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Original Article

Change of *K* value and water state of yellowfin tuna *Thunnus albacares* meat stored in a wide temperature range (20°C to –84°C)

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SUMMARY: The study on *K* value change at low temperature storage had been carried out down to a temperature of –40°C, however, there was no evidence about this reaction rate if the temperature were lowered below the storage temperature normally used especially for tuna meat (–60°C). The rate of *K* value change (k_f) of yellowfin tuna (*Thunnus albacares*) meat was determined during storage at a wide temperature range (20°C to –84°C). The logarithm of *K′* (= 100 – *K* value) was used in this study and its plot against storage time yielded a straight line, which indicated an apparent first-order reaction for all temperature storage. Furthermore, physicochemical characterization of water in tuna muscle was carried out using Differential Scanning Calorimetry (DSC) at very low temperature. The temperature dependence of this reaction was analyzed by an Arrhenius's plot that resulted in two break points. The first break point occurring at freezing point might be due to the freeze effect. The second break point was at –10°C. The reaction rate change steeply declined at the temperature range of –70°C to –84°C, and was thought to be related to glass transition which may occur in the fish sample.

KEY WORDS: **ATP-related compound, freshness deterioration rate, glass transition, *K* value, kinetic parameter.**

INTRODUCTION

The *K* value is a biochemical index for fish quality assessment based on nucleotide changes, 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. This value has been widely used in Japan as one of the freshness indices to evaluate the quality change of raw fish after catch (i.e. unfrozen fish).[1–3] However, it has been generally considered that using the *K* value as a quality index of frozen–thawed fish meat is limited, since the *K* value change may be small during frozen storage and the measured *K* value would reflect the quality of fish meat just before frozen. Many tons of fish, which were frozen soon after catch and then thawed, have been actually consumed as sashimi. Rather than *K* value changes, the quality deterioration of frozen–thawed fish meat would be mainly affected by oxidation of lipid and protein denaturation during the freezing process and storage. Apart from these discussions, Miki and Nishimoto[4] investigated the change of *K* value and color for skipjack, mackerel and red sea-bream meats based on the kinetic theory with storage temperature ranging from 20°C to –40°C. They found that the *K* value does change even at low temperature (~–40°C), suggesting that enzymatic reactions could still progress in frozen fish meat. Moreover, based on this result, it could be assumed that the other enzymatic and chemical reactions could also proceed in frozen fish meat.

It is well known that among many commercial fish species, tuna meats have to be kept at special temperature condition of below –60°C for long-term storage. Despite this situation, it is mysteri-

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ous that there are no reports with respect to the efficiency of such special storage condition. The ultimate purpose of our study is to provide scientific evidence and clarify the suitable conditions for long-term storage of tuna. As mentioned above, many changes such as discoloration would take place during frozen storage. However, we initially selected to measure the *K* value change among many changeable factors, because its related enzymes and reaction mechanism are relatively well understood. In addition, such physicochemical information on the phase state of water or enzymes are also necessary to understand enzymatic or chemical reactions in frozen food.

Recently, many studies have focused on the relation between stability (i.e. the diffusion-controlled rate of many reactions) and the glass transition of frozen food.[5,6] When food is frozen to very low temperatures, the residual substance is concentrated with precipitating ice. This process is called 'freeze concentration'. According to the recent glass transition theory, the residual concentrated substance in the cooling process turns to a glassy state (i.e rubbery solution changing to amorphous solid) when the temperature reaches the so-called glass transition temperature, T_g. The mobility of molecule in glassy state substance is strictly limited, so molecular translational diffusion causing various reactions is also restricted. Therefore, a glassy state food is considered to be very stable. However, there is still a lack of data on physicochemical properties of tuna meat for preserving at low temperatures, especially at temperatures below –60°C.

This study was conducted to determine any changes in *K* value, pH and water state including the glass transition of tuna meat during storage at various temperatures.

MATERIALS AND METHODS

Fish sample

Fresh yellowfin tuna *Thunnus albacares*, consisting of two lots was 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 conducted at 5°C.

