EFFECTS OF WATER STATE UPON FRESHNESS CHANGE OF RAW TUNA MEAT DURING STORAGE

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ABSTRACT

Freshness change of raw tuna meat was investigated in a storage temperature range of 20°C to -84°C by using the *K* value as a freshness indicator. Physicochemical characterization of water in the sample was also performed using Differential Scanning Calorimetry (DSC). The plot of logarithm of (100-*K* value) against storage time yielded a straight line, which indicated an apparent first-order reaction for all temperature storage. The temperature dependence of this reaction was analyzed by an Arrhenius plot, resulting in two breaking points. The first breaking point was close to the freezing point of the sample, suggesting that this breaking point was due to the freezing effect. The second occurred was at -10°C. The reaction rate change steeply declined at the temperature rage of -70°C to -84°C and was thought to be related to glass transition which may occur in the fresh sample.

INTRODUCTION

Frozen storage has been widely utilized for preserving quality of fresh fish meat. Generally, by decreasing temperature, the physicochemical changes of water state occur, such as formation of ice crystals, freeze concentration of the residual substances with precipitating ice, and glass transition of the concentrated residual substances below the temperature so-called glass transition temperature (T_g). It is expected that these changes affects stability of fish meat quality during storage. In this study, firstly the freshness change of fresh fish meat was investigated in a storage temperature range of 20°C to -84°C. Secondly, physicochemical characterization of water in the sample was performed using Differential Scanning Calorimetry (DSC). And finally, comparing both experimental results, effects of water state upon the freshness change of fish meat was examined.

1 MATERIALS AND METHODS

1.1 Sample

Fresh yellowfin tuna *Thunnus albacares* was used as a sample. Two lots of the samples were purchased at different times from a local fish retailer as a raw fillet (dorsal ordinary muscle). These fillets were cut into cubes (~1.5×1.5×1 cm) and then placed individually in a polyethylene bag prior to storage. All preparations were carried out at 5°C.

1.2 Storage Condition

The first lot was used for storage at -84°C, -70°C, -46°C (in freezers with a precision of ±1°C) and the second lot for storage at -10°C, -3°C, -0°C, 5°C, 10°C and 20°C (in an incubator with a precision of ±0.1°C; LTI-600SD.1000SD; Eyela, Tokyo. Japan). From each of storage treatment, the samples were taken out in duplicate at different intervals for analysis of freshness.

1.3 Freshness Measurement

The *K* value was used as a freshness indicator. It is a biochemical index based on nucleotide degradation, which is expressed as a percentage of the amount of inosine (HxR) and hypoxanthine (Hx) to the total amount of adenosine 5-tri-, di-, mono-phosphates (ATP, ADP, AMP), inosine mono-phosphate (IMP), HxR and Hx. After death, ATP and ATP-related compounds are converted through enzymatic reactions as the following route:

$$\text{ATP} \rightarrow \text{ADP} \rightarrow \text{AMP} \rightarrow \text{IMP} \rightarrow \text{HxR} \rightarrow \text{Hx}$$

That is, the *K* value increases continuously after death; the smaller the *K* values, the fresher the sample is. The *K* value has been widely used in Japan as one of the freshness indices to evaluate the quality change of raw fish after catch because many tons of raw fish have been consumed as *sashimi* in Japan (Ehira and Uchiyama, 1987; Kennish and Kramer, 1987; Saito *et al.*, 1959; Miki and Nishimoto, 1984).

In this study, based on the modified method of Ryder (1985), the *K* value was determined by high performance liquid chromatography.

1.4 Physicochemical Characterization of Water in Samples

The freezing and glass transition temperatures of fish muscle were evaluated using DSC analysis. Sample fish (10-20 mg) were weighted into an aluminum DSC pan, hermetically sealed and then loaded onto the Shimadzu (DSC-50, Kyoto, Japan) instrument at room temperature. The sample was then cooled at 3°C per min to -60°C and heated up at the same rate to 40°C. To observe glass transition of the sample, DSC experiment at lower temperature, down to -130°C, was conducted at 10°C per min. The obtained DSC curves were analyzed using Shimadzu software (TA60). In these DSC analyses, fresh samples were used.

