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Assessment of Commercial Quality Evaluation of Yellowfin Tuna *Thunnus albacares* Meat Based on Myoglobin Properties

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Four quality grades (excellent, good, acceptable, and "not acceptable") of yellowfin tuna meat (*Thunnus albacares*), as judged by a professional appraiser, were compared based on the red/ox state and extractability of myoglobin (Mb). As a result, the metMb ratio of the "not acceptable" grade of meat was significantly higher than that of the other higher-grade samples. In contrast, the highest ratio of oxyMb was found in the "excellent" meat, followed by good > acceptable > "not acceptable" meats. Color measurement revealed significant differences in *a value between the different grades of meat, but showed essentially no difference in *L** and *b** values. Both *a** value and redness index (*a**/*b**) showed high correlation coefficients with metMb ratio. Mb extractability tended to be higher in the higher grade of meat. In conclusion, the commercial appraisal of tuna meat quality was demonstrated to be reliable.**

Keywords: tuna, meat, quality, color, myoglobin

Introduction

Seven species of tunas are consumed around the world. Yellowfin tuna (*Thunnus albacores*), whose meat is characterized by a light red color and less fat, is one of the most commercially important and favored tuna species. This scombridae species inhabits tropical and subtropical waters around the world. Therefore, this species is generally exported to Japan from tropical countries, such as Indonesia, Taiwan, and so on (MMAF, 2012). A quality check is frequently performed before processing and transportation. Sensory analyses performed by experienced appraisers are generally carried out to determine freshness as a composite of qualitative traits. Color is one of the most important factors affecting consumer preferences. The meat color of tuna is closely related with myoglobin (Mb) content and the red/ox state of the heme iron.

Mb is a small globular protein whose biological function is basically to temporarily store oxygen in skeletal and cardiac muscles for facilitation of respiration (Livingston *et al.*, 1983), though new functions of Mb have been recently reported, such as nitrogen oxide scavenging activity (Cossins and Berenbrink, 2008; Flögel *et al.*, 2010; Helbo *et al.*, 2012). Generally, Mb content changes dependent on animal species, muscle part, age, and diet of the animal. Pale colored meat, such as chicken and pork, generally contain lower concentrations of Mb compared with red colored meat such as beef. Oxidative muscles, namely, slow skeletal muscle (including the dark muscle of fish) and heart muscle, contain abundant Mb. In scombridae fish such as tuna, abundant Mb is also found in fast skeletal muscle (also referred to as ordinary muscle, light muscle or white muscle).

An iron atom of heme is placed in the hydrophobic region of Mb, binding the imidazole group of proximal histidine directly and distal histidine through a coordinate bond (Phillips and Schoenboen, 1981). The binding of oxygen, as well as the state of an iron atom in the heme pocket, contributes to the meat color; the Fe^{2+} form appears bright red and purple in color while the Fe^{3+} one appears brown.

Many factors, such as catch method, slaughter technique, handling, and storage conditions, influence tuna meat quality. Many attempts have been made to investigate the quality of fish and meat (Chow *et al.*, 1985, 2009; Trout, 1989; Chen and Chow, 2001; Duran *et al.*, 2008; Faustman, 2010;

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Imamura *et al.*, 2012). However, the reliability of quality ranking by sensory evaluation has not yet been well documented to date. Thus, the objective of this study was to investigate the reliability of quality grading of yellowfin tuna meat by measuring color and examining the characteristics of Mb derivatives.

Materials and Methods

Sample preparation Fresh yellowfin tuna specimens, caught by long-line fishing in the Celebes Sea and landed at the Port of Bitung, Sulawesi, Indonesia, in August of 2009, were obtained. Samples were ranked into four categories by sensory evaluation (mostly according to appearance, odor, and finger touch) of a professional qualified appraiser belonging to a tuna meat processing company (Nutrindo Fishery Co.); namely, excellent, good, acceptable, and "not acceptable" grades, the basic grading for Japanese and US markets (Fig. 1). Ten different individuals were obtained for each group. The meat (fast skeletal muscle) was sampled from the dorsal part of the fish, wrapped in polyethylene film and immediately transported on ice to our laboratory in Tokyo (via jet plane within one day). Samples were stored at −80°C until used for the experiment (for up to 6 months). Just before the experiment, the meat in the vinyl bag was thawed in tap water and immediately subjected to the following analyses.

