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Rate of Degradation of Nucleotides in Cooling-stored Carp Muscle*1

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Abstract

The rates of degradation of adenosine phosphates (Adp), inosinic acid (IMP) and total nucleotides (Nd) in the carp muscle cubes held at various temperatures between +2～+25°C were investigated. 1) The rate of degradation of IMP was affected more greatly by the variation of temperature than that of Adp, while the degradation rate was smaller in IMP than that in Adp. Arrhenius plots for IMP and Nd degradation gave a curve with increasing slope below about 15°C. 2) The lower the storage temperature was, the higher the level of maximum value of IMP in the muscle cubes was and the longer the time to reach the maximum was. 3) Both the keeping-quality time and its relative extension for each 1°C reduction in storage temperature, which were calculated by the application of linear equation between temperature and square root of Nd degradation rate, increased with an approach of storage temperature to 0°C.

Recently, the nucleotides degradation sequence in fish muscle has been evaluated to be more useful as an index of fish quality[1-5]. The sequence was also suggested to be an important factor in assessment of frozen-stored fish quality[6]. On the other hand, many species of fish are kept in the ice box or the refrigerated room during their transportation and storage. Therefore, the information on the rate of nucleotides degradation at different temperatures above 0°C is required for keeping-quality of the fish. However, the problem appears to have been little studied, though the microbiological loss of fish quality in the region of temperature above 0°C has been demonstrated by some researchers[7,8].

In the experiment, the rates of degradation of nucleotides in carp muscle held at various temperatures between +2°～+25°C were investigated, and the effect of storage temperature, particularly of its small variation on the time of keeping-quality was discussed.

Materials ane Methods

Seven cultured carp (*Cypinus carpio* L.) were obtained from a commercial nursery (25–38 cm in length, 0.8–1.5 kg in weight). One fish was used for each different experiment. From the fish immediately after being killed by decaptitaion, ordinary

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muscle alone was taken out and cut into small cubes (ca. 10 mm^3). After the cubes were mixed together enough, proper aliquots of the mixture were taken in a petri-dish and stored at 3 or more different temperatures between $+2°\sim+25°C$.

The nucleotides contents in the cubes were determined by means of ion exchange chromatography or its simplified modification. Duplicate samples were analyzed at a time and the average value was applied for analysis of the results. The content of every nucleotides was expressed as the content of adenosine phosphates (Adp), inosinic acid (IMP) and total nucleotides (Nd), respectively, and the change of the content of each nucleotides of them, were submitted to the calculation of rate or rate-constant of their degradation. The determination and calculation were made similarly as stated in the previous report[9]. In brief, the rate of Nd degradation was calculated by making use of the fact that the relation of the logarithmic value of Nd content to the time was represented in the form of linear equation, and also the rate-constants of Adp and IMP were calculated by employing FROST's method for the consecutive first order reaction. And furthermore, using the rate-constants, the maximum value of IMP and the time required to reach maximum were calculated.

Nucleotides in the muscle cubes ought to degrade faster than those in such a case of the uncut muscle as fillets. However, in the author's another experiment[10], no distinct difference in the relation of nucleotides degradation rate to temperature was found between cubes and fillets, though the rate of degradation was greater in the cubes than in the fillets as stated in NEWBOLD's report[11].

Results and Discussion

Relation of temperature to rate of Adp- and IMP degradation The Arrhenius plots for Adp- and IMP degradation are shown in Fig. 1. Although considerable scatter is seen in the figure, some distinctive feature worthy of attention are to be observed. The rate-constant of Adp was larger than that of IMP, whereas the slope of plot was remarkably greater in IMP than in Adp, particularly its extent being large in the range of temperature below the transition point, about 15°C. The existence of transition point seems to coincides with FRASER's results[12], where the rate of IMP degradation in mackerel increased sharply at about 10°C or above. Apparent activation energy below the transition temperature was approximately 25 kcal/mole. The value was not far from the values obtained by DYER et al.[13] for IMP degradation in the frozen-stored swordfish and also was almost equal to the values obtained in the author's separate test[14] for the activity of IMP dephosphorylation of carp muscle extracts. The results show that the temperature dependence of degradation of IMP was greater than that of Adp, though IMP degraded more slowly than did Adp. Such difference between IMP and Adp appears to have been based on the difference in their biochemical role and significance.

