

Analysis of Metmyoglobin Formation Rates in Frozen Tuna Meat during Frozen Storage

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Summary

Formation of metmyoglobin (metMb) in frozen tuna meat stored at -90, -60, -40, -30, -20, and -10°C for approximately 6 months was investigated. The reaction rate of metMb formation was estimated from a linear plot of $\ln([M_\infty - M_t] / [M_\infty - M_0])$ and storage time (t) for each storage temperature (T_s) (M_∞ , M_t , and M_0 are metMb contents at times $t = t_\infty$, t , and 0, respectively). When M_∞ was assumed to be 100%, the rate of metMb formation followed the first-order reaction only during the early stage of storage period. MetMb formation, however obeyed the first-order reaction for all test temperatures even during long-term storage when M_∞ was assumed to be dependent on storage temperature ($M_\infty(T_s)$). A discontinuity was observed in the temperature dependence of $M_\infty(T_s)$ at storage temperature range between -60 and -40°C, which was attributed to the glass transition of protein system. On the other hand, the temperature dependence of metMb formation did not show a significant change over all storage temperatures.

Key words : Reaction rate, Metmyoglobin formation, Glass transition, Tuna, Frozen storage

1. Introduction

The discoloration of tuna meat from a desirable pink to an undesirable brown color results from oxidation of ferrous myoglobin (Mb) derivatives to ferric metmyoglobin (metMb)^{1,2)}. Occurrence of such phenomena is affected by various factors, such as temperature, pH, oxygen consumption, and ionic strength³⁾. The fish industry sector, to a large extent, has relied on the utilization of prolonged low-temperature storage method to preserve the desired meat color. MetMb formation in frozen fish meat has been shown by previous studies to proceed despite frozen storage at -18°C, which is the

temperature commonly used for storage of frozen foods^{4,8)}. However, storage of meat at $\leq -40^\circ\text{C}$, was found to significantly inhibit metMb formation^{4,6,8)}. Notwithstanding the number of researches which have previously investigated the effects of storage temperature on metMb formation, only few studies were conducted based on the reaction rate. Analysis of reaction rate is important to simulate and predict the effect of temperature on the quality changes of fish meat during manufacture, transportation, and storage.

In a study conducted by Matsuura *et al.*⁹⁾ on metMb formation rate in fish meat, it was reported that the autoxidation of Mb isolated from two

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species of tuna and skipjack followed the first-order reaction in an ambient temperature range of 20 to 40°C. However, no remarkable difference on the resulting rate constants was observed among species. A similar study was done by Miki and Nishimoto⁷⁾ in which the rate of metMb formation in skipjack muscle kept at the storage temperatures ranging from 20 to -40°C for 2 weeks was investigated. Similarly to the study of Matsuura et al., the rate of metMb formation was found to be a first-order reaction, and its rate was temperature-dependent at all temperatures. Considering that both of these studies were conducted for short storage period and at temperature range higher than typical keeping temperature for tuna meat (< -40°C) during storage and transportation to local markets¹⁰⁻¹¹, these findings may not be sufficiently applied to actual fisheries industry. Furthermore, with the recent rise in the demand for high-quality fresh and frozen tuna in the world market¹²⁾, greater understanding of fish meat quality as affected by changes in temperature during fish meat handling and long-term storage is very important.

This study was conducted to investigate the rate of metMb formation in tuna meat stored at -90, -60, -40, -30, -20, and -10°C for approximately 6 months, and to understand the rate process relating to the physico-chemical state of fish meat during low-temperature storage.

2. Materials and methods

2.1 Sample preparation

Fresh bluefin tuna (*Thunnus thynnus*) was purchased from a local fish retailer as raw fillet. Since the tuna fillet was obtained from a single portion of ordinary dorsal muscle, the effect of initial freshness on metMb formation rate was assumed to be negligible. Tuna fillet was cut into pieces having dimensions of 1x4x6 cm, and then packed individually in polyethylene bags (75 µm) prior to storage. Tuna meat samples were directly stored at -90, -60, -40, -30, -20, and -10°C (±1°C) for approximately 6 months without pre-freezing.

Analyses of the frozen samples kept at each storage temperature were done in duplicate at designated sampling times.