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 precision of ±0.1°C: LTI-600SD.1000SD; Eyela, Tokyo, Japan). From each storage temperature treatment, the samples were taken out in duplicate at different intervals for analyses (pH and *K* value).

K value

The *K* value was determined by high performance liquid chromatography based on the modified method of Ryder.[7] One gram of fish muscle tissue was homogenized with 4 mL each of chilled 10% and 5% perchloric acid. The homogenate was centrifuged at 2000 *g* for 10 min at 5°C and supernatant was immediately neutralized to pH 6.8 with 1 N and 10 N KOH. The neutralized mixture was centrifuged again at 2000 *g* for another 10 min and the supernatant was diluted to 20 mL with neutralized perchloric acid solution and then filtrated prior to storage at –46°C for subsequent analysis.

Separation of ATP-related compounds was conducted on a reverse-phase column (GS-320 HQ; Asahipak, Kanagawa, Japan). The mobile phase of 200 mM sodium dihydrogenphosphate dehydrate (pH 2.8) was used at a flow rate of 1 mL per min and temperature 30°C. The eluant was monitored at 258 nm for each ATP-related compound. The concentration of each compound was calculated from its peak height.

pH value

The pH value was measured by a pH meter (M-8; Horiba, Kyoto, Japan) at the same intervals as *K* value measurement.

Thermal analysis

The freezing and glass transition temperatures of fish muscle were determined using DSC analysis. Sample fish (10–20 mg) were weighed 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. In order to observe glass transition of the sample, the experiment on DSC at a lower temperature, down to –130°C, was carried out at 10°C per min. The obtained DSC curves were then analyzed using Shimadzu software (TA60). In these thermal analyses, fresh samples were used.

Data analysis

Instead of the K value, the value of K' ($=100-K$ value) is used 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 as used by Miki and Nishimoto.[4] The analytical approach for calculating and predicting food quality deterioration involves a kinetic model in which it is based on the deterioration process rate.[8] The apparent total process rate can be experimentally expressed by the following first-order reaction equation even though its process would be so complicated.

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

where a is constant, k_f is the reaction rate constant, and t is time of storage. The reaction rate k_f can be correlated with environmental and composition factors.[9] Temperature of storage is one of the main environmental factors that has a major impact and influence on quality loss rate.[10] The most common and generally valid assumption is that temperature dependence of the deterioration rate follows the Arrhenius equation[9], that is:

$$k_f = k_0 \cdot \exp(-E_a/RT) \qquad (2)$$

where k_f is the apparent reaction rate constant of K' value change, k_0 is frequency factor, E_a is energy of activation, R is gas constant (1.986 cal·mol^{-1}K^{-1}) and T is absolute temperature. This equation is frequently used as a theoretical basis for the development of a mathematical model, which describes the temperature sensitivity of the food to be analyzed.[11]

RESULTS

Based on the changes in concentrations of ATP and its related compounds observed in this study, yellowfin tuna was considered as an inosine (HxR)-forming type fish as shown in Fig. 1. This result was in agreement with the reported value by Ehira[12] for yellowfin tuna which had a ratio of 7 : 1 for HxR : Hx.

The K' values decreased with prolonged storage time for all temperature treatments, except at –84°C (Fig. 2). The plot of logarithm of K' values versus storage time yielded a straight line at all temperature treatments, even though some deviations existed below –46°C as seen in Fig. 2b. This means that the reaction indicated a first-order reaction. These results were in agreement with the results hitherto reported.[4,13] The initial K' values in Fig. 2 were quite different. This might be due to the difference of the two lots as described earlier. On considering the reaction rate for the first-order reaction, however, the initial value is recognized not to be important. The apparent reaction rate constant k_f was calculated from the slope of straight lines by linear least square method, and the values and its correlation coefficients are listed in Table 1.

It is reasonable that the reaction occurred faster with an increasing storage temperature. For example, to attain the K value of 30% on storage at 5°C, 0°C and –3°C, samples required different storage times for 3.85 days, 7.2 days and 15.5 days, respectively.