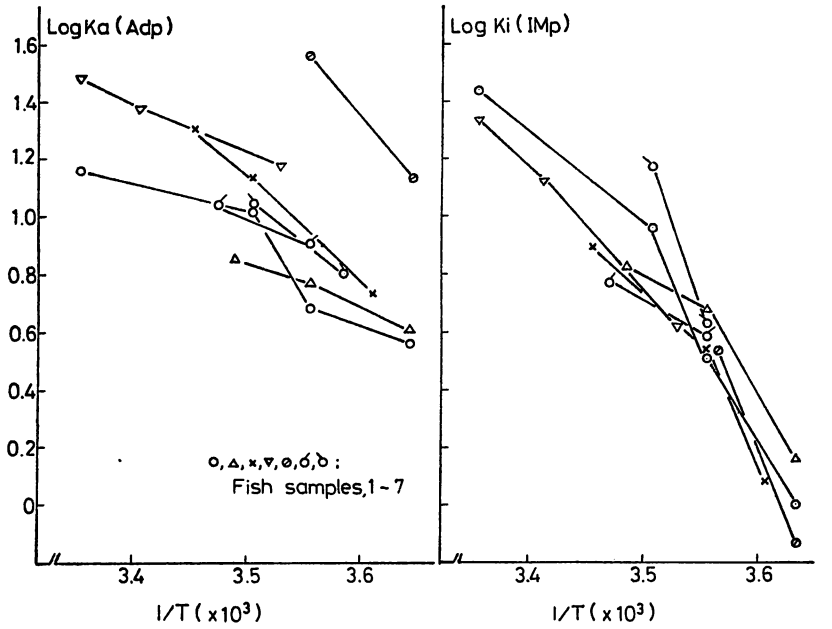

Fig. 1. Arrhenius plots of log rate-constant vs temperature. Ka and Ki represent rate-constants of degradation of adenosine phosphate and inosinic acid, respectively.

Relation of temperature to both the maximum value of IMP and the time to reach its maximum It has been known that IMP in fish muscle increases with the sharp slope of decrease of Adp to reach its maximum and subsequenly

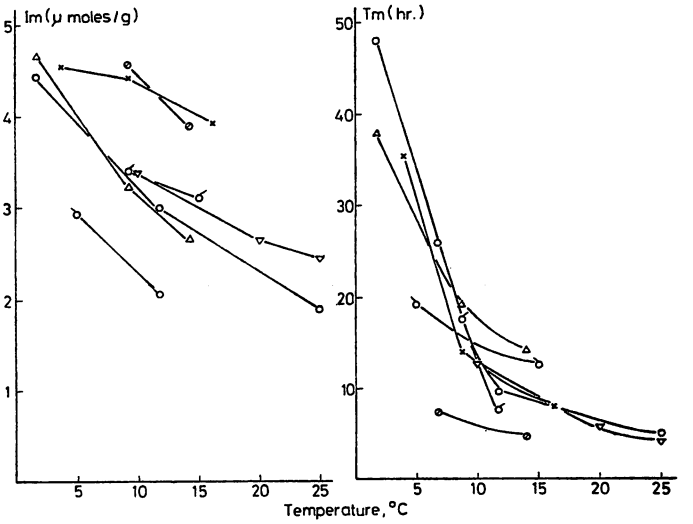

Fig. 2. Maximum values of IMP (Im) and the time required to reach them (Tm). Symbols are the same as in Fig. 1.

decreases slowly. Both the level of maximum value and the time to reach its maximum will be affected by the rate of IMP degradation and, accordingly, by the storage temperature. As seen in Fig. 2, the level of maximum value of IMP increased as the storage temperature was lowered. And the time to reach maximum was prolonged with the lowering of storage temperature, which was noticeable particularly in the region of temperature below about 15°C.

In brief, judging from the points of both the level of IMP content and the time of keeping its level, to keep the fish muscle at lower temperature may be considered to be relatively more effective.

Relation of temperature to the total nucleotides degradation The Arrhenius plots for the total nucleotides degradation are shown in Fig. 3. The plots were, as in the case of IMP degradation mentioned above, the curve with increasing slope below about 15°C, and the activation energy in the range below the transient temperature was approximately 28 kcal/mole.

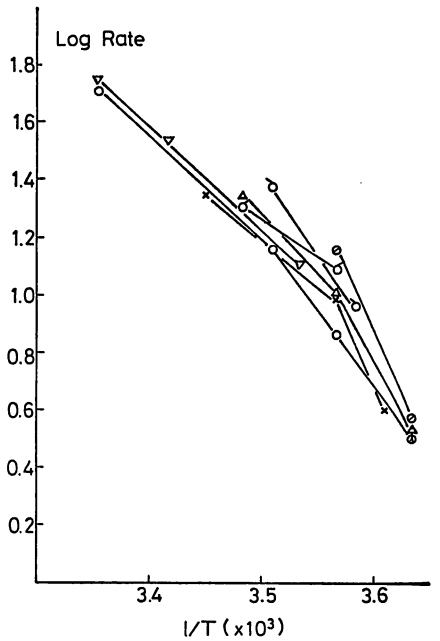

Fig. 3. Arrhenius plots for the total nucleotides degradation. Symbols are the same as in Fig. 1.