Chemicals All the chemicals used in this study were of reagent grade, purchased through Wako Co., Otsu, Japan.

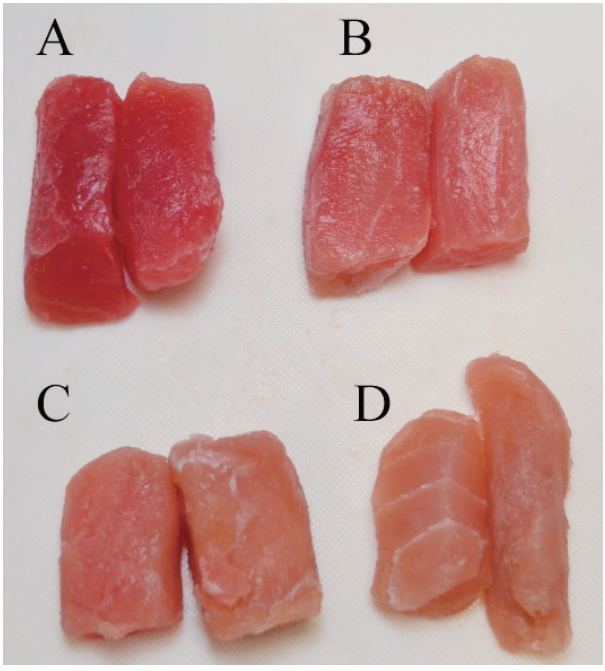

Fig. 1. Appearance of the tuna meat slice of each grade. A, excellent; B, good; C, acceptable; D, "not acceptable". Note that the redness is more enhanced in the higher quality meat.

Determination of Mb derivatives The following procedures were carried out at 0 − 4°C unless otherwise stated. The meat slice samples were homogenized with 7 volumes of ice cold water under moderate speed for 1 min using a homogenizer (Polytron model PT 10 − 35, Kinematica AG, Littau, Switzerland). The supernatants obtained by centrifugation at 3000 g for 15 min were filtered through filter paper (No.2, Advantec Co., Tokyo, Japan), and subsequently filtered through a cartridge filter (0.20 μm pore size, RC 15; Sartorius, Goettingen, Germany). Visible spectra were measured in the range of 380 nm to 780 nm using a spectrophotometer (V-630 Bio, Jasco, Tokyo, Japan). The ratio of Mb derivatives (deoxyMb, oxyMb and metMb) was calculated based on absorbance at 380 and 780 nm (Tang *et al.*, 2004). The measurement was performed in duplicate for ten meat samples for each grade.

Electrophoresis SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on the water-soluble fraction basically according to Laemmli (1970). After the run, the gel was stained with Coomassie Brilliant Blue (CBB) R-250. Equal volumes of the extracts from the different grades of meat were applied to the gel.

Measurement of Mb extractability The water-soluble fraction (prepared as described above) was used for the measurement of Mb extractability, as a parameter for the extent of Mb insolubilization (Chen and Chow 2001; Chow *et al.*, 2009). To 1 mL of the extract was added 0.5 mL of 25 mM potassium buffer (pH 7.0) and gently mixed. Next, 25 μL of 5 % $NaNO_3$ was added, followed by addition of 25 μL of 1 % KCN. After incubation of the mixture at room temperature for 1 min, the absorbance of the mixture was measured at 540 nm (A_{540}). To calculate the concentration of Mb, the molecular extinction coefficient (11300) and molecular weight (16000) were used to facilitate calculation (Chow *et al.*, 2009). Mb concentration was calculated using the following equation.

$$\text{Extractability of Mb (mg/100 g)} = A540 \times 2 \times 16000$$

Meat color The color of the meat slice samples was measured with a colorimeter (NF333, Nippon Denshoku Co., Tokyo, Japan). The tristimulus (L^*, a^*, and b^*) values (Hunt, 1977) were measured in triplicate for each sample meat slice. The redness index (a^*/b^*) was also adopted to evaluate the meat color according to Chen *et al.* (1997). The correlation between metMb ratio with a^*, and metMb ratio with a^*/b^* were examined as described by Ochiai *et al.* (1988).

