Doctoral Dissertation

ELECTRON PARAMAGNETIC RESONANCE STUDY ON METMYOGLOBIN AND NONHEME IRON FORMATIONS IN FROZEN TUNA MEATS

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博士学位論文内容要旨

Abstract

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論文題目 Title	Electron Paramagnetic Resonance Study on Metmyoglobin and Nonheme Iron Formations in Frozen Tuna Meats		

In this study, a novel quantitative measurement of metmyoglobin (metMb) and nonheme iron formed in tuna meat during frozen preservation was investigated by means of Electron Paramagnetic Resonance (EPR).

Subzero temperature storage is an important and convenient method for preserving fresh fish for a long period of time. During handling and storage of fish, changes in biochemical, chemical and microbiological properties occur. Metal ions, e.g., ferrous ion (Fe^{2+}), act as a catalyst for those deteriorations. The metal ions are in two forms, that are heme and nonheme irons. The heme iron is an iron molecule binding with a porphyrin ring which is found in myoglobin (Mb) and hemoglobin. On the other hand, the nonheme iron is a free iron molecule, which does not bind with a porphyrin ring. An oxidation of Mb and its derivatives to metMb results in an undesirable color change of fish meat from red to brown during storage. Also, a formation of nonheme iron occurs. A measurement of color change in tuna meat during subzero temperature storage is hard to do because thawing of meat and pigment extraction are required, leading to the erroneous result.

Electron Paramagnetic Resonance (EPR) is a spectroscopic technique that allows direct and non-invasive detection of paramagnetic species consisting of one or more unpaired electrons in complex and non-transparent samples. These species include short living or stable free radicals and transition metal ions, such as Mn^{2+}, Fe^{3+} and Cu^{2+}. EPR spectra are characterized by the g-factor. The EPR spectra of metMb (Fe^{3+}) appear at g=6 and g=2, on the other hand, the spectrum of mononuclear nonheme iron (Fe^{3+}) appears at g=4.3.

The objective of this study was to investigate efficiency of non-destructive EPR method to evaluate nonheme iron content compared to other conventional

destructive spectrophotometric method. In addition, the mechanism of metMb formation and its degradation in high and low fat tuna meat was investigated. Finally, mathematical models of the kinetics reaction were used to predict the change of heme pigment formation.

In Chapter 2, nonheme iron content in tuna meat was examined using EPR compared with spectrophotometric method. The content of nonheme iron estimated by EPR was lower than that observed by spectophotometric method. This is because the EPR method detected only Fe^{3+}, but the spectophotometric method measured either Fe^{3+} or Fe^{2+}.

In Chapter 3, the metMb and nonheme iron in tuna meat stored at -10, -20, -30, -40, -60, and -90 °C were measured by EPR. The metMb content in tuna meat stored at -10 and -20 °C increased until reaching the maximum value, and then decreased. On the other hand, the metMb content slightly increased for tuna meat stored at -30 °C, and the value did not change for the samples stored at temperatures $\leq$ -40 °C. For nonheme iron, the content slightly increased in the sample stored at -20 and -30 °C after 4 months. Only the sample stored at -10 °C increased until reaching the maximum value and remained stable.

In Chapter 4, the metMb and nonheme iron in two kinds muscle (chu-toro and akami) of tuna meat stored at -5, -10, and -15 °C for 4 months were investigated. At the storage temperature above -15 °C, the metMb content in chu-toro initially increased until reaching the maximum and then gradually decreased with the prolongation of storage. However, at -15 °C the metMb content increased monotonically. Chu-toro nonheme iron gradually increased, except these stored at -15 °C where it increased slightly. The metMb content in akami stored at -5 and -10 °C initially increased until reaching a plateau. Only small amount of nonheme iron was detected in akami, which increased slightly irrespective of the storage temperature. The rate of metMb formation at the early period in chu-toro was higher than akami at each temperature.

In Chapter 5, the rates of metMb formation and degradation in chu-toro bigeye tuna meat stored at -5, -10, and -15 °C for 4 months were investigated. The reaction rate of metMb formation was estimated from the consecutive first order reaction at different storage temperature and time. The results revealed that the

mechanism of metMb formation and degradation at all given temperatures followed the consecutive first order reaction.

In conclusion, this study demonstrated that the application of EPR technique accurately detected metMb and nonheme iron contents in tuna meat. The formation of metMb and nonheme iron was different between chu-toro and akami samples. The formation and degradation of metMb and nonheme iron followed the consecutive first order reaction.