1.5 Data Analysis

The value of *K*' (=100 – *K* value) was analyzed in this study, which represents the ratio of the remaining amount of ATP, ADP, AMP and IMP to the total amount of ATP-related compounds in the same way as Miki and Nishimoto (1984) used. The analytical approach to calculate and predict food quality deterioration needs a kinetic model in which it is based on the deterioration process rate (Arabshahi and Lund, 1985). The apparent total process rate is given by the following first-order reaction equation, though its process would be so complicated.

$$K'(=100 - K \text{ value}) = a \cdot \exp(-k_f \cdot t) \quad (1)$$

where a is constant, k_f is the reaction rate constant, and t is storage time. The reaction rate k_f is correlated with environmental and composition factors (Saguy and Karel, 1980). Storage temperature is one of the main environmental factors that has a major influence on quality loss rate (Cohen and Saguy, 1985). The most common and generally valid assumption is that temperature dependence of the deterioration rate follows the Arrhenius

equation (Saguy and Karel, 1980). That is to say,

$$k_f = k_0 \cdot \exp\ (-E_a/RT) \qquad (1)$$

where k_f is the apparent reaction rate constant of K' value change, k_0 is frequency factor, E_a is activation energy, R is gas constant and T is absolute temperature. This equation has been frequently used as a theoretical basis for the development of a mathematical model to describe the temperature sensitivity of foods (Labuza and Riboh, 1982).

2 RESULTS (Agustini *et al.*, 2001)

Figure 1 shows the plots of log (100–K value) *vs.* storage time. The K' values decreased with increase in storage time for all temperature treatments, except at -84°C. The plots showed linear relationship at all temperature treatments, though some deviations were observed below -46°C as seen in Fig. 1(b). This indicates that the reaction showed a first-order reaction behavior. These were agreed with the previous results (Miki and Nishimoto, 1984; Tomioka *et al.*, 1985). The apparent reaction rate constant k_f was calculated from the slope of the plots by linear least square method, and the values and their correlation coefficients are shown in Table 1, including the data of -84°C. It is reasonable that the reaction rate was larger with an increasing storage temperature.

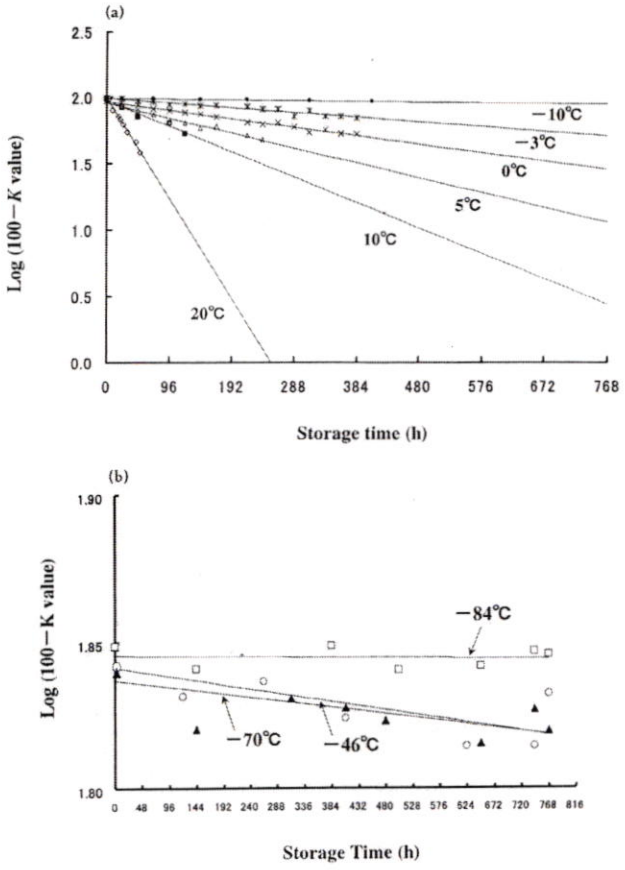

Fig. 1 (a) Changes of K value of tune meat during storage from 20°C to -10°C. (b) Changes of K value of tuna meat during storage from -46°C to -84°C.