Statistical analysis JMP 7.0.2 (SAS Institute, Cary, NC, USA) was used for all the statistical analyses. Experimental results were provided as mean values with standard deviations. The data were analyzed using one-way analysis of

variance (ANOVA). Differences among the different grades of tuna meat were analyzed using the Tukey-Kramer test (Steel and Torrie, 1988), and statistical significance was determined at $P < 0.01$ and $P < 0.05$ levels.

Results and Discussion

As shown in Fig. 1, high quality meat is distinguishable by its vivid red color, but as deterioration proceeds, the redness is reduced and a whitish color becomes pronounced. The quality of yellowfin tuna meat was found to remain basically unchanged during storage at −84°C for up to approx. one month, as demonstrated by parameters such as K value and pH (Agustini *et al.*, 2001). However, slight discoloration of tuna meat during freezing/thawing has been reported (Chow *et al.*, 1988). Moreover, the lower grade meat also emitted a fishy odor (data not shown).

Mb derivatives ratio In this study, the relation between quality and Mb derivatives of yellowfin tuna meat was investigated. The deterioration of meats is partly induced by metMb formation, resulting in browning of the meat. The ratio of Mb derivatives, namely, deoxyMb, oxyMb, and metMb was determined based on the spectrophotometric absorption of the meat extract. The grade of meat was significantly correlated with metMb ratio (%) (Fig. 2a); *i.e.*, the value was the lowest in the excellent grade meat, and gradually increased as the grade of meat decreased. However, the values for the good and acceptable grades were similar to each other, with no significant difference between them.

The oxyMb ratio was inversely related with metMb ratio (Fig. 2b). Both metMb and oxyMb ratios could be good parameters indicative of the quality of tuna meat. As far as the deoxyMb ratio is concerned, no significant correlation with meat quality was recognized, except for the excellent and "not acceptable" meats (Fig. 2c). The ratio of deoxyMb tended to be higher, probably because of the exposure of the meat samples to the ambient air during transportation to the lab, even though the samples had been kept in vinyl bags and ice-stored as described above. This value corresponds to the value observed at partial oxygen pressure of ~ 9 mm Hg (Faustman and Cassens, 1990), suggesting a slight decrease of deoxyMb during handling of the samples in the present study.

Oxygen binds to the sixth coordination site of ferrous heme, appearing bright red color on the surface. Conversion of ferrous oxyMb to ferric metMb, via autoxidation, causes browning of the meat and is considered undesirable by consumers. Therefore, maintenance of the bright red meat color, representing freshness, occurs mainly through managing the red/ox state of an iron atom by reducing the Mb autoxidation rate. In this study, metMb ratio of the excellent grade tuna meat was approximately 18%, suggesting this meat grade was very fresh. The value is comparable to that of bigeye tuna (*Thunnus obesus*) meat frozen by air-blast freezing at −70°C for 48 h and stored at −55°C, which was reported to be 20 − 25% (Imamura *et al.*, 2012).

SDS-PAGE Figure 3 shows the SDS-PAGE patterns of the water-soluble fractions of yellowfin tuna meat of different quality grades. The molecular weight of yellowfin tuna Mb without posttranslational modification is 15574, as reported by Lee *et al.* (2003). This band (Mb) is highlighted in the figure with an arrow. Irrespective of the quality grade, the protein fraction showed similar electrophoretic patterns, except for the densities of the bands X and Y indicated in the figure. Band X, which is considered to be a decomposed

