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EVALUATION OF FISH FRESHNESS DETERIORATION OF YELLOWFIN TUNA *(Thunnus albacares)* BASED ON KINETIC STUDY

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The rate of fish freshness deterioration in yellowfin tuna (*Thunnus albacares*) during storage was investigated from K value based on nucleotide degradation. The study for low temperature storage had been carried out up to temperature of –40°C, however there is no evidence how fish freshness deterioration rate will be, if the temperature is lowered below the storage temperature used especially for tuna (-60 °C). This study was conducted to evaluate the freshness deterioration rate (k_f) during storage at different temperatures, from 20°C to–84°C. The value of K'= (100-K value) was used in this study and its plot against storage time yielded a straight line, which indicated a first-order reaction.

The temperature-dependence of fish freshness change was analyzed by Arrhenius theory. The first break point of reaction rate occurred at the freezing point. The second breakpoint was at –10°C and the third break point was at –70°C, which considered due to the formation of glassy state in fish meat substance.

KEY WORDS: ATP-related compound, K value, freshness deterioration rate, kinetic parameter

Introduction

The evaluation of fish freshness based on nucleotide degradation has been used for over last 40 years.[1] The biochemical index of fish quality assessment based on nucleotide changes called K value was formerly introduced by Saito *et. al.* in 1959.[2] This value is expressed as a percentage of the amount of inosine (HxR) and hypoxanthine (Hx) to the total amount of adenosine 5-tri-,di-,mono-phosphate (ATP, ADP, AMP), inosine mono phosphate(IMP), HxR and Hx. K value is widely used in Japan as freshness index. The rate of its change differs between species of fish. This means that each species of fish have their own K value-changing rate. Therefore, K value can not be used as a universal measure of fish freshness[3,4] However, as far as the species is limited, this index is useful to estimate the fish freshness during autolysis before the initial bacterial spoilage begins.[5]

Many methods, for example ion exchange chromatography[6], HPLC[3,7], enzymatic assay and biosensor,[8] have been used for quantitative determination of ATP and its breakdown products. High performance liquid chromatography (HPLC) method was considered as the most reliable and appropriate method for analysis of K value[7] judging from its speed of analysis, resolution, sensitivity and accuracy.[1,3]

The analytical approaches for calculating and predicting food quality deterioration involve a kinetic model in which it is based on the deterioration process rate.[9] Instead of K value, the value of K'(=100-K value), which represent the ratio of remaining amount of ATP, ADP, AMP and IMP to the total amount of ATP related compounds as used by Miki and Nishimoto[10] is used in the study. The apparent total process rate can be actually expressed by the following first order reaction equation experimentally (see equation 1) even though its process would be so complicate.

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

where a is constant, k_f is reaction rate constant, t is time of storage. The reaction rate k can be correlated with environmental factors and composition factors.[11] Temperature of storage is one of the main environmental factors, which has a major impact and influence on quality loss rate.[12] The most common and generally valid assumption is that temperature-dependence of the deterioration rate will follow Arrhenius equation[11], that is:

$$k_f = k_o \bullet \exp(-E_a/RT), \qquad \text{(Eq.2)}$$

where k_f is the apparent reaction rate constant of fish freshness deterioration, k_o is frequency factor, E_a is energy of activation, R is gas constant (1.986 cal•g•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.[13]

There are few studies dealing with kinetic of fish freshness deterioration. Miki and Nishimoto[10] had conducted on such kinetic studies by using skipjack, mackerel and red sea bream with storage temperatures ranging from 20°C to –40 °C. But there is still lack of data for physicochemical properties of tuna stored

at very low temperature, especially at temperature lower than –60°C, that is the commercial temperature normally used for tuna. Therefore, this study was conducted to observe the change of chemical and physicochemical parameters of yellowfin tuna meat as affected by storage temperature.

The specific objects of this study were (1) to investigate the rate of freshness deterioration in yellowfin tuna meat as affected by storage temperature using biochemical index K value, (2) to determine kinetic parameters based on temperature dependence of the reaction.

Material and Method

Fish Sample

Yellowfin tuna (*Thunnus albacares*) was purchased at a local fish retailer as a fillet (dorsal ordinary muscle). These fillets were cut into cubes (ca. 1.5x1.5x1 cm) and then wrapped individually with polyethylene bag prior to storage. All preparation was conducted at low temperature of 5°C.

Storage Condition

The samples were stored at temperature of -84°C, -70°C, -46°C (in freezers), -10°C, -3°C, 0°C, 5°C, 10°C and 20°C (in incubator Eyela LTI-600SD.1000SD) with precision of ±0.1°C. From each storage temperature treatment, the samples were taken out in duplicate at different interval time for analysis (K value and pH value).