The pH value of yellowfin tuna meat did not markedly vary during storage below ice storage temperature, but a significant change occurred at

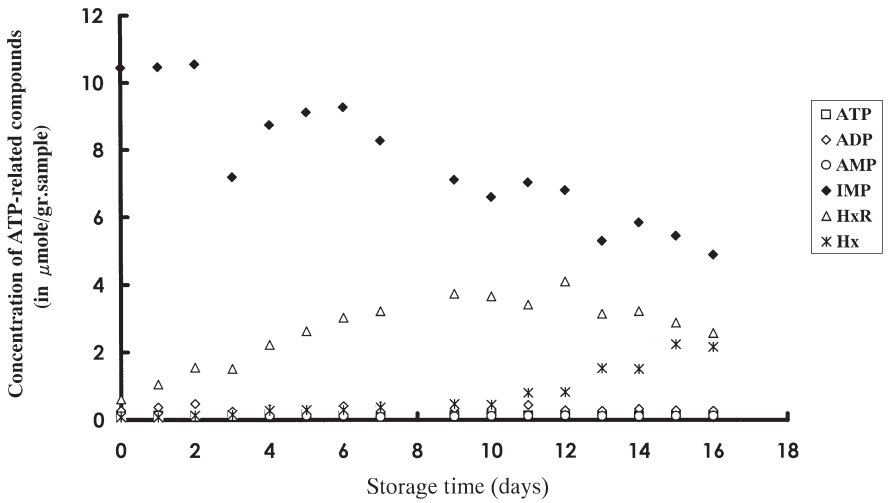

Fig. 1 Changes of ATP and its related compounds in yellowfin tuna *Thunnus albacares* meat stored at 0°C.