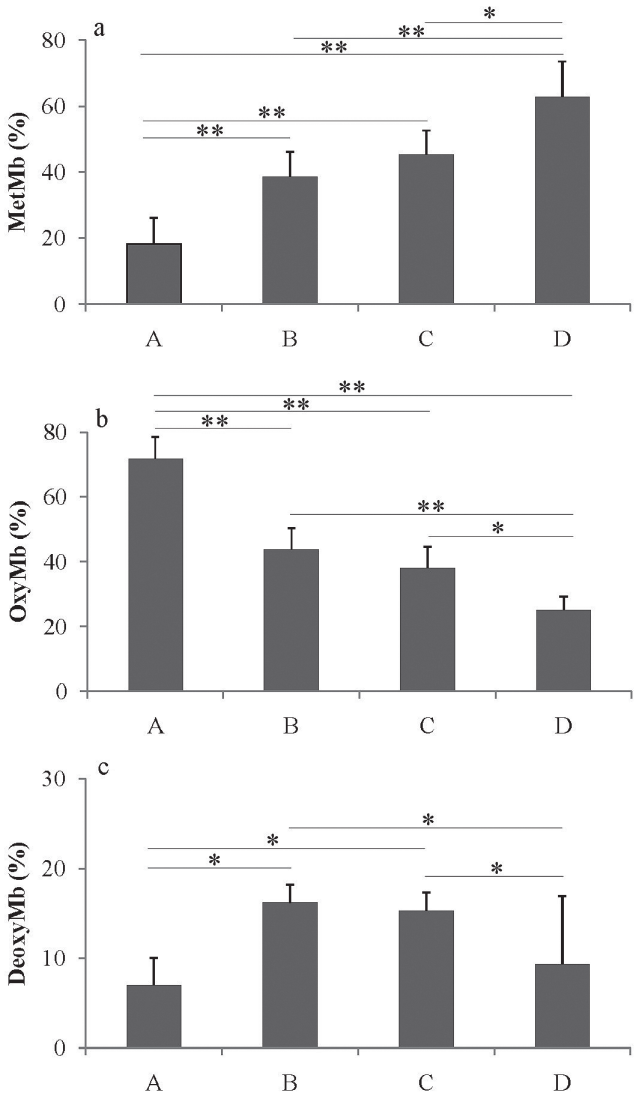

Fig. 2. Mb derivatives ratios of different quality grades of tuna meat. A, excellent; B, good; C, acceptable; D, "not acceptable". a, metMb ratio; b, oxyMb ratio; c, deoxyMb ratio. The asterisks represent significant differences (**$P < 0.01$ and *$P < 0.05$). Data are shown as average with standard deviation ($n = 10$ for each grade of meat).

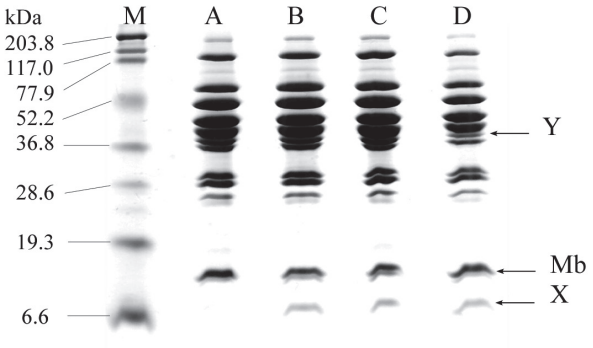

Fig. 3. SDS-PAGE patterns of the water-soluble fractions from different quality grades of tuna meat (light muscle). M: molecular weight marker. A, excellent; B, good; C, acceptable; D, "not acceptable". 15 % gel.

protein, was present except for in the excellent grade meat, while the density of band Y, approx. 40 kDa, (including the one just below this band) was clearly low in the "not acceptable" grade meat. In this grade, there were also other bands of lower staining intensities compared to those of higher-grade meat. This seems to be the result of protein denaturation in the low quality meat. Stagg *et al.* (2012) observed protein degradation triggered by acidification and high temperature in the muscle of skipjack (*Katsuwonus pelamis*). It was concluded that meats of excellent and "not acceptable" quality grades are clearly distinguishable from each other.

The results further confirmed the reliability of sensory evaluation, although differences between meats of good and acceptable grades were not recognized. In this context, in the case of bluefin tuna (*Thunnus orientalis*), the protein band of about 50 kDa, which is considered to be creatine kinase, decreased in low quality meat or burnt meat (Ochiai, 2010). In the case of yellowfin tuna, band Y could be a parameter of meat quality. Creatine kinase has already been characterized as an indicator of physical stress and muscle damage in livestock animal production (Daroit and Brandelli, 2008).