K Value

K value was determined by high performance liquid chromatography based on modified method of Ryder.[7] One gram of muscle tissue of fish was homogenized with 8 ml of chilled 10% and 5% perchloric acid. The homogenate was centrifuged at 2,000g for 10 minutes at 5°C and supernatant immediately neutralized to pH 6.8 with 1N and 10N KOH. The neutralized mixture was centrifuged again at 2,000g for another 10 minutes and the supernatant was diluted to 20 ml with neutralized perchloric acid and then filtrated prior to storage at –46°C for subsequent analysis.

Separation of ATP-related compound was achieved on a reverse-phase column Asahipak GS-320 HQ. The mobile phase of 200 mM Sodium dihydrogenphosphate dihydrate (pH 2.8) was used at a flow rate 1ml/minute and temperature 30°C. The eluant was monitored at 258 nm for each ATP-related compound. The concentration of each compound was determined based on its peak height.

pH Value

pH value was analyzed by Horiba M-8 pH meter at the same interval time as K value measurement.

Thermal Analysis

The glass transition temperature of fish meat was measured by DSC analysis. Sample fish was weighed into aluminum DSC pan (10-20 mg), hermetically sealed and then loaded onto the Shimadzu DSC-50 instrument at room temperature. Sample was then cooled at the rate of 10°C /min to –130°C and heated it up at the same rate to 40°C. The obtained DSC curve was then analyzed by using software of Shimadzu TA60. In this thermal analysis, fresh sample was used.

Result and Discussion

Rate of Fish Freshness Deterioration

The value of K'=(100-K value), which represents the ratio of remaining amount of ATP, ADP, AMP and IMP to the total amount of ATP-related compounds as used by Miki and Nishimoto,[10] was used in this study. From the results, it is shown that the value tends to decrease with a prolongation in storage time for all temperature treatments. The plot of logarithm of K' versus storage time yields a straight line as shown in Fig. 1.This finding clearly indicates a first order reaction, which agrees well with the result reported hitherto.[10] The apparent reaction rate constant k_f was calculated from the slope of straight lines by linear least square method. From the result, it can be seen that the higher the storage temperature is, the faster the reaction rate becomes. This means that the higher storage temperature is, the faster the fish will deteriorate. For example, in order to reach the K value of 30%, sample stored at 5°C, 0°C and –3°C required different storage time for 3.85 days, 7.2 days and 15.5 days, respectively.

The changes of the pH value of yellowfin tuna flesh during storage below ice storage temperature were very little recognized, but a significant change occurred at above ice storage temperature (see Fig. 2). These increasing in pH were mainly due to microbiological activity.

The Effect of Temperature on the Rate of Quality Changes

The relation of rate constant k_f against temperature is performed on Fig. 3. From this figure, it appears that the kinetics of freshness deterioration, calculated by Arrhenius equation, changed at temperature close to the freezing point (–2°C), at –10°C and at about –70°C, respectively.

This first change in kinetic took place at temperature close to freezing point, may be happened due to the change of water phase in yellowfin tuna muscle during freezing (freeze concentration effect). Theoretically the process of freezing is accompanied by a gradual increase in the concentration of all soluble material in the residual liquid phase which is considered as freeze concentration.[14,15]

Based on the Arrhenius theory, kinetic parameters of

activation energy (E_a) and frequency factor (k_o) are determined and these values are shown in the Table 1. The activation energy increased sharply at the freezing point and the value obtained was comparable to that of mackerel and red sea bream determined by Miki and Nishimoto.[10] By decreasing temperature below –10°C, there was a little change in the rate of deterioration. The reason why this event happened was not conclusively determined yet, but this may probably be related to the presence of unfrozen water in the sample fish. According to Nagashima and Suzuki,[14] when beef was frozen at temperature of -2°C, all free water present was frozen and after reached temperature of -20°C, nearly 90% of free water and bound water that has weak bounding to the protein was frozen but nearly 10% of bound water with strong bounding could not be frozen. This phenomenon may have the similar impact on fish. Therefore, decreasing temperature below -10°C during freezing has a little effect on kinetic of fish freshness deterioration.

Table 1. Kinetic parameters of fish freshness deterioration of yellowfin tuna (*Thunnus albacares*) during storage.

Temperature range (°C)	E_a (Kcal/mol)	$k_o(h^{-1})$
> –2	19.3	$2X10^{15}$
–2 to –10	38.5	$5 X10^{30}$
–10 to –70	1.6	$1 X10^{62}$
–70 to –84	16.8	$3 X10^{16}$

However, further decrease in temperature up to –70°C, another change in the rate of deterioration was observed and this was considered due to formation of glassy state in the sample.