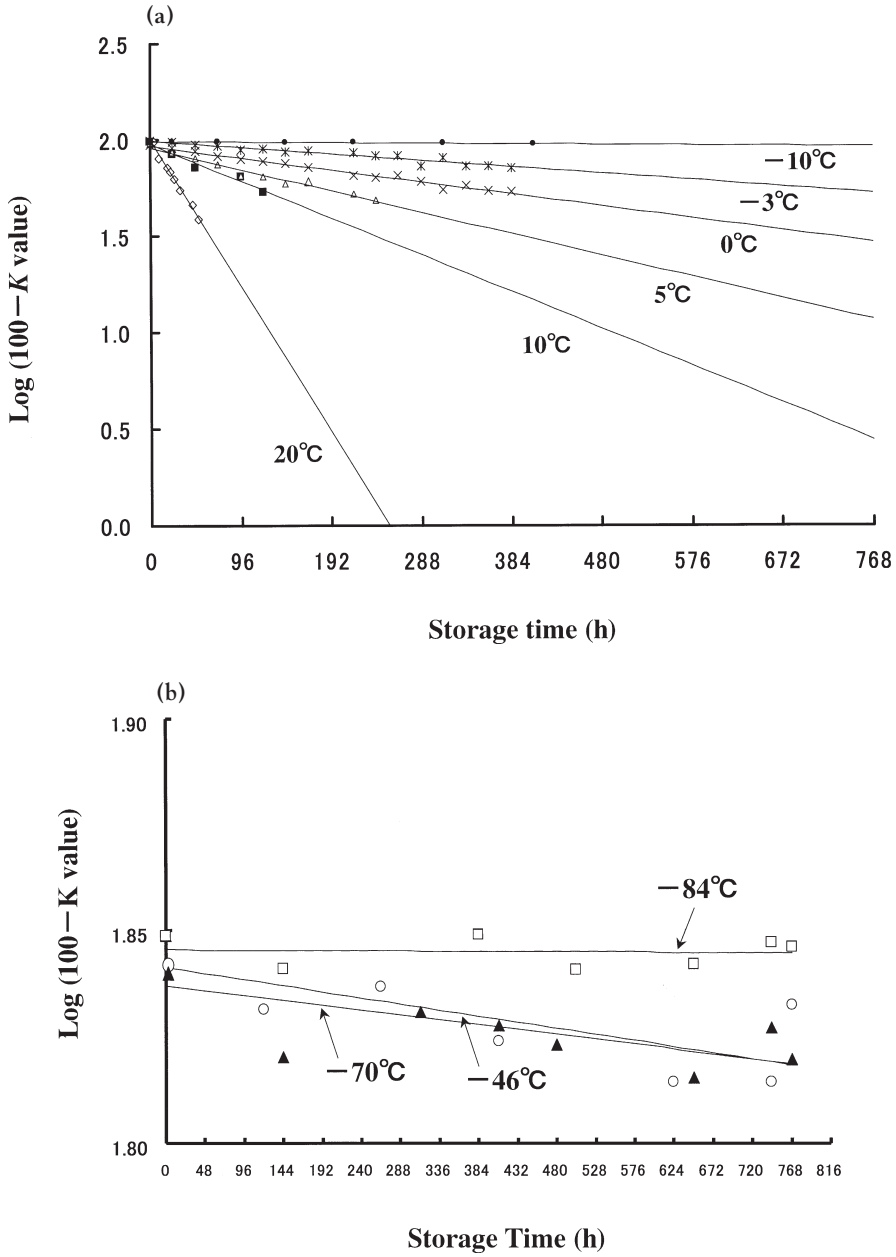

Fig. 2 (a) Changes of *K* value of yellowfin tuna *Thunnus albacares* meat during storage from 20°C to –10°C. (b) Changes of *K* value of yellowfin tuna *Thunnus albacares* meat during storage from –46°C to –84°C.

above ice storage temperature (Fig. 3). These increases in pH might be mainly due to microbiological action.

Based on the rate constant of K' value changes, the Arrhenius analysis was performed by plotting the logarithm of the apparent rate constant k_f against the reciprocal absolute temperature of storage (Fig. 4). The plot of ln k_f versus $1/T$ did not exhibit one straight line over measured temperature range, but some straight lines with different slopes depending on the temperature range. It is obvious that the plots above –3°C gave a straight line, but the plot below –3°C exhibited complex behavior. Although a linear relationship seems to be recognized between –10°C and –70°C, it is not certain since the rate constants k_f at these temperatures were not so accurate, as seen in Fig. 2b. The behavior of k_f at low temperature will be discussed in detail later.

Figure 5 presents the DSC heating curve of tuna meat where a large endothermic peak was observed corresponding to the melting of ice. From

Table 1 Apparent reaction rate constant k_f of freshness deterioration in yellowfin tuna *Thunnus albacares* 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.