Table 1 Apparent reaction rate constant k_f of freshness deterioration in tuna meat stored at different temperatures

Storage Temperature (°C)	20	10	5	0	−3	−10	−46	−70	−84
$k_f \times 10^3$ (h^{-1})	18.19 (0.99)	4.61 (0.96)	2.76 (0.97)	1.61 (0.98)	0.92 (0.94)	0.12 (0.98)	0.07 (0.54)	0.05 (0.43)	0.0021 (0.01)

Values in parentheses are coefficient correlation.

Based on the rate constant of K' value changes, the Arrhenius analysis was carried out by plotting ln k_f against the reciprocal absolute temperature of storage (Fig. 2). The plot did not exhibit one straight line over experimental temperature range, but some straight lines with different slopes depending on the temperature range; there seems to be break points around -3°C and -10°C. Considering the results, the apparent activation energies of each linear regime were determined to give tentative criteria (Table 2). The activation energy values of other fishes reported previously are also listed in Table 2. Here the k_f value at -84°C was excluded for calculation of the activation energy because it might be inaccurate value; the correlation coefficient was quite smaller than those at other temperatures. However, it is certain that the reaction rate changed steeply declined at the temperature rage of -70°C to -84°C.

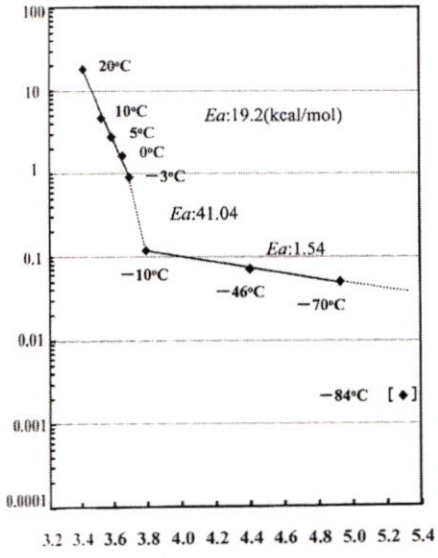

Fig. 2 Effect of temperature on reaction rate on K' value change of tuna meat.

Table 2 Kinetic parameter of some fishes at different temperatures

Fish species	E_a (kcal/mol)			$k_0(h^{-1})$		
	>−3°C	−3 to −10°C	<−10°C	>−3°C	−3 to −10°C	<−10°C
Yellowtail	22_a	71_a	–	–	–	–
Cod	29_a	57_a	–	–	–	–
Skipjack	14.0_b	50.9_b	54.8_b (to −40°C)	$7.3\times10^{8}{}_b$	$5.3\times10^{38}{}_b$	$8.0\times10_b$
Sea bream	15.8_b	33.6_b	–	$4.8\times10^{9}{}_b$	$7.2\times10^{11}{}_b$	–
Mackerel	16.2_b	41.4_b	–	$2.3\times10^{10}{}_b$	$4.9\times10^{30}{}_b$	–
Tuna	19.2_c	41.04_c	1.54_c (to −70°C)	$3.9\times10^{12}{}_c$	$1.6\times10^{30}{}_c$	$2.3\times10^{-3}{}_c$

[a]From Tomioka et al. (1987). [b] From Miki and Nishimoto (1984). [c]Observed value from this study.

Fig. 3 shows the DSC heating curve of tune meat. A large endothermic peak was observed, corresponding to the melting of ice. From the onset temperature of the peak, the freezing point (T_f) was evaluated to be -5°C. The DSC heating curve of tuna meat at very low temperatures shows a clear base line shift to endothermic (Fig. 4). This shift is considered to be a typical glass transition phenomenon. From the mid point of the shift, the glass transition temperature of yellowfin tuna meat was determined to be -63°C. The onset and end-point temperatures were observed to be -75°C and -47°C, respectively.

