Japan Journal of Food Engineering, Vol. 11, No. 3, pp. 133 – 138, Sep. 2010

◇◇◇ Original Paper ◇◇◇

Electron Paramagnetic Resonance Study of Metmyoglobin and Nonheme Iron Formation in Frozen Tuna Meats

Pantira HEMPATTARASUWAN[1], Chotika VIRIYARATTANASAK[2], Shingo MATSUKAWA[1], Manabu WATANABE[1], and Toru SUZUKI[1,†]

[1]*Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Konan 4–5–7, Minato–ku, Tokyo 108–8477, Japan*
[2]*Department of Food Engineering, Faculty of Engineering, King Mongkut's University of Technology Thonburi, 126 Pracha u–tid Road, Tungkru, Bangkok 10140, Thailand*

Two types of bigeye tuna meats, fatty muscle (chu–toro) and lean muscle (akami), were stored at −5, −10, and −15℃ and the effects of temperature and fat content on metmyoglobin (metMb) and nonheme iron (Fe^{3+}) formation were determined using Electron Paramagnetic Resonance (EPR). For the sample meats, two high spin iron peaks on the EPR spectra were detected. The first peak appeared in the range of 90–130 mT and was identified as a signal of high–spin metMb, while the second peak, which appeared in the range of 140–170 mT, was determined to be a signal of high–spin nonheme iron. Peak intensity and half–width were used to determine metMb and nonheme iron content. The rates of metMb and nonheme iron formation in chu–toro and akami were slower as the storage temperature was lower. Chu–toro exhibited a quick initial increase in metMb, however, the metMb content turned decreasing after reaching a maximum, while nonheme iron increased monotonically. Meanwhile, for akami, the metMb exhibited a slower initial increase before reaching a maximum value, and remained constant during the storage period investigated, while the nonheme iron exhibited a small increase. These results suggest that once metMb is formed during frozen storage, iron ion (Fe^{3+}), i.e., nonheme iron, is subsequently released from the porphyrin rings of a proportion of the metMb. That is, a type of compensatory reaction is occurring. Therefore, metMb content during frozen storage would exhibit time course curves with peak values. Notably, in case of high fat tuna meat, this phenomenon might be of significance.

Key words: Metmyoglobin, Nonheme iron, Electron Paramagnetic Resonance, Frozen storage, Bigeye tuna

1. Introduction

Frozen storage is an important and convenient preservation method, effectively preserving fish and fish products for long periods of time. Tuna is usually frozen on board the fishing vessel and stored at frozen condition after landing. During handling and storage of fish, changes in biochemical, chemical and microbiological properties occur [1]. The rate of such chemical reactions in frozen storage slows down significantly and the growth of microorganisms completely stops at −18℃. However, it is well known that the discoloration of red tuna meat continues even at −18℃, which has been usually evaluated by the content of metMb that is autoxidized from myoglobin [2].

Bito [3] previously reported the continued metMb formation in tuna meat during frozen storage at approximately −20℃ for 6 months, while storage at −35℃ to −78℃ resulted in minimal discoloration. Furthermore, metMb formation in frozen tuna meat was considered to be independent of freezing rate [4]. Hashimoto and Watabe [5] also reported that a storage temperature of −40℃ prevented discoloration due to metMb formation. However, the mechanism of discoloration is still unclear due to the complexity of the state of iron ions related to myoglobin, despite the many proposed views. For example, Gómez–Basauri and Regenstein [6] pointed out that nonheme iron in cod and mackerel muscle increases while heme iron decreases during frozen storage. Schricker *et al.* [7] suggested that the increase in nonheme iron might be induced as a result of the release of iron from the heme iron complex by oxidation of the porphyrin ring.

