博士学位論文

Rate Process of Metmyoglobin Formation in Frozen Tuna Meat and Thermo Physical Property in Low Temperature

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博士学位論文内容要旨

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論文題目　：**RATE PROCESS OF METMYOGLOBIN FORMATION IN FROZEN TUNA MEAT AND THERMO PHYSICAL PROPERTY IN LOW TEMPERATURE**

ABSTRACT

Color of tuna meat strongly influences on consumer's purchase decision. To prolong the color shelf life of fresh tuna, tuna is always kept in frozen state immediately after harvest until transportation to local market. Effects of frozen storage temperature on color stability in tuna meat were intensively studied; however, the mechanism of discoloration is not completely understood. In addition, the effects of biochemical factors such as pH have been well researched. Nevertheless, physicochemical factors, such as phase transitions or the mobility of molecules in tuna meat during frozen storage, have received far less research attention and understanding of these presents the greatest potential for prolonging color stability in fish meat during frozen storage. The objective of this study is to investigate mechanism of discoloration in tuna meat during frozen storage in physicochemical aspect.

Chemistry behind the color and color changes of fresh meat and meat during storage was reviewed in Chapter 1. In Chapter 2, the physical state changes in tuna meat during holding at different storage temperatures were studied with differential scanning calorimetry (DSC). Two apparent transition temperatures were observed in frozen tuna. One was −71°C, which was suggested to be the glass transition temperature of water molecules in the vicinity of protein. The other lay in the range between −40 to −30°C, which is probably caused by whether the glassification of protein backbone or the beginning of ice melting. Our results were in a good agreement with the previously published glass transition temperatures for fish flesh. According to literatures, some studies have reported glass transition temperatures of around −70°C while over 70% of all published glass transition temperatures for fish flesh were higher than –40°C. These observed transition temperatures could be applied to explain the change of discoloration rate during frozen storage.

The rate of metmyoglobin (metMb) formation in tuna meat during long-term frozen storage at −90 to −10°C was investigated in Chapter 3. 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). The results revealed that 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 ($M_\infty(T_s)$). Although, the temperature dependence of metMb formation rate did not show a significant change over all storage

temperatures, a discontinuity was observed in the temperature dependence of $M_\infty(T_s)$ at storage temperature range between −60 and −40°C, which was in according to the apparent transition temperatures in the range between −40 to −30°C observed in Chapter 2. These results implied that whether glass transition of protein backbone or the beginning of ice melting; in the other words, the mobility protein or water molecule, has strongly influences on the rate of metmyoglobin formation in frozen tuna meat.

In chapter 4, the temperature-dependence of mobility of iron atom in metMb molecule was investigated with electron paramagnetic resonance (EPR) spectroscopic. The results revealed that the similar EPR spectra for both metMb solution and tuna meat sample indicated that EPR has a potential in investigation of metMb in tuna meat. According to temperature dependence of EPR signal, below −90°C the iron atom still mobile while the globin protein and water in its vicinity were already frozen. In addition, the observed transition above −15°C is suggested to be relating to the melting of ice.

Base on the measurement in Chapter 4, EPR study in metMb have no required the light transmission of sample, and must be operating at very low temperature (<−90°C). Also, the individual information of metMb is easily obtained from the analysis of EPR spectra. Therefore, EPR measurement has a potential as a quantitative determination of metMb formation in frozen fish meat without pigment extraction and thawing prior color measurement. In chapter 5, the potential of EPR method was examined and compared with a conventional visible spectrophotometry. The tuna meat was directly subjected to EPR measurement at −150°C without pigment extraction. Its metMb concentration was evaluated from the calibration curve which was linearly plotted between I_S / I_R and the known metMb concentrations. I is the EPR intensities. S and R represent sample and reference, respectively. I_S and I_R are proportional to $w_S^2 h_S$ and $w_R^2 h_R$, respectively, where w and h are the half-width and height of signal, respectively. The results revealed that there was a good correlation between the metMb concentrations determined from EPR and those from a visible spectrophotometry (R^2 = 0.975). The metMb concentrations in tuna flesh determined from the EPR method using the calibration curve were 1.268 fold higher than those from the conventional method by visible spectrophotometry. These results suggest that EPR method is a suitable technique for quantitative measurement of metMb in tuna meat. Additionally, it will help in further investigating the color stability in a molecular level in tuna meat during frozen storage.

In conclusion, the color stability in tuna meat during frozen storage is strongly affected by whether the glass transition of freeze-concentrated solution containing various mineral compounds or the beginning of ice melting, which was observed in the range −40 to −25°C by DSC. An increased temperature higher than this temperature range accelerates the rate of metMb formation by increasing the mobility of low-molecular-weight solutes surrounding the myoglobin protein. On the other hand, below −70°C the soluble protein such as myoglobin and water molecules in its vicinity became vitrified while iron atom in metMb molecule still mobile. These mobilities of unfrozen water molecule or iron atom in heme group seem to be not relevant to the rate of pigment oxidation. Thus, long-term storage of raw tuna meat below −40°C could therefore be sufficient for prolonging the color shelf-life of fresh tuna meat.