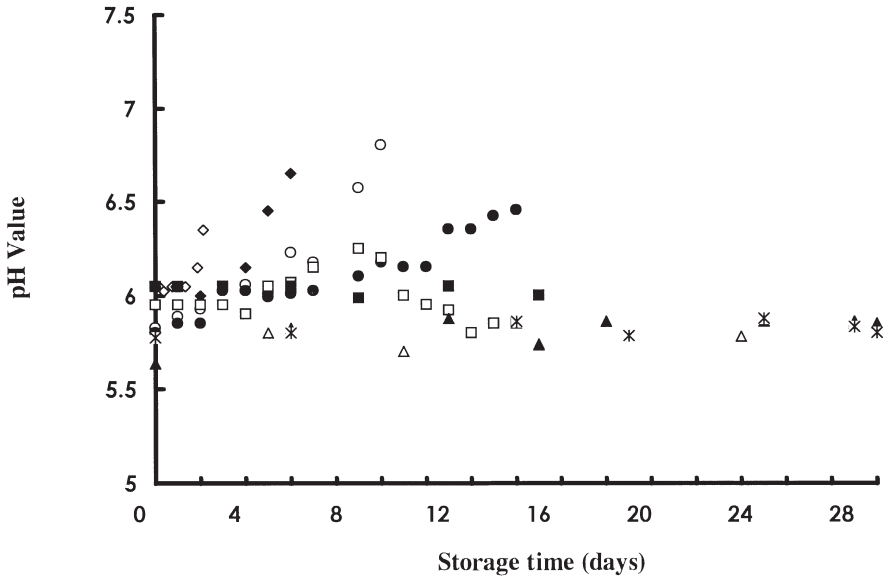

Fig. 3 The pH changes of yellowfin tuna *Thunnus albacares* meat during storage at different temperatures. ◇, 20°C; ◆, 10°C; ○, 5°C; ●, 0°C; □, −3°C; ■, −10°C; △, −46°C; ▲, −70°C; ⁎, −84°C.

the onset temperature of the peak, the freezing point (T_f) was estimated to be −5°C. The DSC heating curve of tuna meat at very low temperatures indicated a clear base line shift to endothermic as shown in Fig. 6. This shift is considered a typical glass transition phenomenon. From the midpoint of the shift, it is confirmed that the glass transition temperature of yellowfin tuna meat is −63°C. The onset and end-point temperatures were observed to be −75°C and −47°C, respectively.

DISCUSSION

As previously noted, the apparent rate constant k_f of the K value change exhibited a complex behavior below −3°C. Tomioka *et al.*[13] reported that an Arrhenius plot of dephosphorylation of nucleotides in cod and yellowtail exhibited a break point at around −2°C, and that the apparent activation energy below −2°C gave a higher value. Miki and Nishimoto[4] also reported on freshness lowering of some fishes, which gave a break point at around −2°C on the Arrhenius plot. They also reported that there was one more break point at −10°C and the Arrhenius plot in the range of 20°C to −40°C could be separated to three linear regions (i.e. 20°C ~ −2°C, −2°C ~ −10°C and −10°C ~ −40°C). It should be noted that the calculated activation energy in the range of −2°C ~ −10°C gave the highest value. The break point at around −2°C was accounted for by the phase change of water in fish meat. The process of freezing is accompanied by a gradual increase in the concentration of all soluble materials in the residual liquid phase, which is considered as the freeze concentration.[14,15] From our results, there was a lack of data on kinetic parameters between temperatures of −3°C and −10°C. However, Miki and Nishimoto[4] reported the k_f change of some fishes at three different temperatures of −3°C, −5°C and −10°C gave a linear line on the Arrhenius plot. Therefore, it would be reasonable to consider that a similar trend can be also observed between −3°C to −10°C in the tuna fish meat. It can be assumed that for our results, the plot of k_f versus $1/T$ in the temperature range of 20°C to −10°C would have a break point at around −3°C, whereby this temperature is close to the freezing point of −5°C as determined by the DSC experiment.

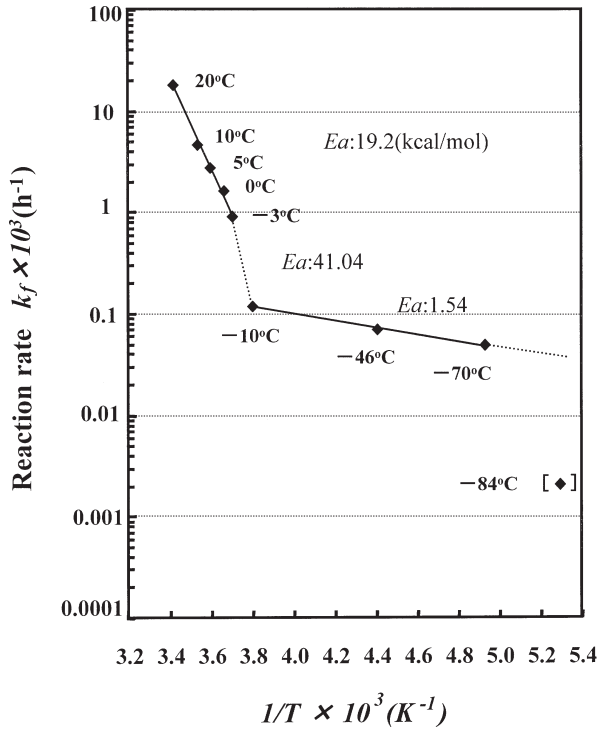

Fig. 4 Effect of temperature on reaction rate of *K* value change of yellowfin tuna *Thunnus albacares* meat.