(Received 28 May 2010: accepted 28 Jul. 2010)
† Fax:+ 81–3–5463–0585, E–mail: toru@kaiyodai.ac.jp

Another reason that the discoloration mechanism is still unclear is that analytical methods for assessing metMb and nonheme iron are immature. At the present time, a spectrophotometric method is widely used for determining the content of metMb and related compounds in meat extract. However, a pigment extraction procedure is required prior to measurement, which often introduces considerable error to these methods [8]. Furthermore, the method is inconvenient for concomitantly evaluating both nonheme iron and metMb. On the other hand, it should be noted that the Electron Paramagnetic Resonance (EPR) method is an effective spectroscopic method for analyzing metal ion components such as metMb and Fe^{3+} without any preparation procedure [9], since the EPR spectroscopy technique has been employed in the study of coordination structures of heme proteins a few decades ago. Svistunenko *et al.* [10] reported that the high-spin heme in metMb provides a large EPR signal at 90–130 mT (g=5.85: zero-line crossing) and a tiny signal at g=2. In comparison, it is generally known that nonheme iron in biological samples is in the mononuclear high spin Fe^{3+} ion form, which shows a large signal at approximately 140–170 mT (g=4.3) [11, 12]. However, EPR spectroscopy can not always detect all iron ions; e.g., signals are not detected from some Fe^{2+} complexes [13]. Therefore, only the ferric state (Fe^{3+}) of metMb and nonheme iron can be determined using EPR.

Recently, we reported that by using a large EPR signal in the range of 90–130 mT (g=5.8) representing the high-spin heme signal, the quantitative evaluation of metMb in frozen tuna meat is possible [9]. Here, EPR was used to study the effects of fat content and storage temperature on the formation of metMb and nonheme iron in frozen tuna meat.

2. Materials and Methods

2.1 Tuna samples and standard solutions

Two types of fresh bigeye (*Thunnus obesus*) tuna fillets were purchased from a supermarket as raw material without frozen. One tuna fillet was cut from fatty muscle (chu-toro), and the other was from lean muscle (akami). Total fat content was determined by the acid-hydrolysis method [14].

For the estimation of metMb content in tuna from EPR spectra, various concentrations of standard metMb solutions were prepared from Equine skeleton Mb (Sigma-Aldich Co., Tokyo, Japan) according our previous report [9]. Exact concentrations were assayed by spectrophotometer measurement prior to EPR measurement.

For the estimation of nonheme iron (Fe^{3+}), standard solutions (8.96 to 107.46 μM) were prepared from 10 to 60 μl of an iron standard solution (Wako Pure Chemical Industries Ltd., Osaka, Japan). Prior to EPR measurement, the sample solutions were diluted in 1:3 glycerol/water (v/v) in order to adjust the half-width of the nonheme iron EPR spectra of the standard iron solutions so that they were similar to those of tuna samples, according to method of Bou-Abdallah and Chasteen [12].

2.2 Storage condition and EPR spectrum measurement

A small amount of tuna meat was randomly sampled by piercing a plastic cylindrical pipe (approximately 3 mm internal diameter) into the fish fillet. The fish sample was then removed from the pipe and immediately frozen in liquid nitrogen. The randomly selected frozen tuna meat samples were packed into EPR tubes to a height of 3.7±0.1 cm (approximately 0.4 cm^3) and subsequently kept in liquid nitrogen prior to EPR measurement. The actual weight of the samples in each EPR tube was recorded. The samples, which was frozen in EPR tubes, were stored at −5, −10, and −15℃ after EPR measuring for zero day storage. At appropriate time duration, EPR measurements for these samples were conducted. EPR spectrum measurements were conducted according to a previously reported method [9].

For measurement of the standard solutions for metMb and nonheme iron, about 0.4 cm^3 of each standard solutions was introduced into the bottom of a quartz EPR sample tube (approximately 5 mm external diameter), and frozen slowly in liquid nitrogen in order prevent fracture of the EPR tube. The EPR tube containing the frozen solution was kept in liquid nitrogen.

EPR spectra were measured on an EPR spectrometer, JES-TE300 (JEOS Co., Tokyo, Japan) under the following conditions: microwave frequency, 9.11±0.1 GHz (X-band); microwave power, 1 mW; modulation width, 1 mT; spectral range, 60 − 360 mT; sweep time, 4 min; time constant, 0.1 sec; number of scans, 1; temperature, −150℃. A Mn^{2+} marker (JEOL Co., Tokyo, Japan) was used as a reference for the magnetic field and the peak intensity. EPR spectra for the samples and the Mn^{2+} marker were simultaneously recorded. Measurements were performed in triplicate.