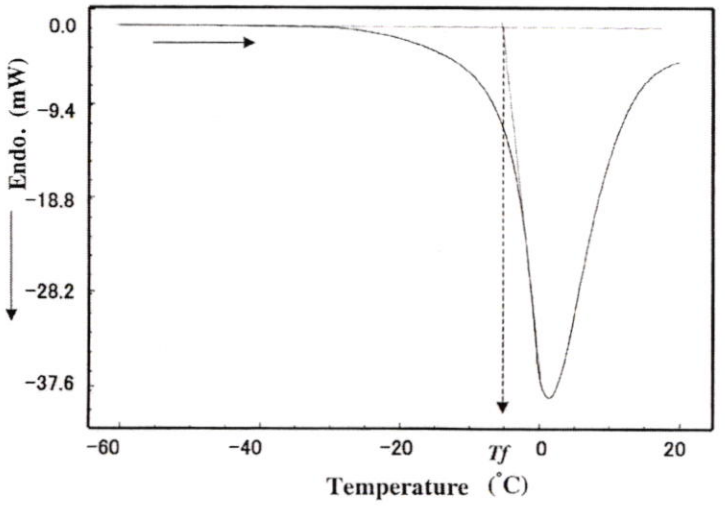

Fig. 3 DSC heating curve of tuna meat.

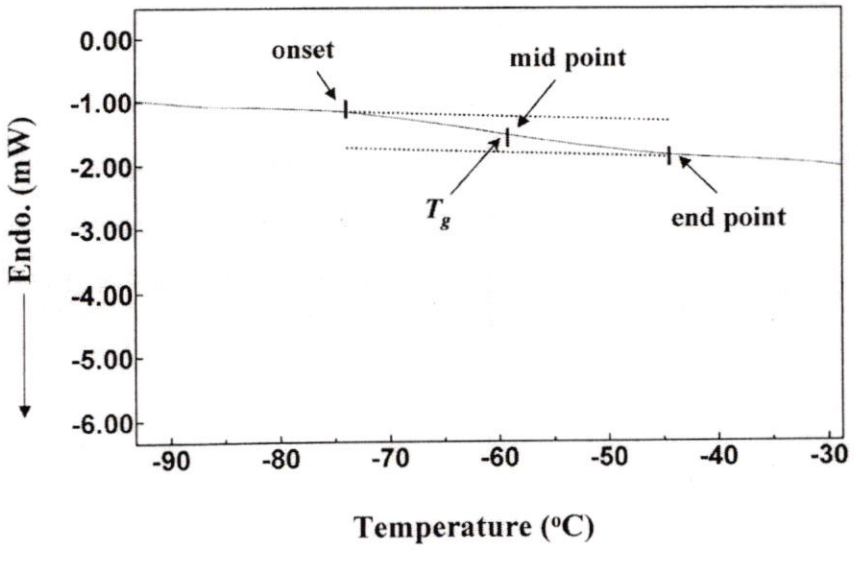

Fig. 4 DSC heating curve of tuna meat at very low temperature.

3 DISCUSSION

As shown in Fig. 2, the Arrhenius plot of the apparent rate constant k_f showed a complex behavior over experimental temperature range. Tomioka *et al.* (1987) reported that the Arrhenius plot of dephosphorylation of nucleotides in cod and yellowtail exhibited a break point around -2°C, and that the apparent activation energy below -2°C gave a larger value. Miki and Nishimoto (1984) also reported on freshness lowering of some fishes, which gave a break point around -2°C on the Arrhenius plot. Additionally, they observed that there was one more break point at -10°C and the Arrhenius plot in the range of 20°C to -40°C was separated to three linear regions (i.e.20°C ~ -2°C, -2°C ~ -10°C and -10°C ~ -40°C). They concluded that the break point around -2°C was accounted for by the phase change of water in fish meat. The freezing process is accompanied by a gradual increase in the concentration of all soluble materials in the residual liquid phase, which is referred as the freeze concentration (Franks, 1985; Brake and Fennema, 1999).

From our result, there was a lack of data on kinetic parameters between temperatures of -3°C and -10°C. However, Miki and Nishimoto (1984) reported the k_f change of several fishes at -3°C, -5°C and -10°C showed linear behavior on the Arrhenius plot. Therefore, it would be reasonable to assume that a similar tendency is also observed between -3°C and -10°C in the tuna meat. For our results, the plot of k_f *vs.* $1/T$ in the temperature range of 20°C to -10°C would have a break point around -3°C, whereby this temperature is close to the freezing point of -5°C as evaluated by the DSC analysis. This suggested that the breaking point around -3°C was due to the freezing effect.