2.2 Determination of metmyoglobin content

Analysis of metMb content was performed using the modified method of Bito¹⁾. Frozen tuna muscle (6g) was minced immediately after sampling and then mixed with 20 ml of cold distilled water (4°C). The tuna meat slurry was centrifuged at 10000 rpm for 5 min at 4°C, using a RS-18GL centrifuge (Tomy Seiko Co., Ltd, Tokyo, Japan). The pH of supernatant was adjusted to 6.8 - 7.0 with 1N NaOH, and subsequently centrifuged at 15000 rpm for 10 min at 4°C. The supernatant was filtered with 0.3 µm porous filters (Nihon Millipore Kogyo K.K., Yonezawa, Japan). 0.2M phosphate buffer (pH 7.0) was then added to the filtered supernatant at a ratio of 1:1 (v/v). The absorbance was read at 503 and 540 nm using a U-1100 spectrophotometer (Hitachi, Japan). Metmyoglobin content (metMb%) was calculated from the relationship between the relative concentration of metmyoglobin to total myoglobin, and the ratio of the absorbance at 540 to 503 nm, which was reported by Bito¹⁾.

3. Results and discussion

3.1 Changes in metmyoglobin content

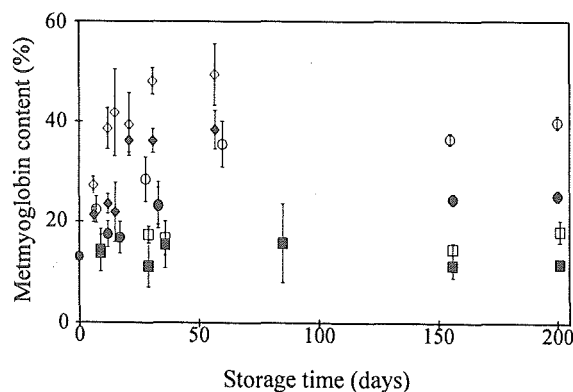


Fig.1 Changes in metmyoglobin content of bluefin tuna meat during storage at -10°C (◇), -20°C (◆), -30°C (○), -40°C (●), -60°C (□), and -90°C (■).

Figure 1 illustrates changes in metMb content of

bluefin tuna meat during frozen-storage at different temperatures for approximately 6 months. A sharp increase in the metMb content was observed in tuna meat stored at -20 and -10°C , while the rate of the metMb formation was relatively slower when meat was stored at -30°C for prolonged storage time. In comparison, the metMb formation in tuna meats stored at $\leq -40^{\circ}\text{C}$ exhibited only a slight increase in metMb values during the initial storage time, and then remained constant until the end of the storage period. The results obtained in this study are quite consistent with the findings of similar studies previously conducted⁴⁻⁸.

The increase in metMb content in tuna meats corresponding to storage time was attributed to greater degree of Mb oxidation^{1,13}. Results of this study, therefore, indicate that Mb could undergo oxidation even at low temperature storage, and stability of Mb is improved when tuna meat is stored at $\leq -40^{\circ}\text{C}$.

3.2 Rate analysis of metmyoglobin formation

Based on previous studies, autoxidation of Mb obeys a first-order reaction^{7,9}, with the rate process expressed in equation:

$$-d[\text{Mb}]/dt = k[\text{Mb}] \quad (1)$$

Practically, Mb autoxidation in fish meat is easily determined by the analysis of metMb formation rather than the depletion of Mb. Therefore, the process of Mb autoxidation in fish meat presumes to show a straight line in a plot of $\ln([M_{\infty} - M_t]/[M_{\infty} - M_0])$ and storage time (t) at a constant storage temperature (T_s), where M_{∞} , M_t , and M_0 are metMb contents at times $t = t_{\infty}$, t , and 0 , respectively. From the slope of the straight line, the first-order rate constant (k) was determined.

Previous studies on metMb formation typically assumed M_{∞} to be 100%^{7,9}. Based on these studies, results presented in Fig. 1 were expressed in $\ln([100 - M_t]/[100 - M_0])$ form and were plotted against storage time as shown in Fig. 2. Results showed that linearity was observed only in the initial period of storage. At prolonged storage time, however, large deviations in the value of $\ln([100 - M_t]/[100 -$

$M_0])$ was observed for all test storage temperatures. The observed distinct linearity of metMb formation during the initial storage period (Fig. 2), is comparable with the results obtained by Miki and Nishimoto⁷. Similarly, they reported that the plot of $\log(100 - M_t)$ against storage time yielded straight lines when the storage time was over 14-day period in range of -40 and 20°C .