2.3 Estimation of metMb and nonheme iron contents

From obtained EPR spectra, the ratio of intensities for characteristic peaks from the sample (e.g., metMb) and reference (Mn^{2+} marker) signals (I_S/I_R) was calculated using the following equation, according to a previous report [9]:

$$I_S/I_R = W_S^2 h_S / W_R^2 h_R$$

where W is the half-width and h is the height of the line for the characteristic signal for sample and marker signal, as shown in Fig. 1.

The metMb content was calculated from a calibration curve that plots the relationship between I_S/I_R obtained from EPR and metMb content obtained from the spectrophotometry method of the standard metMb solution. The calibration curve was linear with a correlation coefficient $R^2=0.98$. The nonheme iron content was also calculated from a calibration curve that plots the relationship between I_S/I_R obtained from EPR and nonheme iron content obtained from an iron standard solution. The calibration curve was linear with a correlation coefficient $R^2=0.98$.

3. Results and Discussion

The total fat content of bigeye tuna meat was measured by the acid hydrolysis method and was 7.4±1.2% (wet basis) for chu-toro and 0.8±0.2% for akami. Koriyama *et al.* [15] reported that fat content in bigeye tuna muscle chu-toro and akami are 6.6% and 0.5%, respectively. The fat content in our samples is consistent with bigeye tuna muscle categorized as chu-toro and akami.

Figures 2 and 3 show the changes in EPR spectra during storage at −10℃ for chu-toro and akami, respectively. The peaks from 300–350 mT in each EPR spectra are from the Mn^{2+} marker signal. The first peak, in range of 90–130 mT, in the EPR spectra of both samples, is the high-spin metMb signal, as described in the introduction. While, the second peak, in range of 140–170 mT, in the EPR spectra of both samples is thought to be the high-spin nonheme iron signal, according to the literature [11, 12].

As seen in Fig. 2, the intensity of the metMb peak for chu-toro increased until reaching a maximum, then decreased as the storage time increased until the peak was no longer detected. Meanwhile, the intensity of nonheme iron peak increased monotonically with increasing storage time. For the akami, as seen in Fig. 3, the intensity of metMb peak increased monotonically with storage time; that is, there was no decreasing trend. Meanwhile, the intensity of nonheme iron peak did not show a significant increase.

Figure 4(a) shows the changes in metMb of akami stored at −5, −10, and −15℃. The metMb content in akami stored at −5 and −10℃ initially increased until reaching a plateau at approximately 0.10 μmol/g. However, the rate of increase in metMb in akami stored at −15℃ was much lower, and as a result, the metMb levels could not reach a plateau. Only small amounts of nonheme iron were detected in akami, as shown in Fig. 4(b), which increased slightly throughout the storage time. The nonheme iron content stored at −10 and −15℃

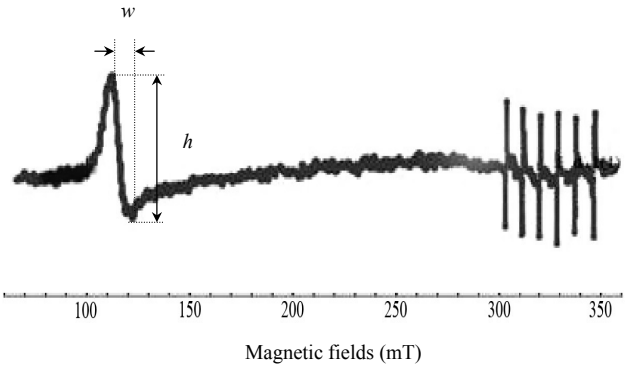

Fig. 1 EPR spectra of metMb of equine metMb solution. W and h represent the half-width and the height of the line of EPR signal of both metMb and nonheme iron, respectively.

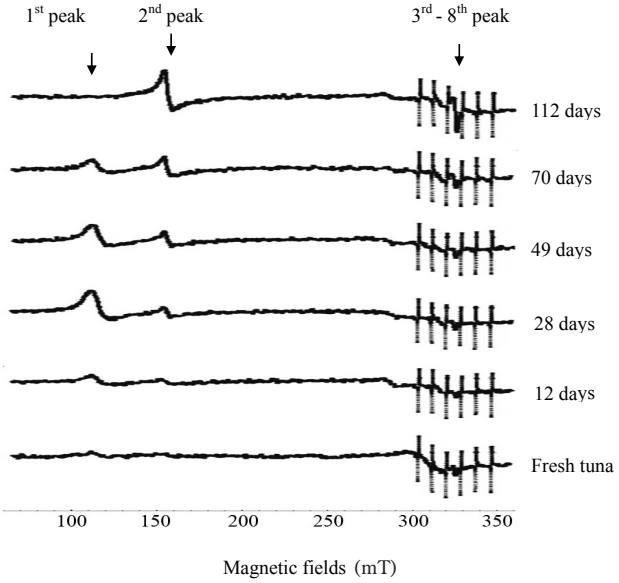

Fig. 2 Changes in EPR spectra of chu-toro stored at −10℃ for 0–112 days.

