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The Effect of Freshness on Puffer Fish Toxin, Tetrodotoxin

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Abstract

K value (ratio of nucleotide decomposition) of dorsal muscle and TTX (tetrodotoxin) concentration in dorsal and tail muscles, skin and liver were investigated at regular time intervals during storage at 5℃ for 0 to 4 days in brown-backed toad fish (*Lagocephalus wheeleri*) and vermiculated puffer fish (*Takifugu vermicularis*) using biofresh analyzer and tissue biosensor, respectively. K value increased gradually whereas TTX concentration almost stable in puffer fishes body parts during storage period. The highest concentration of TTX was found in liver. No significant difference in TTX concentration was found between TTX content of dorsal muscle and that of tail muscle.

Key Words: K value, Tetrodotoxin(TTX), Biofresh analyzer, Tissue biosensor, Puffer fish

1. Introduction

Seafood quality and safety have been concerning researchers for many years since freshness supplies an important source of protein for nations(1). Freshness as one of the quality index has been intensively investigated from different points of view(2). As a result, parameters have been suggested to evaluate the freshness, and many techniques have been proposed to perform freshness parameters(3). The seafood industry is taking a turn for the worse not only from the pollution caused by heavy metals and agricultural chemicals but also due to the determination of the fish caused by bad handling present safety levels and hygiene of seafood are at a very low level. In this current environmental out breaks of seafood poisoning are always likely to occur. Therefore, the safety and hygiene of traditional seafoods should be reexamined.

Recently a great concern is paid on the safety of specific types of seafood due to existence of tetrodotoxin(TTX) in their tissues. Toxicity of seafoods with TTX is gained a special interesting mainly from Japanese researchers since TTX has been first identified in puffer fishes which are consumed in Japan(4, 5, 6). In the current study fish freshness was reviewed from ATP decomposition K value indicator, factors affecting K value and K value

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determination methods, while TTX was reviewed from its occurrence, chemistry and methods of determination(7). However, little information is presently available about freshness value of puffer fishes. Moreover, K value and TTX have been studied separately(8, 9, 10). Since puffer fishes are consumed in raw and cooked dishes it is required to know whether freshness state does have any effect on the toxicity of puffer fishes(9). Therefore, in this study the changes of K value and TTX concentration in puffer fishes stored at 5℃ were investigated to clarify the effect of fish freshness on TTX toxicity(10).

2. Materials and Methods

Enzyme columns and standard solutions of biofresh analyzer was purchased from New Japan Radio Co. Ltd(11). Biofresh buffer solution was prepared from 100 ml of 1 M Tris-HCl, pH 8, 0.02 g of NaN_3, 0.54 g of KH_2PO_4, and 1.21g of $MgCl_2$ mixed together and completed to one liter of deionized water. Working solution consisted of 50% of biofresh buffer solution saturated with oxygen for one hour. Two groups of frozen and one group of fresh brown-backed toad fishes (*Lagocephalus wheeleri*) were purchased from local markets in Tokyo on October, 1999. Frozen fishes were stored at -50℃ to -75℃ until used. Another group of fresh vermiculated puffer fishes (*Takifugu vermicularis*) was purchased from local market in Tokyo on September 1999. The weight of fishes was 220g to 320g and the length was 22cm to 26cm. Frozen fishes were thawed under running water for about 30 min. Dorsal and tail muscles, skin and liver were separated from each other and stored in polyethylene bag at 5℃ for 0 to 4 days. Samples were analyzed as described below at regular intervals. A 5g to 7 g of dorsal muscle was heated in microwave oven for 5 to 10 sec. Muscle fluid was extracted and diluted 10 times with 50% of biofresh analyzer buffer solution and filtered. Twenty microliters of the sample extract was injected to biofresh analyzer. K value was calculated automatically by biofresh analyzer. One gram each of dorsal and tail muscles, skin and liver was mixed with 2 ml of 0.1% acetic acid heated in boiling water for 10 minutes, cooled and centrifuged at 5000 rpm, at 15℃ for 10 minutes. Then the sample was mixed again with 0.6 ml of 0.1% acetic acid and 1.3 ml of 30% sodium chloride, recentrifuged as above, filtered and diluted to 5 ml of deionized water. Fifty microliters of the sample extract was injected to tissue biosensor. Tissue biosensor was prepared and used as described by (8). A representative calibration curve was shown in Fig. 1. TTX concentration was calculated from the calibration curves of authentic TTX.