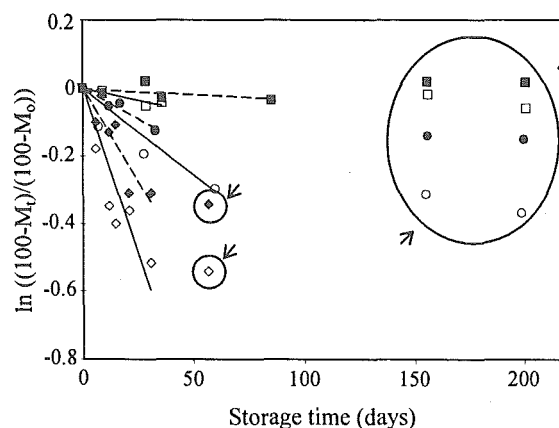


Fig. 2 Changes in metmyoglobin content of bluefin tuna meat during storage at different temperatures when M_{∞} was 100. \diamond — \diamond , -10°C ; \blacklozenge — \blacklozenge , -20°C ; \circ — \circ , -30°C ; \bullet — \bullet , -40°C ; \square — \square , -60°C ; and \blacksquare — \blacksquare , -90°C . The arrows show the metMb formation did not follow the first-order reaction when storage time was prolonged.

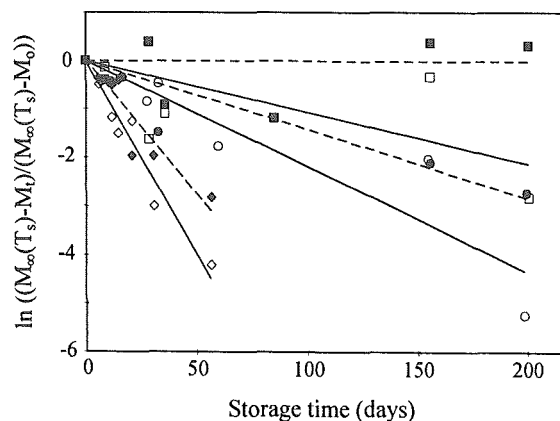


Fig. 3 Changes in metmyoglobin content of bluefin tuna meat during storage at different temperatures when metMb is assumed to be a function of storage temperature $M_{\infty}(T_s)$. \diamond — \diamond , -10°C ; \blacklozenge — \blacklozenge , -20°C ; \circ — \circ , -30°C ; \bullet — \bullet , -40°C ; \square — \square , -60°C ; and \blacksquare — \blacksquare , -90°C .

In this study, it was found that assumption of $M_{\infty} = 100\%$ is only valid for short-term storage period. With prolonged frozen storage, metMb formation was negligible and seemed to remain constant for storage time longer than 30 days (Fig. 2). From the results, we therefore proposed an alternative assumption in which M_{∞} is dependent on storage temperature (T_s), expressing M_{∞} as function of T_s ($M_{\infty}(T_s)$).

$M_{\infty}(T_s)$ was estimated as to be an adjustable parameter from the best linear fit of the plot between $\ln([M_{\infty}(T_s) - M_t] / [M_{\infty}(T_s) - M_0])$ and storage time for each T_s . Fig. 3 presents the plot of $\ln([M_{\infty}(T_s) - M_t] / [M_{\infty}(T_s) - M_0])$ against storage time. The plot exhibited linearity for all storage temperatures, and the reaction rate constant (k) was calculated from the slope of each straight line. The observed good linear fit supports our assumption that $M_{\infty}(T_s)$ is indeed a function of T_s . $M_{\infty}(T_s)$ and corresponding k values for each T_s are shown in Table 1.

Table 1 MetMb content at infinite time ($M_{\infty}(T_s)$) and reaction rate constant (k) of metMb formation in bluefin tuna meat stored at different temperatures.

Storage temperature (°C)	$M_{\infty}(T_s)$ (%)	k (1/days)
-10	50	0.079
-20	40	0.0549
-30	40	0.0218
-40	26	0.0142
-60	18.5	0.0107
-90	17	0.0001

Fig. 4 comparatively illustrates the fitting curves assuming $M_{\infty} = 100\%$ (Fig. 4A) and $M_{\infty}(T_s)$ as a function of T_s (Fig. 4B). These figures further validates the assumption that $M_{\infty}(T_s)$ in tuna meat during frozen storage is a function of storage temperature (T_s).

In this study, previously reported metMb formation data were likewise analyzed utilizing rate process based on both assumptions: $M_{\infty} = 100\%$ and $M_{\infty}(T_s)$ as a function of T_s . MetMb formation data reported by Suzuki *et al.*⁵⁾ and Miki and Nishimoto⁷⁾

followed the first-order reaction when $M_{\infty} = 100\%$ was assumed. On the other hand, using data reported by Bito⁴⁾, Hashimoto and Watabe⁶⁾, and Suzuki *et al.*⁵⁾, a good fit of the metMb formation resulted with the assumption that $M_{\infty}(T_s)$ is a function of T_s . However, the rate of metMb formation did not obey the first-order reaction for either of the two assumptions when data reported by Chow⁸⁾ was utilized.