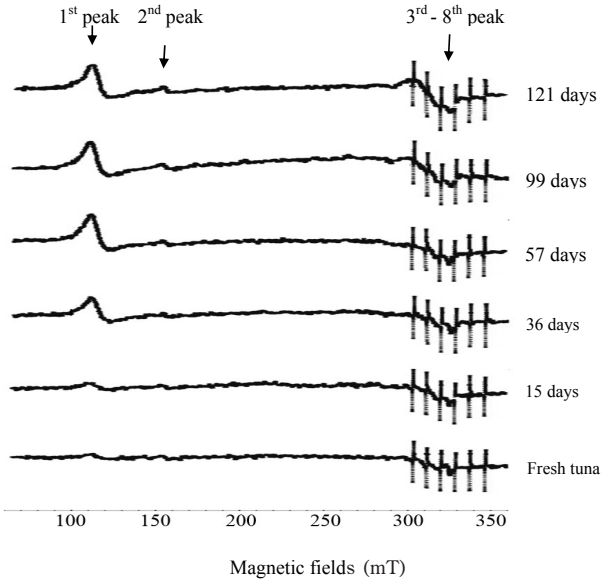

Fig. 3 Changes in EPR spectra of akami stored at −10℃ for 0–121 days.

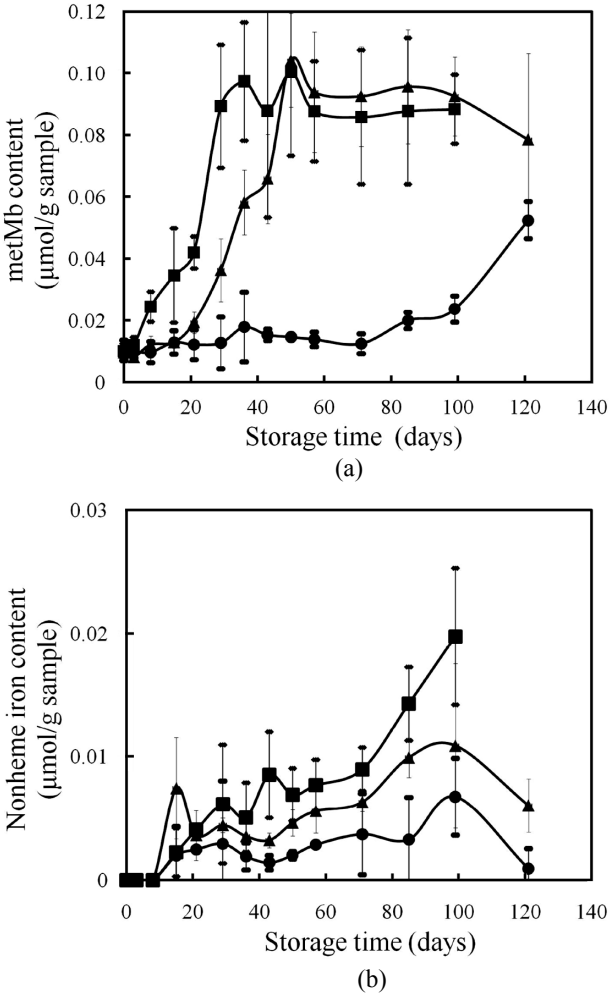

Fig. 4 Changes in metMb (a) and nonheme iron (b) content in akami during frozen storage at different temperatures where —■—, −5℃; —▲—, −10℃; —●—, −15℃.
The ranges of error bars indicate the highest and lowest values in triplicate measurements.

seems to decrease after the 120 days, however, it is thought insignificant because the values are within experimental error. The oxidation of myoglobin, in the early period of the storage, depends on the temperature. Although this trend agrees with many reports, the plateau appearance has not been previously reported. Previously, other researchers have studied autoxidation of myoglobin over short time periods, and have not been able to report changes in metMb content after reaching its maximum value [16,17]. In this study, we found that the metMb content in akami was relatively stable after reaching its plateau.