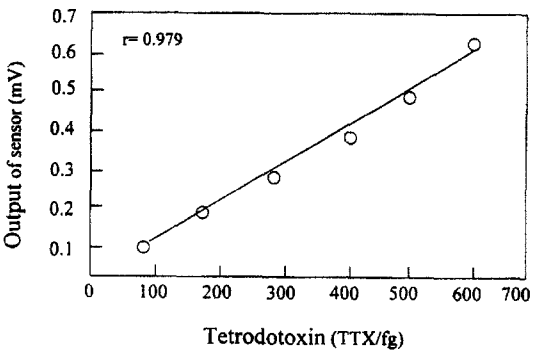

Fig. 1. Calibration curve for determination of tetrodotoxin in the tissue biosensor.

3. Results and Discussion

K value increased gradually in *Lagocephalus wheeleri* and *Takifugu vermicularis* during storage at 5℃ for 0 to 4 days. In the first group of *Lagocephalus wheeleri* (Fig. 2), K value increased from 18% at the beginning of storage (0 hr) to 25% at the end of storage (48 hr). In the second group of *Lagocephalus wheeleri* (Fig. 3) K value increased from 37% at the beginning of storage

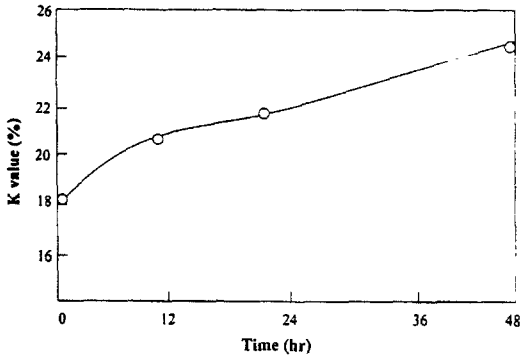

Fig. 2. Changes of K value in the first group of *Lagocephalus wheeleri* fish duringstorage at 5℃ (points are mean for 5 specimens).

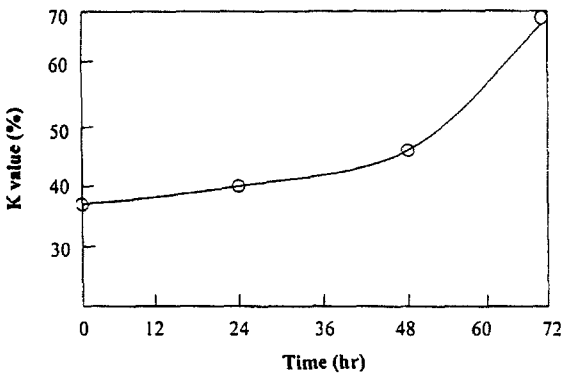

Fig. 3. Changes of K value in the second group of *Lagocephalus wheeleri* fish during storage at 5℃ (points are mean for 5 specimens).

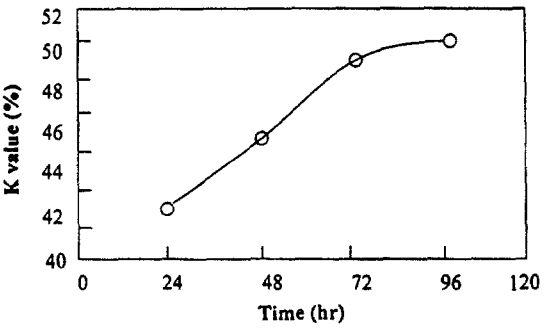

Fig. 4. Changs of K value in the third group of *Lagocephalus wheeleri* fish during storage at 5℃ (points are mean for 2 specimens).

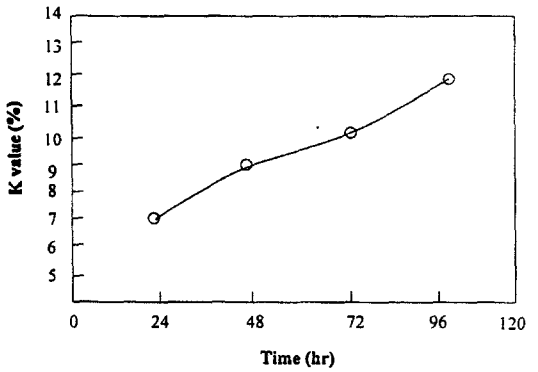

Fig. 5. Changes of K value in *Takifugu vermicularis* during storage at 5℃ (points are mean for 2 specimens).