Furthermore, as pointed out by Miki and Nishimoto[4] that the Arrhenius plot of *K* value change of some fish meat gave one more break point at –10°C, tuna meat could also give a similar result. Below –10°C, the *K′* value change in the temperature range down to –70°C was detectable, even though it took a longer period of storage as shown in Fig. 2b. However, since the *K′* value change for tuna meat was quite small, the difference of rate constants k_f at –10°C, –46°C and –70°C seemed to be within deviation, so the activation energy obtained from the Arrhenius plot might be inaccurate. Considering these factors, we attempted to determine the activation energy to give tentative criteria and these values are listed in Table 2. Here, it should be noted that the k_f at –84°C was quite different from those above –70°C as shown in Fig. 4. So k_f at –84°C was excluded for calculation of the activation energy.

The activation energy values obtained were comparable to those of other fishes reported hitherto.[4,13] As for the temperature from –10°C to –70°C, the rate constant k_f had some substantial values even if they were small. Although we could not obtain any physicochemical information on water or other substances from the DSC analysis, the following information was already available. According to an NMR experiment conducted by Nagashima and Suzuki,[16] when beef was frozen all free water present was not frozen at a temperature of –2°C, and after the temperature reached

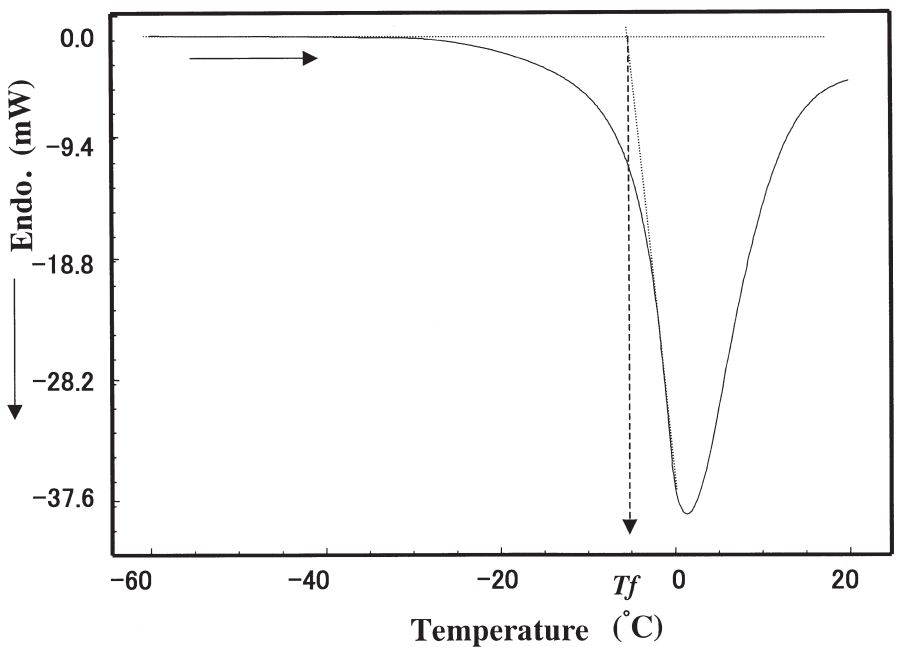

Fig. 5 Temperature and heat flow of yellowfin tuna *Thunnus albacares* meat obtained by DSC analysis.