Figure 5(a) shows the changes in metMb of chu-toro stored at −5, −10, and −15℃. At storage temperatures above −15℃, the metMb content initially increased until reaching its maximum content and then gradually decreased as storage time was increased. However, at −15℃, metMb increased monotonically. The change in chu-toro nonheme iron content during frozen storage is shown in Fig. 5(b). Chu-toro nonheme iron content significantly increased from 0.010 to 0.035 μmol/g, except when stored at −15℃, where it increased only slightly. There was no similar report as for tuna meat, however, Gómez-Basauri and Regenstein [6] suggested that the degradation of heme during frozen storage in cod and mackerel is responsible for the increase in nonheme iron. That is, damage to globin molecules may affect the stability of the heme molecule, leading to the subsequent release of iron [18]. Gómez-Basauri and Regenstein [6], Benjakul and Bauer [19] and Chaijan *et al.* [20] also reported that the decrease in heme iron content in muscles for several kinds of fish except for tuna meat is inversely related to nonheme iron content. Therefore, as seen in our results, the decrease in chu-toro metMb content after peaking is considered to be due to the release of free iron from heme proteins as same as other fishes.

The difference in behavior of metMb and nonheme iron between in chu-toro and akami may be associated with the fat content in the samples. It was reported for general meat products and food models that lipid oxidation involving the degradation of polyunsaturated fatty acids and the generation of free radicals leads to the deterioration of proteins and the oxidation of heme pigments [21]. Also, hydrogen peroxide (H_2O_2) formation during lipid oxidation as well as free radicals can oxidize heme iron and release nonheme iron in oxymyoglobin solution system [22]. Furthermore, Igene *et al.* [23] reported that unheated extraction of beef heme pigment with H_2O_2 induced an increase of nonheme iron.

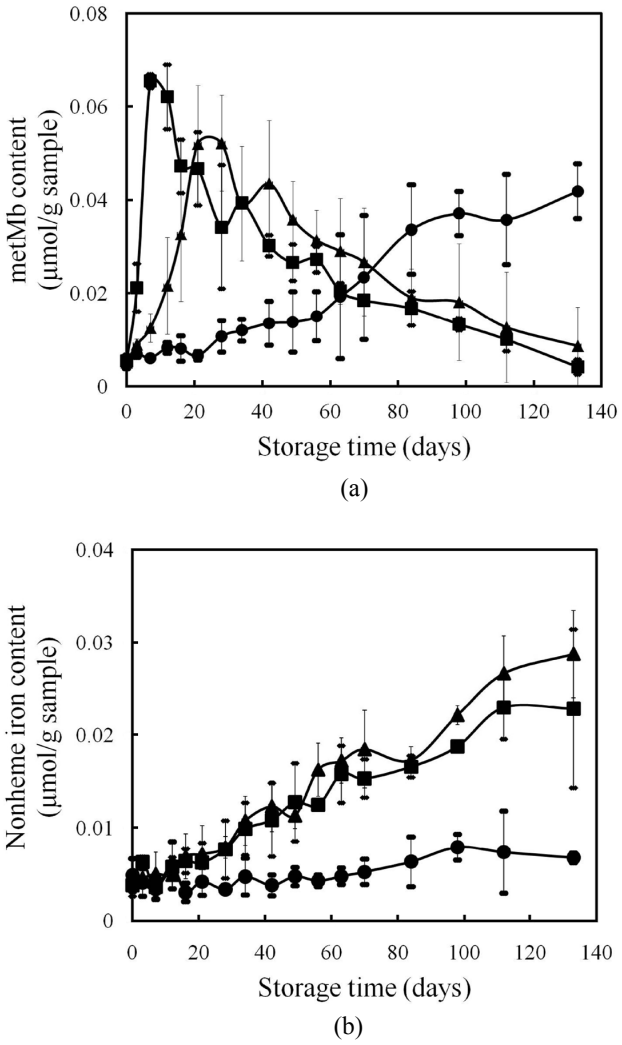

Fig. 5 Changes in metMb (a) and nonheme iron (b) content in chu-toro during frozen storage at different temperatures when —■— , -5°C; —▲— , -10°C; —●— , -15°C.
The ranges of error bars indicate the highest and lowest values in triplicate measurements.