(0 hr) to 45% at 48 hr of storage, then it increased quickly to 70% at the end of storage (72 hr). This sharp increase however was noticed only in this group.

In the third group of *Lagocephalus wheeleri* (Fig. 4), K value increased from 41% after 24 hr of storage to 50% at the end of storage (96 hr). Similarly, in *Takifugu vermicularis* (Fig. 5), K value increased gradually from 8% after 24 hr of storage to 12% at the end of storage (96 hr).

TTX almost did not change in the muscles, skin and liver of both species during storage at 5℃ for 4 days. In the first group of *Lagocephalus wheeleri* (Fig. 6), TTX content of dorsal muscle, tail muscle, skin and liver at the beginning of storage (0 hr) were 180 fg/g, 200 fg/g, 420 fg/g, and 600 fg/g, respectively and their contents at the end of storage (48 hr) were 180 fg/g, 210 fg/g, 490 fg/g and 630 fg/g, respectively. In the second group of *Lagocephalus wheeleri* (Fig. 7), TTX content of dorsal muscle, tail muscle, skin and liver at the beginning of storage (0 hr) were 203 fg/g, 222 fg/g, 400 fg/g and 797 fg/g, respectively, and their contents at the end of storage (70 hr) were 251 fg/g, 251 fg/g, 465fg/g, and 772 fg/g, respectively. In the third group of *Lagocephalus wheeleri* (Fig. 8), TTX content of dorsal muscle, tail muscle, skin and liver after one day of storage were 159 fg/g, 215 fg/g, 307 fg/g, and 857 fg/g, respectively, and their contents of TTX at the end of storage four day were 189 fg/g, 273 fg/g, 302 fg/g, and 851 fg/g, respectively.

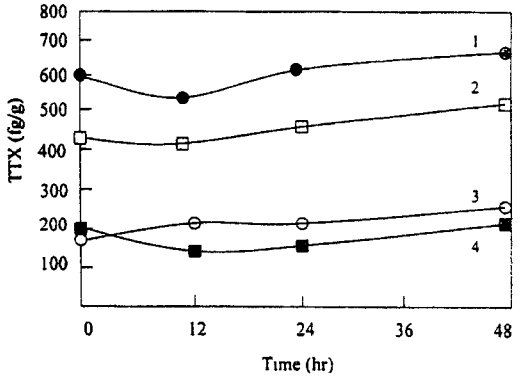

Fig. 6. Changes of TTX concentration in the first group of *Lagocephalus wheeleri* during storage at 5℃ (points are mena for 5 specimens). 1: Liver, 2: Skin, 3: Tail muscle, 4: Dorsal muscle.

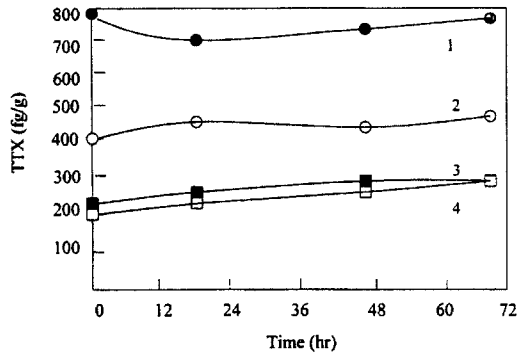

Fig. 7. Change of TTX concentration in the first group of *Lagocephalus wheeleri* during storage at 5℃ (points are mean for 5 specimens). 1: Liver, 2: Skin, 3: Tail muscle, 4: Dorsal muscle.

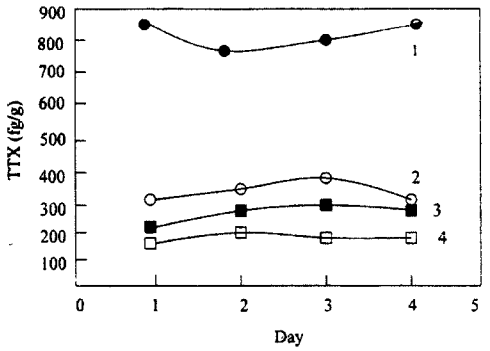

Fig. 8. Changes of TTX concentration in the first group of *Lagocephalus wheeleri* during storage at 5℃ (points are mena for 2 specimens). 1: Liver, 2: Skin, 3: Tail muscle, 4: Dorsal muscle.