Color measurement The L^*, a^* and b^* values of the different quality grades of tuna meat are shown in Fig. 4. The L^* value did not differ significantly between the different meat quality grades (Fig. 4a). The values were around 30, with no significant differences, and therefore, did not reflect meat quality. On the other hand, the a^* value tended to be smaller in the lower grades of meat (Fig. 4b). The values were significantly different ($P < 0.01$) between any combinations of meat quality grades. The result is quite reasonable, since the value is roughly proportional to the optical manifestation of meat redness (Fig. 1). It was concluded that a^* value is an excellent parameter to distinguish meat quality. In the case of b^* value, there were almost no significant differences between the meat of different quality grades (Fig. 4c). Previous studies on bluefin tuna meat disclosed that Mb

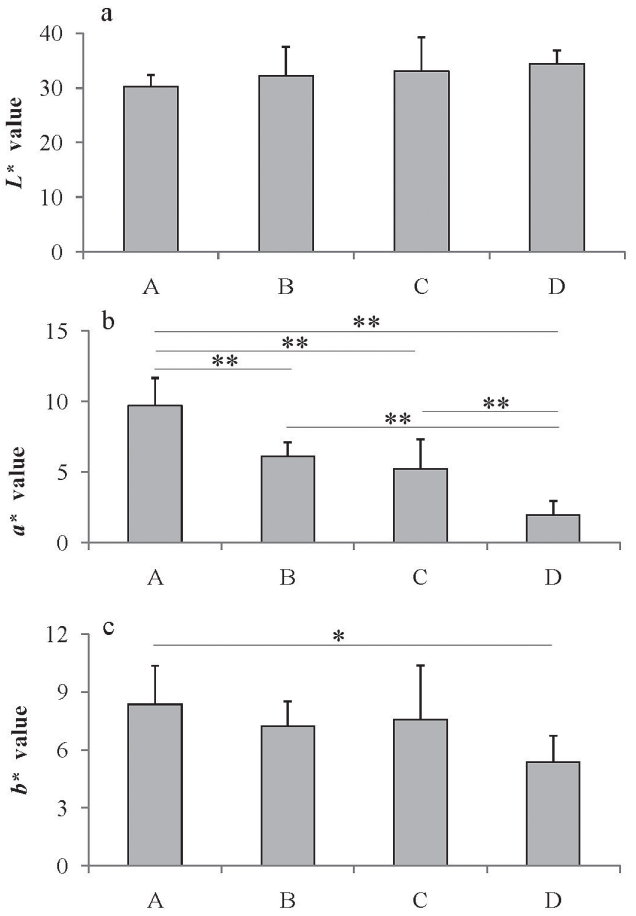

Fig. 4. Differences in the tristimulus values of different quality grades of tuna meat. A, excellent; B, good; C, acceptable; D, "not acceptable". a, L^* value; b, a^* value; c, b^* value. The asterisks represent significant differences (**$P < 0.01$ and *$P < 0.05$). Data are shown as average with standard deviation ($n = 10$ for each grade).

oxidation resulted in increased L^* and decreased a^* values (Chow *et al.*, 1990, Chen and Chow, 2001). This discrepancy might be due to the lower Mb content of yellowfin tuna meat.

Next, the redness index (a^*/b^*) was calculated using the data in Fig. 4, and was compared between the different grades (Fig. 5). The values were found to be significantly different between the different meat quality grades. Even between the B and C meat grades, a significant difference ($P < 0.05$) was observed. This value clearly represented differences in the quality of tuna meat.

The correlations of a^* value and redness index against metMb ratio (%) value are shown in Fig. 6. The correlation coefficients were −0.999 and −0.997 for a^* and redness index against metMb ratio, respectively. These results are quite similar to previous reports on fish Mb (Chow *et al.*, 1988; Ochiai *et al.*, 1988; Chen and Chow, 2001).