Although these reports did not discuss about tuna meat, it can be considered that lipid would has similar affects for tuna meat. Therefore, damage to the porphyrin ring during storage induces the release of iron; thus, the low fat content in akami (0.76%) is thought to attenuate the oxidation of heme pigments after reaching the plateau. When comparing myoglobin autoxidation in the early period, before decreases in metMb were observed, the rate of myoglobin autoxidation in both samples decreased at lower storage temperatures (−15℃<−10℃<−5℃). Furthermore, the rate of autoxidation in the chu-toro was higher than the akami. Other researchers have reported similar results even for other meats or fishes. For example, Chan *et al.* [24] and Lee *et al.* [25] reported that metMb formation in horse and porcine solution is positively correlated to lipid oxidation. Also, Sohn *et al.* [26] reported that the rate of metMb formation, during ice storage for 48 h, in yellowtail dark muscle with lipid content of approximately 12.3 g/ 100 g was higher than in ordinary muscle with lipid content of approximately 4.1 g/ 100 g.

4. Conclusions

We have used the EPR method to simultaneously detect metMb and nonheme iron in nondestructive frozen tuna meat. From the result of EPR measurements, we demonstrated that metMb in tuna meat during frozen storage does not always exhibit monotonic increases, but occationally exhibits a decreasing phenomena after reaching maximal levels. The fact that at the same time the nonheme iron in tuna meat increases supports the contention that the mechanism involved in nonheme iron formation, subsequent to the formation of metMb during frozen storage, may be due to release from porphyrin rings of some of the metMb. Therefore, the total iron content from metMb and nonheme iron in tuna meat may be constant. In case of high fat content tuna meat, this phenomenon was highly remarkable. This suggests that the fat in tuna meat not only accelerates autoxidation of metMb but also affects the damage to the porphyrin ring and/or globin protein, causing the release of iron. However, in case of akami, the metMb content is constant after reaching maximum while nonheme iron content slightly increased. This may be due to the oxidation and further conversion of other iron compounds, such as insoluble fraction, ferritin, and low-molecular weight fraction.

From the perspective of meat color, our results suggest that the fatty tuna meat during long-term frozen storage may lose its dark red color. For a long time, the mechanism involved in the change in tuna meat color while frozen was unexplained despite being studied by many researchers. While the precise mechanism remains unclear, our results may provide a method by which this mysterious phenomenon can be elucidated. Furthermore, nonheme iron in meat has recently been focused on from a nutritional perspective, since it is known that heme iron can be absorbed more readily in *vivo* than nonheme iron [27]. Thus, our result showing the increases in nonheme iron during frozen storage is interesting. Further studies relating to nonheme iron, using EPR, are expected in the future.