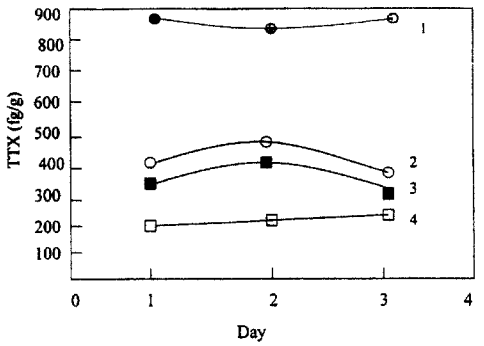

Fig. 9. Changes of TTX concetration in *Takifugu vermicularis* during at 5℃ (points are mean for 2 specimens). 1: Liver, 2: Skin, 3: Tail muscle, 4: Dorsal muscle.

In *Takifugu vermicularis* (Fig. 9), TTX content of dorsal muscle, tail muscle, skin and liver after one day of storage were 201 fg/g, 345 fg/g, 407 fg/g, and 880 fg/g, respectively and their contents of TTX at the end of storage three day were 255 fg/g, 320 fg/g, 398 fg/g, and 876 fg/g, respectively.

pH of dorsal muscle, skin and liver of *Lagocephalus wheeleri* at the beginning of storage (0 hr) were 7.08, 6.82, and 6.51, respectively and at the end of storage (72 hr) were 7.07, 6.95, and 6.58, respectively during storage at 5℃ (Fig. 10). The pH of dorsal muscle, skin, and liver was almost between pH 6 and pH 7, during storage at 5℃ for 3 days which means that TTX was not affected by the pH of the fishes.

TTX is expected to be stable during storage period. In all tested puffer fish groups, the highest concentration of TTX was found in the liver, this result agreed with the result of other workers. TTX content of dorsal muscle did not differ significantly from that of tail muscle, which may indicate that TTX is not affected by the type of muscle(7).

On the other hand K value which is affected by enzyme activity increased during storage. In all tested puffer fishes groups the highest concentration of TTX was found in the liver, this result agreed with the result of other workers(7). TTX content in the dorsal muscle did not differ

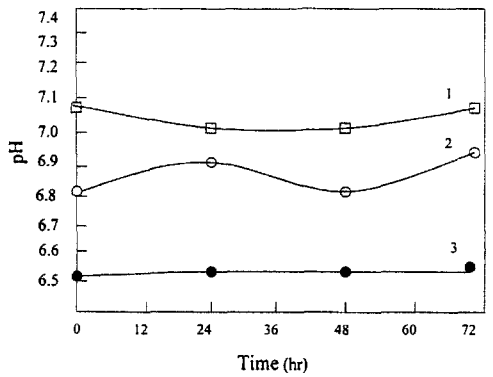

Fig. 10. Changes of pH in *Lagocephalus wheeleri* during storage at 5℃. 1: Dorsal muscle, 2: Skin, 3: Liver.

significantly from tail muscle which indicates that TTX is not affected by the type of muscle. K value increased gradually in puffer fishes, TTX content remained almost unchanged during the storage period. Since TTX has been found not to be peptide neither fish endogenous enzymes nor bacterial enzymes can affect TTX content during storage, as a result, TTX concentration did not change during storage while K value which is affected by enzyme activity increased during storage(12, 13). From the results of this study it was concluded that neither K value nor freshness changes did affect TTX content of the puffer fishes and each parameter had different pattern during storage. K value increased gradually in frozen and thawed (slacked) and fresh *Lagocephalus wheeleri* and *Takifugu vermicularis* whereas TTX content was almost stable in the both species of puffer fish during storage at 5℃ for 4 days. The highest concentration of TTX was found in liver and no significant difference in TTX content was found between dorsal and tail muscle. The difference in TTX concentration among each experimental result appears to be due to individual sample variability. It has been well established that many marine fish and puffer fish contain TTX. The food safety implication of these toxins has also been widely reported. Therefore, development of TTX monitoring system has been anticipated. Further research in our laboratory aims to establish the effect of marine environmental conditions on TTX monitoring. It is envisioned that this sensor system may be used for future TTX monitoring within the marine environment.

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