The relation between the temperature (θ°C) and the rate of nucleotides degradation (R_θ) was observed to be curvilinear. The equation obtained by regression analysis, was as the following; $R_\theta=0.0053\theta^2+0.0455\theta+0.318$ (Fig. 4). However, the relation between the square root of rate and the temperature was found to be

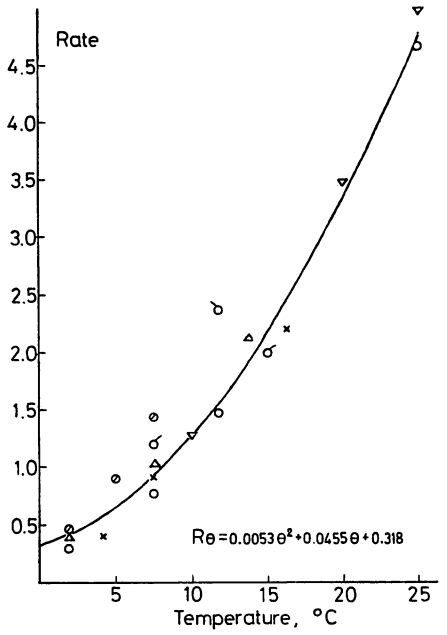

Fig. 4. Relation of rate of nucleotides degradation (R_θ) and temperature (θ). Symbols are the same as in Fig. 1.

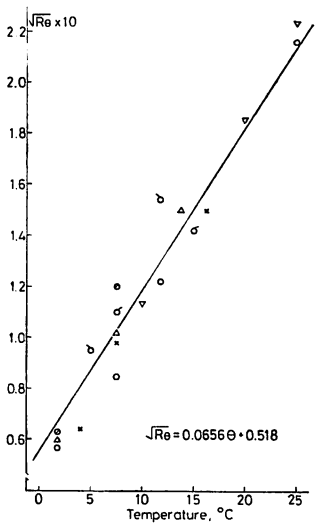

Fig. 5. Relation of the square root of rate of total nucleotides degradation ($\sqrt{R_\theta}$) and the temperature (θ). Symbols are the same as in Fig. 1.

nearly linear, its relation equation being $\sqrt{R_\theta}=0.0656\theta+0.518$ (Fig. 5). The rates calculated from the equation agreed with them from the above secondary equation within the allowance of $\pm 5\%$.

Effect of temperature on the keeping-quality time Using the relation equation mentioned above, the effect of temperature on the time of keeping fish-quality was discussed quantitatively. The time length of keeping-quality (D_θ), in which the degradation reaches a specified critical level at the arbitrary temperature θ°C, is to be expressed as follows; $D_\theta=S/R_\theta$, where S=quality magnitude, difference between initial quality and critical value, R_θ=rate of nucleotides degradation at θ°C. Then, substituting the above equation, $\sqrt{R_\theta}=0.0656\theta+0.518$, gives an approximate relation between keeping-quality time and temperature, $D_\theta=D_0/(1+0.127\theta)^2$, where $D_0=S/R_0$=keeping-quality time (in days) at 0°C.

From the above equation, both the relative value of keeping-quality time at the arbitrary temperature to that at 0°C and the relative extension in keeping-quality time for 1°C reduction were calculated as shown in Table 1. The results show that the effect of temperature on keeping-quality time increased with the lowering of temperature, particularly its extent was noticeable around 0°C. For exsample, the relative extension was about 30% around 0°C while about 7% at 20°C.

Thus it is confirmed that the approaching 0°C the storage temperature was, the

Table 1. Keeping-quality time and its extension with 1°C reduction at different temperatures.

Storage temperature °C	Relative time of keeping-quality (20°C, 1.0)	Relative extension for 1°C reduction of storage temperature
0	12.53	0.312
5	4.69	0.175
10	2.43	0.122
15	1.48	0.094
20	1.00	0.075
25	0.72	0.064

greater the effectiveness of low temperature on the keeping of fish-quality, as measured with the degree of nucleotides degradation, was, and moreover the keeping time around 0°C was noticeably affected by small variation of temperature.

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