References

1) M. N. O'Grady, F. J. Monahan, N. P. Brunton; Oxymyoglobin oxidation and lipid oxidation in bovine muscle–mechanism studies. J. Food Sci., **66**, 386–391 (2001).
2) Y. Thiansilakul, S. Benjakul, M. P. Richards; Isolation, characterisation and stability of myoglobin from Eastern little tuna (*Euthynnus affinis*) dark muscle. Food Chem., **124**, 254–261 (2011). in press.
3) M. Bito; Studies on the retention of meat color of frozen tuna–II. Effect of storage temperature on preventing discoloration of tuna meat during freezing storage. Nippon Suisan Gakkaishi. **31**, 534–539 (1965).
4) M. Bito; Studies on the retention on meat color of frozen tuna–V. Effect of freezing rate. Nippon Suisan Gakkaishi. **34**, 608–612 (1968).
5) K. Hashimoto, S. Watabe; Changes in color and water holding capacity of tuna meat during freezing storage. Nippon Suisan Gakkaishi, **49**, 203–206 (1983).
6) J. V. Gomez–Basauri, J. M. Regenstein; Processing and frozen storage effects on the iron content of cod and mackerel. J. Food Sci., **57**, 1332–1336 (1992).
7) B. R. Schricker, D. D. Miller, J. R. Stouffer; Measurement and content of nonheme and total iron in muscle. J. Food Sci., **47**, 740–743 (1982).
8) W. C. Franke, M. Solberg; Quantitative determination of metmyoglobin and total pigment in an intact meat sample using reflectance spectrophotometry. J. Food Sci., **36**, 515–519 (1971).
9) C. Viriyarattanasak, S. Matsukawa, N. Hamada–Sato, M. Watanabe, T. Suzuki; Quantitative measurement of metmyoglobin in tuna flesh via electron paramagnetic resonance. Food Chem., **111**, 1050–1056 (2008).
10) D. A. Svistunenko, M. A. Sharpe, P. Nicholls, M. T. Wilson and C. E. Cooper; A new method for quantitation of spin concentration by EPR spectroscopy: application to methemoglobin and metmyoglobin. J. Magn. Reson., **142**, 266–275 (2000).
11) G. Portilio, G. Federici, L. Calabrese, M. Costa, D. Cavallint; An Electron Paramagnetic Resonance study of the nonheme iron of cysteamine oxygenase. J. Biol. Chem., **245**, 6235–6236 (1970).
12) F. Bou–Abdallah, N. D. Chasteen; Spin concentration measurements of high–spin (g=4.3) rhombic iron(III) ions in biological samples: theory and application. J. Biol. Inorg. Chem., **13**, 15–24 (2008).
13) M. C. R. Symons; "Chemical and Biochemical Aspects of Electron Spin Resonance Spectroscopy" Wiley, London, 1978, pp. 1–90.
14) AOAC, Official Methods of Analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists, Arlington, 1990, p. 871.
15) T. Koriyama, T. Kohata, K. Watanabe, H. Abe; Chemical components of Bigeye tuna muscle and the effects of lipid on the taste. Nippon Suisan Gakkaishi, **66**, 462–468 (2000). (In Japanese)
16) C. Viriyarattanasak, M. Watanabe, T. Suzuki; Analysis of metmyoglobin formation rates in frozen tuna meat during frozen storage. Refrigeration, **24**, 227–233 (2007).
17) C. –J. Chow, Y. Ochiai, S. Watabe; Effect of frozen temperature of autoxidation and aggregation of bluefin tuna myoglobin in solution. Food Biochem., **28**, 123–134 (2004).
18) M. Estévez, R. Cava; Lipid and protein oxidation, release of iron from heme molecule and colour deterioration during refrigerated storage of liver pâté. Meat Sci., **68**, 551–558 (2004).
19) S. Benjakul, F. B Bauer; Biochemical and physicochemical changes in catfish (*Silurus glanis* Linne) muscle as influenced by different freeze–thaw cycles. Food Chem., **72**, 207–217 (2001).
20) M. Chaijan, S. Benjakul, W. Visessanguan, C. Faustman; Changes of pigments and colour in sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) muscle during iced storage. Food Chem., **93**, 607–617 (2005).
21) J. Kanner; Oxidative processes in meat and meat products: quality implications. Meat Sci., **36**, 169–186 (1994).
22) Y. Satoh, K. Shikama; Autoxidation of oxymyoglobin. J. Biol. Chem., **256**, 10272–10275 (1981).
23) J. O. Igene, J. A. King, A. M. Peason, J. I. Gray; Influence of heme pigments, nitrite, and nonheme iron on development of warmed–over flavor (WOF) in cooked meat. J. Agric. Food Chem., **27**, 838–842 (1979).
24) W. K. M. Chan, C. Faustman, M. Yin, E. A. Decker; Lipid oxidation induced by oxymyoglobin with involvement of H_2O_2 and superoxide anion. Meat Sci., **46**, 181–190 (1997).
25) S. Lee, A. L. Phillipps, D. C. Liebler, C. Faustman; Porcine oxymyoglobin and lipid oxidation in vitro. Meat Sci., **63**, 241–247 (2003).
26) J. H. Sohn, H. Ushio, N. Ishida, M. Yamashita, M. Terayama, T. Ohshima; Effect of bleeding treatment and perfusion of yellowtail on lipid oxidation in post–mortem muscle. Food Chem., **104**, 962–970 (2007).
27) T. H. Bothwell, R. W. Charlton, J. D. Cook, C. A. Finch; "Iron Metabolism in Man" Blackwell Scientific Publications, Oxford, 1979, p 256.