Extractability of Mb Mb concentrations of the water extracts, namely the extractability of Mb from the different

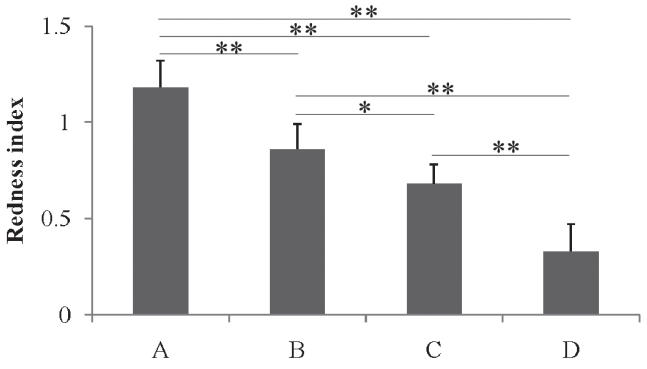

Fig. 5. The redness index (a^*/b^*) of different quality grades of tuna meat. A, excellent; B, good; C, acceptable; D, "not acceptable". The asterisks represent significant differences (**$P < 0.01$ and *$P < 0.05$). Data are shown as average with standard deviation ($n = 10$ for each grade).

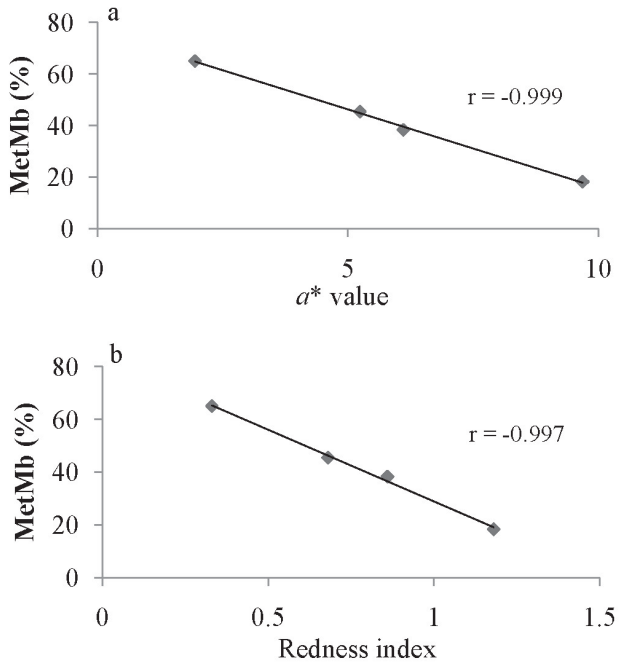

Fig. 6. Correlation between color values and metMb ratio (%). a, between a^* value and metMb ratio (%); b, between the redness index (a^*/b^*) and metMb ratio (%).

grades of meat, are shown in Fig. 7. Significant differences were found between any combinations of meat grades. Mb concentration is also an excellent parameter of meat quality grade. Mb concentration should be very similar in a given species (Brown, 2006); therefore, it is likely that Mb was partially denatured and insolubilized. The extractability of Mb was reduced in lower grades of meat. The absolute contents of oxyMb, calculated based on the data of Figs. 2 and 7, were 157 ± 26.5, 63.7 ± 11.5, 50.6 ± 8.0 and 22.6 ± 5.5 mg/100 g for the excellent, good, acceptable and "not acceptable" meats, respectively. The values seem to appropriately demonstrate quantitatively the quality of each grade. As shown in Fig. 8, a close relationship was observed between the a^* value of meat and the Mb concentration of the water extract (r = −0.990).

These significant differences in Mb derivatives ratio could have been due to differences in the freshness of the fish. Namely, in the case of long-line fishing, the time required for harvesting fish on board the ships differs greatly, because there are so many hooks on the line. Some fish are harvested alive, while others are dead and remain in the warm seawater for a long time. For this reason, it is impossible to harvest fish of similar freshness using this fishing method. The ranking of fish quality before shipping is thus very important.

As described above, tuna meat quality deteriorates under sub-optimal storage conditions. In the case of yellowfin tuna, low meat quality is considered to result from prolonged incubation of the catch in the warm seawater postmortem. Body temperature is generally maintained at a higher temperature than that of ambient seawater (Carey and Teal, 1966). Muscle pH is also considered to decrease in postmortem tuna meat (Roy *et al.*, 2011). Tuna Mb is quite unstable at a high temperature range and under low pH (Chow *et al.*, 1989). Preincubation of purified tuna Mb at 40°C and pH 6 resulted in its structural decay (Ochiai, 2011), suggesting that Mb in the yellowfin tuna meat was denatured.