According to the literature and our results, we proposed the assumption that $M_{\infty}(T_s)$ should depend on T_s for evaluation of the rate process of metMb formation in frozen tuna meat during long storage.

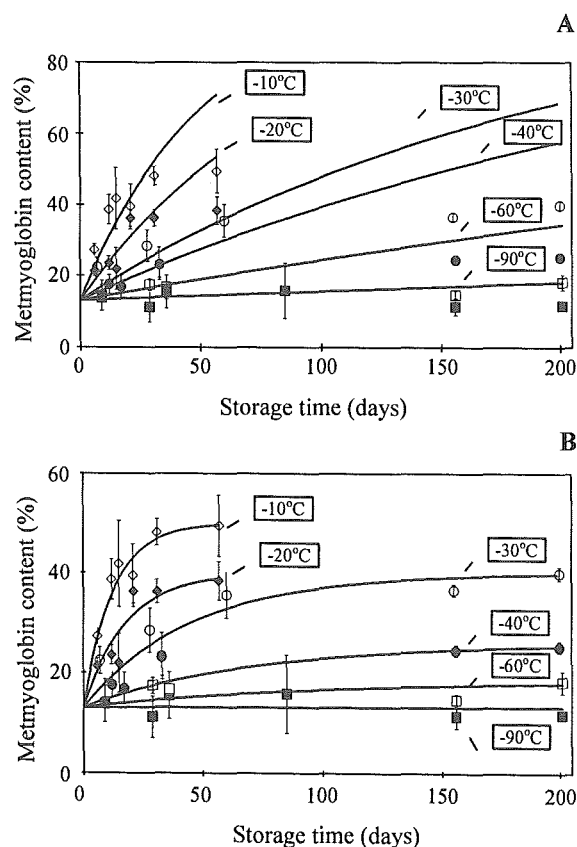


Fig. 4 Fitting curves of changes in metmyoglobin content of bluefin tuna meat during storage at -10°C (\diamond), -20°C (\blacklozenge), -30°C (\circ), -40°C (\bullet), -60°C (\square), and -90°C (\blacksquare) assuming $M_{\infty} = 100\%$ (A) and $M_{\infty}(T_s)$ as a function of T_s (B).

3.3 Temperature dependence of $M_{\infty}(T_s)$

The temperature dependence of the estimated $M_{\infty}(T_s)$ is presented in Fig. 5. Results show that

$M_{\infty}(T_s)$ values drop with a decrease in storage temperature. Moreover, the plot of $M_{\infty}(T_s)$ against T_s exhibits two straight lines with different slopes depending on storage temperature range. Although the decrease of $M_{\infty}(T_s)$ is remarkable at temperature range of -40 and -10°C , the slope of $M_{\infty}(T_s)$ is relatively small at storage temperature $\leq -60^{\circ}\text{C}$. The apparent discontinuity in the temperature range of -40 and -60°C could be attributed to some physical and/or chemical changes occurring in fish meat during frozen storage.

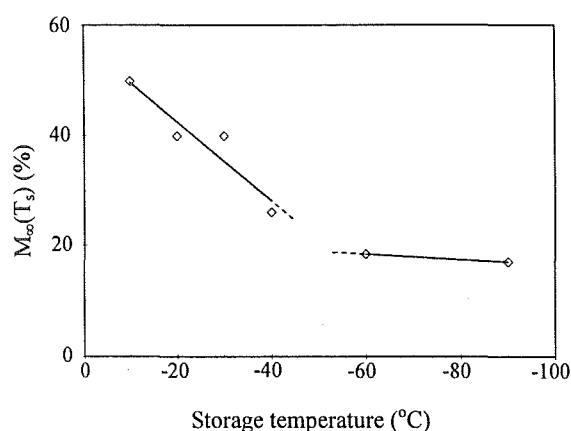


Fig. 5 Relationship between metMb formation, ($M_{\infty}(T_s)$) and storage temperature (T_s).