In Fig 2 the break point at -10°C was also observed, which was similar to the result of some fishes (Miki and Nishimoto, 1984). As for the break point, there is no clear explanation because we were not able to obtain evident physicochemical information on water around -10°C from the DSC analysis. However, the following information was already available, which may give some hint to account for the break point around -10°C. According to an NMR experiment conducted by Nagashima and Suzuki (1986), when beef was frozen all water present was not freezing at a temperature of -2°C and after the temperature reached to -20°C almost all of freezable water, which amounted to 90% of water in the sample, was frozen. Since similar phenomenon can be also observed in fish, it is suggested that the break point around -10°C in the tuna meat could be related to the temperature at which all of freezable water was frozen.

As for the temperature from -10°C to -70°C, the rate constant k_f had some substantial values, though they were small. This suggested that enzymes still have mobility and maintain their activity in the frozen state up to -70°C. We could not find any reports on enzymatic reaction in the frozen state below -70°C, however, there was a report in the field of cryobiology on sperm's cryostability at very low temperatures. When sperm of cow was stored above -80°C, the activity was lost. However, storage at temperature below -80°C resulted in permanent preservation of its activity (Shirayama and Iritani, 1987). This indicates that biological activity such as enzymatic reaction remains at -80°C and stops below -80°C. Our result showed that K' value change also stopped at -84°C. This behavior around -80°C, which was first recognized in this experiment, will be explained from the standpoint of glass transition in next section.

Recently, there have been several studies related to the glass transition temperature (T_g) of fish muscle. For example, T_g of mackerel and cod were reported by -13.3±0.5°C and -11.7±0.6°C, respectively (Brake and Fennema, 1999). However, for cod, other reported values of T_g are -77°C (Nesvadba, 1993), -40°C (Simatos and Blond, 1993) and -15°C (Levine and Slade, 1989). For tuna, different T_g values are reported; -68°C to -71°C (Inoue and Ishikawa, 1997) and -11.5°C to -18°C (Levine and Slade, 1989). The reason for the differences in the reported values of T_g for some fishes is still unclear. It may be due to the difference in analytical procedure and, moreover, complex products such as plant or animal tissues have been known to exhibit more than one T_g (Brake and Fennema, 1999). Even now, the T_g values for meat products are not fixed; however, there is a reliable result that some pure native proteins show

a glass transition temperature of ~-70°C (Green *et al.*, 1994). The glass transition temperature obtained for yellowfin tuna meat in this study was evaluated to be -63°C. This value seems to be reasonable compared to that of native proteins. However, the range of glass transition temperature was wide, from -47°C to -75°C. This indicates that the phase state is gradually changing in the temperature range and hence it can be said at temperatures lower than -75°C, yellowfin tuna meat was in the perfect glassy state. That is to say, below temperatures of -75°C, there was a metastable and unreactive solid state formed within the unfrozen phase in the sample. Therefore, it can be concluded that the step-like change of k_f occurring from -70°C to -84°C is due to the formation of the glassy state in the sample fish meat. The glassy state formation will decrease the rate of molecular motion in the meat significantly, and results in a considerable decrease of ATP degradation in the sample, which makes the sample stable for a much longer time. This finding may be useful for determining the adequate temperature to stop or retard deterioration reactions in frozen meat.

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Effets de l'état d'eau sur le changement de la fraîcheur de la viande brute de thon pendant le stockage

RESUME :Le changement de la fraîcheur de la viande brute de thon était examiné au domaine de températures de 20°C à -84°C en employant la valeur K comme un indicateur de la fraîcheur. La caractérisation physico-chimique était aussi exécutée sur l'eau dans l'échantillon en employant la Calorimétrie Différentielle Programmée (DSC). Le pointage de logarithme (de la valeur 100-K) contre le temps de dépôt livrait une ligne directe, qui avait indiquée une réaction apparente du premier ordre aux toutes les températures de stockage. La dependence sur la température de cette réaction était analysée par le pointage d'Arrhenius, et deux points de rupture étaient données. Le premier point de rupture était proche au point de congélation de l'échantillon, et ce fait suggère que ce point de rupture était nécessité par l'effet de congélation. Le seconde point se passait à la température de -10°C. Le changement de la vitesse de réaction s'abaissait abruptement dans le domaine de températures de -70°C à -84°C et était regardé comme être en rapport avec la transition vitreuse, qui se passait dans l'échantillon frais.