Glass Transition Effect

The change in kinetic of reaction rate k_f at –70°C of yellowfin tuna was considered due to the glass transition effect. As we fairly known that ATP degradation is an enzymatic-catalyzed reaction and is considered as diffusion-limited event.[17] During freezing, many reaction controlled diffusion can occur within an unfrozen phase even at very low temperature.[16] In order to confirm our result, experiment on glass transition temperature of yellowfin tuna was carried out by using DCS. The DSC curve of tuna meat at very low temperature indicated a clear base line shift to endothermic. This shift is considered as typical glass transition phenomena. From mid point of the shift, it is confirmed that glass transition temperature of yellowfin tuna meat is –63°C (see Fig. 4). The onset and endpoint were observed to be –75°C and –43°C, respectively. This value was comparable to the T_g of big eye tuna determined by Inoue and Ishikawa[18] which was –68°C to –71°C. This finding clearly indicated that in this temperature range, there was an amorphous, metastable and unreactive solid state formed within unfrozen phase in the sample. Glass transition will be tremendously decrease the rate of the molecular motion and considerably decrease the degradation of ATP in the sample fish and rendered the sample stable for much longer period.

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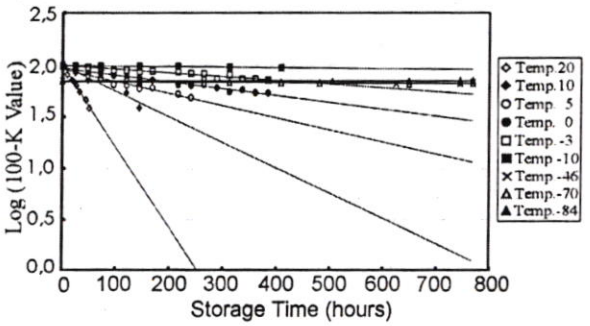

Fig. 1. The changes of fish freshness deterioration of yellowfin tuna (*Thunnus albacares)* during storage at different temperatures.

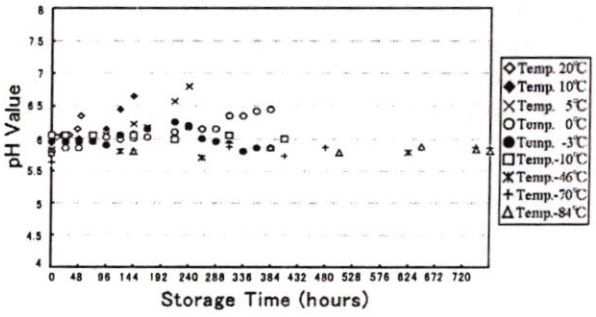

Fig. 2. The pH changes of yellowfin tuna (*Thunnus albacares*) muscle during storage at different temperatures.

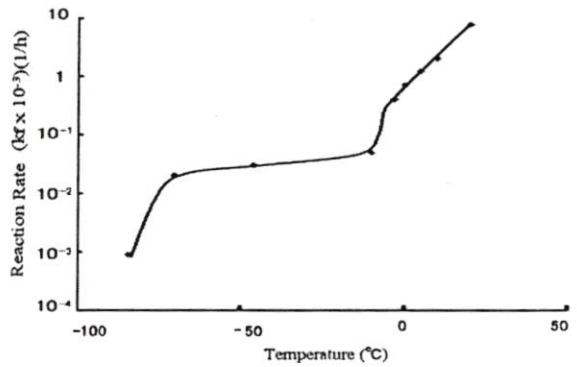

Fig. 3 The relationship between reaction rate of freshness deterioration and temperature of yellowfin tuna (*Thunnus albacares)* muscle.

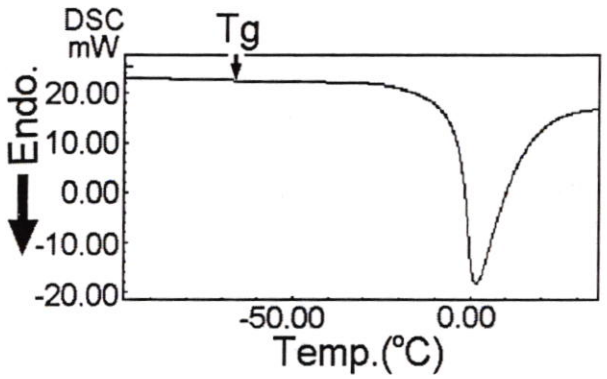

Fig. 4 The DSC traces of yellowfin tuna (*Thunnus albacares*) muscle.