The observed discontinuity in the $M_{\infty}(T_s)$ plot with storage temperature could possibly be attributed to the changes occurring in tuna meat during glass transition stage. As temperature continuously decreases beyond the freezing point, ice formation occurs and the unfrozen solution gradually concentrates. When temperature is sufficiently low, this freeze-concentrated unfrozen solution becomes a solid glass at glass transition temperature, T_g ¹⁴. Theoretically, retardation of various reactions in food occurs at temperatures below T_g , hence food storage at temperatures lower than T_g could prolong its shelf-life. It has even been proposed that T_g may be a good indicator of food stability¹⁵⁻¹⁷. For tuna meat, T_g values have been reported to be in the range of -70 and -40°C ¹⁸⁻²¹, clearly coinciding with the temperature range in which the discontinuity of $M_{\infty}(T_s)$ was observed in

this study.

Frozen storage affects denaturation of Mb which in turn possibly influences the oxidation of Mb to metMb²²⁻²³. It was reported that the denaturation rate of protein such as Mb²⁴ and myofibrillar protein²⁵ decrease correspondingly with decrease in storage temperature. Moreover, the denaturation rate of protein was significantly retarded below -40°C ²⁵. There is a coincidence between temperature retarding protein denaturation ($< -40^{\circ}\text{C}$) and the temperature range in which the discontinuity of $M_{\infty}(T_s)$ observed in this study. The proposed dependence of the observed discontinuity in the $M_{\infty}(T_s)$ plot (Fig. 5) could therefore be more specifically attributed to glass transition of protein system of tuna meat particularly to the freeze denaturation of proteins. In addition, it has been reported that the freshness-lowering rate were likewise retarded considerably when fish meat is stored below -40°C ¹⁹. Long-term storage of raw tuna meat at -40°C could therefore be sufficient for the preservation of tuna meat.

3.4 Temperature dependence of rate constant in metmyoglobin formation

In this study, the temperature dependence of rate constant (k) in metMb formation at a given temperature was estimated using the well-known Arrhenius equation:

$$k = A \exp(-E_a/RT) \quad (2)$$

where k —metMb formation rate constant; E_a —the activation energy (J/mol); A —frequency factor; T —absolute temperature (K), and R —gas constant (8.31 J/K/mol). Apparent activation energy and frequency factor were determined from the slope and intercept of a straight line plot of $\ln k$ versus $1/T$, respectively.

Fig. 6 shows a plot of $\ln k$ against $1/T_s$ when $M_{\infty}(T_s)$ was assumed to be a function of T_s . The values of apparent activation energy (E_a) and frequency factor (A) were 3.18×10^4 (J/mol) and 2.01×10^5 (1/day), respectively. The plot gave a straight line for all storage temperature. This suggests that the reaction mechanism of metMb

formation did not change with storage temperature (-90 to -10°C) despite the discontinuity in the temperature dependence of $M_{\infty}(T_s)$ at storage temperature range of -60 and -40°C (Fig. 5) was observed. The apparent contradiction in the obtained results could not be explained at present and would therefore require further study. Nevertheless, the temperature dependence of metMb formation rate was confirmed.

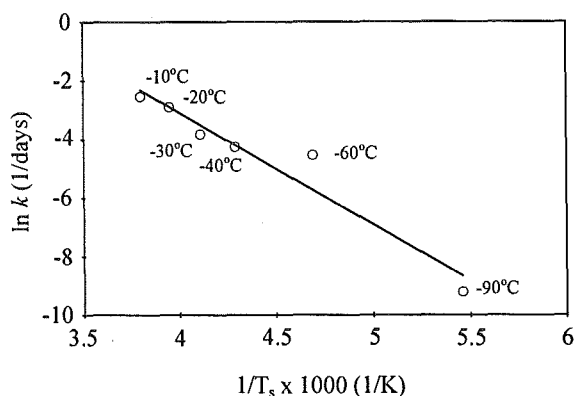


Fig. 6 Effect of temperature on reaction rate of metmyoglobin formation of bluefin tuna meat.

4. Conclusion

This paper presents the results of the study conducted on metMb formation rate in tuna meat during long-term frozen storage at -90 to -10°C. Estimation of the metMb formation rate based on the assumption that M_{∞} is 100% was only valid for short-term frozen storage. On the other hand, metMb formation during long-term storage followed the first-order reaction when $M_{\infty}(T_s)$ was assumed to depend on T_s . A discontinuity in the temperature dependence of $M_{\infty}(T_s)$ was observed between -60 and -40°C storage temperature, which could be attributed to the glass transition of protein system. However, no change in the rate mechanism in metMb formation was observed for all storage temperatures when the dependence of the rate constant of metMb formation with temperature was analyzed. The rate process data generated by this study is deemed important to understand and predict

the quality changes in tuna meat during frozen storage.

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