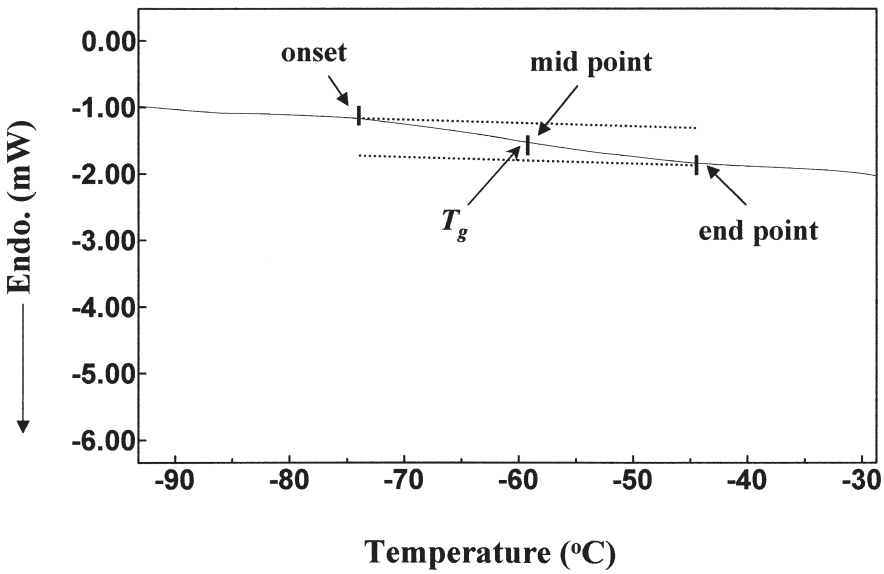

Fig. 6 DSC traces at very low temperature of yellowfin tuna *Thunnus albacares* meat.

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 reference 13.
[b] From reference 4.
[c] Observed value from study.

to –20°C almost 90% of free water and bound water weakly bound to protein molecules were frozen. But even below –20°C, nearly 10% of bound water strongly bounded to protein molecules could not be frozen and had mobility to some extent. This phenomenon can be also observed in fish. If so, enzymes may still have mobility and maintain their activity in the frozen state up to –70°C. Therefore, our finding of the enzymatic reaction progress even at –46°C and –70°C is reasonable. 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 could not be kept. However, storage at temperatures below –80°C resulted in permanent preservation of its activity.[17] This means that biological activity such as an enzymatic reaction remains at –80°C and stops below –80°C. According to our result, K' value change also stopped at –84°C. This extraordinary behavior of the rate constant around –80°C, which was first recognized in this experiment, can be explained further from the standpoint of glass transition.

Recently, there have been some studies related to the glass transition temperature of fish muscle. For example, mackerel and cod[15] have T_g of -13.3 ± 0.5°C and -11.7 ± 0.6°C, respectively. However, for cod, other reported values of T_g are –77°C,[18] –40°C[19] and –15°C.[20] For tuna, reported values are –68°C to –71°C[21] and –11.5°C to –18°C.[20] The reason for the differences in T_g reported for some fishes or same species remains unclear. It may be due to the difference in procedure of analysis and, moreover, complex products such as plant or animal tissues have been known to exhibit more than one T_g.[15] Even now, the T_g values for meat products are not fixed; however, there is a reliable report that some

pure native proteins show a glass transition temperature of ~–70°C.[22] The glass transition temperature (T_g) obtained for yellowfin tuna meat in this study was estimated to be –63°C. This value seems to be reasonable compared to that of native proteins. However, the range of glass transition temperature was quite wide, from –47°C to –75°C. In this temperature range, the phase state is gradually changing, and hence it can be said that at temperatures lower than –75°C, yellowfin tuna meat was in the perfect glassy state. This result clearly indicates that below temperatures of –75°C, there was a metastable and unreactive solid state formed within the unfrozen phase in the sample. Therefore, we can conclude 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 meat. Consequently, the glassy state formation will considerably decrease the rate of molecular motion in the meat, and results in a considerable decrease of ATP degradation in the sample fish meat which renders the sample stable for a much longer period. This finding may be applicable for determining the appropriate temperature to stop discoloration and to retard other related reactions in frozen meat.

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