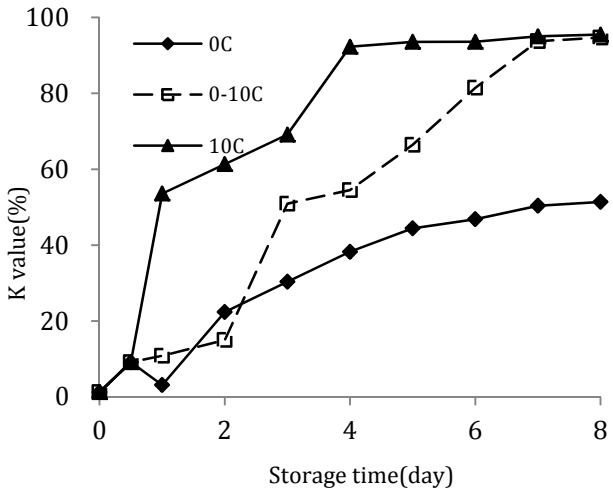

Fig.5 Changes of K value in Sebastes muscle storage at different temperatures.

Summary

The changes of ATP and its degradation products were regular. Storage of Sebastes muscle block at 0℃ accumulated IMP as a major component indicating a quick conversion of ATP to IMP through ADP and AMP. IMP degraded to HxR slowly in an early phase, while it dropped rapidly at the final stage of the storage and at the same time HxR quickly degraded to Hx. As Hx were the main accumulated product during the latter storage, Sebastes belongs to the Hx type.Sebastesthompsoni is the species that shows slow increase in K-value. High temperature(10℃) make metabolic enzymes more active and accelerate the whole process of ATP's degradation but the tendency is similar.0℃ is an appropriate temperature to storage Sebastes and 1-2d after its death is the best time to eat it given both freshness and sensory .

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