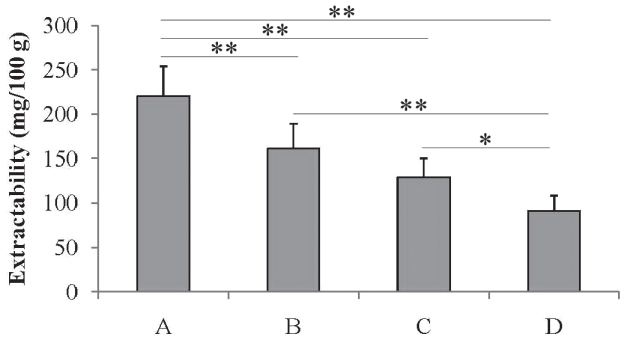

Fig. 7. Mb extractability in meat of different quality grades. A, excellent; B, good; C, acceptable; D, "not acceptable". The asterisks represent significant differences (**$P < 0.01$ and *$P < 0.05$). Data are shown as average with standard deviation ($n = 10$ for each grade).

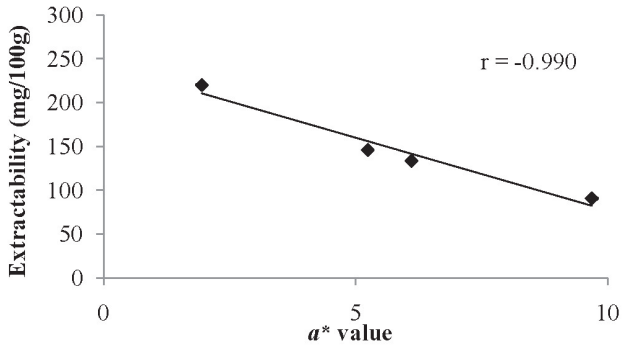

Fig. 8. Correlation between Mb extractability and a^* value.

In order to understand the molecular mechanisms involved in postmortem quality deterioration of red colored meat, it would be advantageous to characterize Mb properties. Mammalian Mbs have eight α-helical segments, designated A through H. However, the D-helix is missing in fish Mbs, as reported by Birnbaum *et al.* (1994). This appears to be the reason why fish Mbs are less stable than their mammalian counterparts (Ochiai *et al.*, 2010). The thermal denaturation of tuna Mb consists of three stages, based on the measurement of α-helical content by circular dichroism (CD) spectrometry (Ochiai *et al.*, 2010). In addition, fish Mb contains a cysteine residue, which is absent in mammalian Mbs (Livingston and Brown, 1981). This could be the reason for the higher aggregation tendency of fish Mbs (Chow *et al.*, 1989; Faustman and Cassens, 1990).

Previous studies reported that the structural stabilities of scombridae fish Mbs clearly differ among species, although the sequence identities of amino acids were in the range of 91 − 99% (Ueki *et al.*, 2004; 2005; 2006). Among them, skipjack tuna Mb was the most thermostable, and bullet tuna (*Auxis rochei*) Mb showed the lowest stability. Thermal stabilities of tuna Mbs, including that of yellowfin tuna, fall in between those of skipjack tuna and bullet tuna Mbs (Ueki *et al.*, 2004). Slight differences in stability have also been reported among tuna Mbs (Chow, 1991). In spite of these facts, tuna Mbs are considered to be more stable than those of stenothermal fish species, as demonstrated by differences in the autoxidation rate (Madden *et al.*, 2004).

The mechanism responsible for Mb aggregation has been partly characterized; Mb itself tends to self-aggregate (Chow *et al.*, 1989), whereas it has been reported that, in postmortem meat, Mb binds to myofibrillar proteins (Chajian *et al.*, 2008) or reacts with fatty acids (Suman *et al.*, 2007).

Conclusions

The quality of yellowfin tuna as evaluated using sensory tests by a professional appraiser was found to be quite reliable based on the results of Mb derivatives ratio, color measurement, Mb concentration, and electrophoretic patterns of the water soluble protein fraction. It follows that even untrained persons can judge the meat quality grade by using these methods. Meanwhile, it is very important to establish how the high quality of meat can be maintained. First of all, the method of harvesting fish should be improved, while handling, storage and transportation conditions also require reconsideration.

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