「日本食品工学会誌」, Vol. 11, No. 3, p. 139, Sep. 2010

◇◇◇ 和文要約 ◇◇◇

冷凍マグロ肉保管中のメトミオグロビンと遊離 Fe^{3+} の消長に関する EPR による研究

Pantira HEMPATTARASUWAN[1], Chotika VIRIYARATTANASAK[2],
松川真吾[1], 渡辺　学[1], 鈴木　徹[1,†]

[1]東京海洋大学食品生産科学科,
[2]*Department of Food Engineering, Faculty of Engineering, King Mongkut's University of Technology Thonburi*

生食用マグロ肉の色調は商品価値にとって重要な因子である．新鮮なマグロ肉はオキシミオグロビンあるいはデオキシミオグロビンにより比較的明るい赤色を呈するが，保存期間中にミオグロビン色素のポルフィリン環の中心部に配座する Fe^{2+} が自動酸化を受け Fe^{+3} に変化し色調が暗褐色に変化するとされる．この現象は一般的な冷凍保管温度−18℃においても比較的急速に進行するため，マグロ流通では−50℃以下の保管温度が利用されている．しかしながら，凍結保管中におけるマグロ筋肉内における色調の変化についてはいまだに不明点が多く残されている．その原因の1つはメトミオグロビンの消長に関連する反応機構の全容が明らかにされていないこと，またメトミオグロビン自体の定量手法の不備が挙げられる．

本研究では，これまで筆者らのグループで取り組んできた Fe^{3+} の検出を可能とする電子スピン共鳴（EPR）による凍結マグロ筋肉中のメトミオグロビンの定量研究［9］を発展させ，凍結保管中のマグロ筋肉内メトミオグロビンの消長，またフリーな Fe^{3+} 量の変動についても同時に検討した．さらにそれらの挙動に与える筋肉内脂質の影響についても検討した．すなわち，未凍結生鮮メバチマグロ肉から脂質含量0.8%の赤身部と脂質含量7.4%の中トロ部を所定のサイズに切り出し−5，−10，−15℃で凍結庫に4ヶ月間保管し，その間，凍結状態（測定時温度−150℃）のまま試料の EPR スペクトルを測定した．EPR 測定には Mn^{2+} を基準物質（マーカー）として用い，JES-TE300（JEOS Co.,）にて X-band で測定を行った．一方，あらかじめ種々な濃度に調整したメトミオグロビンおよびフリーな Fe^{3+} を含む標準溶液 を調製し，試料測定時と同じ条件で EPR 測定を行い，メトミオグロビンおよびフリーな Fe^{3+} 由来のそれぞれのピークの Mn^{2+} ピーク強度に対する相対強度値を求め，それぞれの濃度とピーク強度の検量線を作成した．この検量線を用いて，試料中のメトミオグロビン含量およびフリーな Fe^{3+} の絶対量を求めた．

その結果，−5℃，−10℃保管では赤身，中トロ肉いずれも初期にはメトミオグロビン量は増大するが，赤身肉では30日前後で0.1 μmol/g を上限値として変化が無くなった．一方，中トロ肉ではメトミオグロビン含量は初期に急速な増加を示し，−5℃で約10日，−10℃で20日で最大値を示した後，急激な減少に転じることがわかった．しかし，−15℃保管では，赤身，中トロいずれの場合にも緩慢な上昇を示し，試験期間中には最大値，上限値に至らなかった．また，本研究ではいずれの温度でもフリーな Fe^{3+} が凍結保管中にも増加することが示された．この増加は保管温度が高いほど速いが，とくに脂質の多い中トロでは，その増大が著しく顕著であった．したがって，脂質の多いマグロ筋肉内では，ミオグロビンはいったんメトミオグロビンとなり，さらに脂質の影響により Fe^{3+} の遊離が起きるためメトミオグロビンが減少するといった連続した反応機構があることが示唆された．これら知見は凍結マグロ肉の色調の変化を予測，保管条件を決定する上での基礎となると考えられる．

（受付 2010 年 5 月 28 日，受理 2010 年 7 月 28 日）

1 〒108-8477　東京都港区港南4-5-7
2 126 Pracha u-tid Road, Tungkru, Bangkok 10140, Thailand
† Fax: 03-5463-0585, Email: toru@kaiyodai.ac.jp